



February 19, 2002

TO: Robert Steeves, J.D.
Executive Secretary, DHHS Orphan Products Board

FROM: Acting Director, National Institutes of Health

SUBJECT: Report on the Rare Diseases and Conditions Research Activities of the
National Institutes of Health - FY 2000

The Orphan Drug Act requires the Director, National Institutes of Health (NIH), to submit to the DHHS Orphan Products Board (OPB) a report on the rare diseases and conditions research activities supported by the NIH. I am pleased to submit this report for FY 2000 for your review and submission to Congress. The report highlights rare diseases research advances from both the intramural and sponsored extramural research programs.

Ruth L. Kirschstein, M.D.

Attachment:
Report on the Rare Diseases and Conditions Research Activities of the NIH - FY 2000



February 11, 2002

TO: Acting Director, NIH
Through: Associate Director for Disease Prevention, OD
Barnett S. Kramer, M.D., M.P.H.

FROM: Acting Director, Office of Rare Diseases

SUBJECT: Report on the Rare Diseases and Conditions Research Activities
of the National Institutes of Health - FY 2000

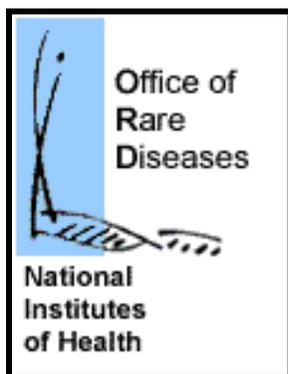
The Orphan Drug Act requires the Director, National Institutes of Health (NIH) to submit to the Department of Health and Human Services' (DHHS) Orphan Products Board a report on the rare diseases and conditions research activities supported by the NIH. The report for FY 2000 is attached for your comment or concurrence prior to submitting it to the Executive Secretary of the DHHS Orphan Products Board.

The Institutes and Centers (ICs) provided this information through each of their Rare Diseases Coordinators, with approval from their respective IC Director.

Henrietta Hyatt-Knorr

Attachment A - Transmittal Memorandum from the Principal Deputy Director of NIH to the Executive Secretary, DHHS Orphan Products Board

Attachment B - Report on the Rare Diseases and Conditions Research Activities of the NIH - FY 2000



Annual Report on the Rare Diseases and Conditions Research Activities of the National Institutes of Health

FY 2000

**Annual Report on the Rare Diseases
and Conditions Research Activities of
the National Institutes of Health**

FY 2000

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Executive Summary

Research on Rare Diseases Supported by NIH: FY 2000

The Orphan Drug Act of 1983 requires the Director of the National Institutes of Health (NIH) to submit a report on the rare diseases and conditions research activities supported by NIH to the Department of Health and Human Services Orphan Products Board. The rare diseases research programs sponsored by NIH Institutes and Centers (ICs) are well-established and well-recognized. These basic, clinical research, and training programs continue to contribute to the development and dissemination of information on the prevention, etiology, diagnosis, and treatment of rare diseases and conditions.

This report presents the contributions and research advances of the Fiscal Year 2000 NIH extramural and intramural research programs and the Office of Rare Diseases (ORD). Responses from the individual ICs and ORD highlight four major areas: 1) an overview of ongoing rare diseases research activities, 2) recent scientific advances in rare diseases research, 3) new or planned rare diseases research initiatives, and 4) rare diseases-related activities such as workshops or symposia.

Many advances presented in this report are the direct result of years of basic rare diseases research sponsored by NIH. Patients with rare diseases have and continue to benefit from the treatment applications realized from the emphasis placed on both basic and clinical research by NIH.

This report uses the definition of a rare diseases set forth in the Orphan Drug Act: a disease or condition with a prevalence of fewer than 200,000 persons in the United States. Prevalence refers to the number of persons alive with the rare disease.

Activities undertaken in 2000 by ORD included co-sponsoring 52 scientific workshops and symposia, all of which are listed in this report. Outcomes of the workshops in past years included establishment of research priorities, development of program announcements, establishment of diagnostic and monitoring criteria, development of animal models, support of patient and tissue registries, development of research protocols and collaborative research arrangements, and dissemination of workshop results.

National Institute on Aging (NIA)

Overview of NIA Rare Disease Research Activities

NIA conducts and supports biomedical, social and behavioral research, training, health information dissemination, and other programs with respect to the aging process. Although NIA does not focus on rare diseases per se, certain rare conditions/diseases are studied as they relate to the process of aging or the diseases of aging. Of particular interest are progeroid syndromes, such as Werner syndrome (WS), Bloom syndrome (BS), and Cockayne syndrome (CS), which have implications for age-related diseases.

Recent Scientific Advances in Rare Disease Research

Werner Syndrome (WS)

WS is a rare autosomal recessive disorder characterized by genome instability and premature onset of several age-related diseases, including atherosclerosis, osteoporosis, type II diabetes mellitus, malignant neoplasm, and cataracts. The cells derived from WS patients also display shorter life spans than those from normal individuals of similar ages. Because of these features, WS has been considered a good model for studies of age-related symptoms.

The gene defective in WS encodes a deoxyribonucleic acid (DNA) helicase that belongs to the RecQ helicase family. Many studies of the WS gene product (*WRN*) have suggested that it plays a role in some aspects of DNA metabolism, although the exact function of the *WRN* within the cell is unknown.

NIA intramural scientists have purified a multisubunit complex from human cells that contain *WRN*. The researchers were able to identify most of the components of this complex, and results suggest there are functions for *WRN* at discrete steps of DNA repair and maintenance. Other studies have demonstrated that *WRN* preferentially unwinds forked-duplex DNA substrates and also actively unwinds triple helical DNA.

A number of important protein interactions have been identified for *WRN*. Intramural investigators have demonstrated the first physical and functional interaction of *WRN* helicase with the single-stranded DNA-binding protein replication protein A (RPA). *WRN* physically interacts with the Ku heterodimer implicated in double strand break repair. These and other protein interactions of *WRN* are currently under investigation and, it is hoped, will provide clues to the molecular-genetic role of *WRN* protein in vivo.

Soon after the *WRN* gene was cloned and sequenced, it was shown that a single nucleotide polymorphism (SNP) at amino acid position 1367 significantly affects the risk of myocardial infarction. In addition, cells obtained from WS patients with mutations in the *WRN* helicase gene exhibit increased chromosome instability and hypermutability. In yeast, loss of the *SGS1* gene, a homolog of the human WRN helicase, reduces life span and causes genomic instability. Using three newly identified temperature-sensitive alleles of *SGS1*, NIA extramural investigator Dr. Steven Brill can now search for potential defects in DNA replication, DNA repair, and recombination due to the thermal inactivation of Sgs1p in both wild-type and *SLX* mutant backgrounds. A total of seven *SLX* genes have now been identified. Mutations in any of the *SLX* genes in combination with *Sgs1* mutations are lethal in yeast cells.

Bloom Syndrome (BS)

BS is a rare autosomal recessive disorder characterized by short stature, sensitivity to sunlight, reduced fertility, and higher incidence of cancer. Like WS, BS can also be described as a genome instability disease. The genomic instability of BS is unique in that it is characterized by a higher rate of sister-chromatid exchange, which is not observed in WS.

The human BS gene encodes a member of the RecQ family of DNA helicases. The gene product, termed *BLM*, has been shown to be able to unwind several types of DNA substrates. It was generally believed that *BLM* functions in certain steps of DNA replication and/or DNA repair, but the exact role of this protein within the cell is unclear. NIA intramural investigators have developed an efficient isolation protocol and purified a multi-protein complex containing *BLM* from human cells. They have successfully identified most components of this complex, and have shown that the complex has some properties distinct from those of the *BLM* protein itself.

BLM shares many similar helicase properties with *WRN*. *BLM* physically and functionally interacts with RPA and is active on a variety of DNA duplex and triplex substrates. Despite the similarities between *WRN* and *BLM*, there are a number of subtle biochemical differences between the two enzymes that may contribute to the distinct cellular and clinical phenotypes of the two genetic disorders. Current studies in this area address the molecular functions of *WRN* and *BLM* proteins in pathways of DNA metabolism that involve other protein factors as molecular matchmakers.

Cockayne Syndrome (CS)

CS is an autosomal recessive disorder with diverse clinical symptoms that include severe mental and growth retardation, microcephaly, progressive neurological and retinal degeneration, skeletal abnormalities, and a hypersensitivity to sunlight. Two genetic complementation groups, *CSA* and *CSB*, have been identified. At the cellular level, CS is characterized by a defect in transcription-coupled repair (TCR) of DNA damage induced by ultraviolet (UV) light and certain forms of oxidative stress.

NIA intramural scientists have investigated the functional significance of conserved motifs in the *CSB* protein in an effort to determine the biological role of *CSB* in DNA repair and transcription. These studies will assist in an understanding of the underlying cellular defects of *CS-B* cells responsible for the clinical symptoms of the syndrome. *CSB* mutant alleles with site-directed mutations were tested for genetic complementation of various phenotypes in both human and hamster *CS-B* null cell lines. Findings indicate that the integrity of the ATPase domain is critical for cellular resistance, ribonucleic acid (RNA) synthesis, and a normal apoptotic response after treatment of cells with UV light. In contrast, a highly acidic region of the *CSB* protein is dispensable for DNA repair. Current studies address the characterization of other regions of the *CSB* protein using genetic and biochemical assays to assess function. The roles of *CSB* in repair of specific oxidative lesions and transcriptional regulation after oxidative stress using cDNA arrays are also being investigated.

Friedreich's Ataxia (FRDA)

FRDA is a disorder that usually manifests before adolescence and is generally characterized by incoordination of limb movements, dysarthria, nystagmus, diminished or absent tendon reflexes, Babinski sign, impairment of position and vibratory senses, scoliosis, pes cavus, and hammer toe. The

triad of hypoactive knee and ankle jerks, signs of progressive cerebellar dysfunction, and preadolescent onset is commonly regarded as sufficient for diagnosis.

NIA grantee Dr. Grazia Isaya has been studying the function of frataxin, the defective protein in FRDA. Dr. Isaya has validated the use of the yeast *Saccharomyces cerevisiae* to study FRDA by showing that the human frataxin gene can replace a defective frataxin homolog in yeast. Furthermore, Dr. Isaya has made the seminal observation that frataxin is an iron-binding protein.

Another NIA investigator, Dr. Gino Cortopassi, has demonstrated that fibroblasts from FRDA are sensitive to oxidative stress, and that this stress may be rescued by iron chelators. Dr. Cortopassi plans to characterize the type of damage that occurs to the mitochondria at a molecular level, in order to evaluate the physiological endpoints and to attempt to rescue the damaged cells by inhibiting the damage-causing pathway.

Fanconi Anemia (FA)

FA is an autosomal recessive disorder characterized by diverse congenital abnormalities and a predisposition to bone marrow failure and cancer, particularly acute myelogenous leukemia (AML). FA patients also display squamous cell carcinomas of the head and neck. The cells derived from FA patients are sensitive to DNA cross-linking agents such as mitomycin and ionizing radiation, suggesting that FA patients are defective in certain aspects of DNA repair.

Cell-fusion studies have shown that FA is caused by mutation in any of seven distinct complementation groups. Six of the FA genes have so far been cloned, but the proteins show little homology to any other known proteins. NIA intramural scientists have developed a rapid and efficient method and purified a complex containing gene products of four FA genes. Several components of this complex have been identified and have demonstrated associated biochemical activity relevant to DNA repair.

Alpha Thalassemia Mental Retardation X-linked (ATRX) Syndrome

ATRX syndrome is an X-linked combination of thalassemia, mental retardation, and multiple associated developmental abnormalities. The gene defective in ATRX has been cloned, and mutations in the same gene have been found to cause several other forms of syndromal X-linked mental retardation. The ATRX gene encodes a gene product containing a SWI2/SNF2-type DNA-dependent ATPase domain. Many proteins with such a domain are present in multi-protein complexes, which often have ATP-dependent chromatin-remodeling activities. It has been hypothesized that ATRX could participate in regulation of gene expression by remodeling chromatin structures. NIA intramural investigators have purified an ATRX-containing multi-protein complex and identified several components of this complex that are consistent with a role in chromatin dynamics.

Blepharophimosis – Epicanthus inversus – Ptosis Syndrome (BPES)

BPES affects eyelid formation, with a characteristic extra fold of skin and drooping eyelid. A number of BPES patients also have premature ovarian failure. This past year, NIA intramural investigators have shown that, in every family and sporadic case that has been analyzed, the syndrome is caused by mutations in a forkhead winged helix transcription factor (*FOXL2*) identified at the disease gene locus on chromosome 3. Consistent with its etiological role during development, *FOXL2* is expressed only in nascent eyelids and in ovarian follicles. Some mutations result only in the eyelid phenotype, and others

provoke both the eyelid trait and associated ovarian failure. In all families studied, the degree of loss of function of one allele confers dominant inheritance of the disorder, because the remaining intact gene copy cannot produce enough intact protein to control eyelid formation and enough ovarian follicles for a full reproductive life span.

Ectodermal Dysplasia (Anhidrotic) (EDA)

Males affected by EDA, an X-linked disorder, show very little hair, few sweat glands, and rudimentary teeth. The gene disrupted to cause EDA was isolated by NIA intramural scientists. This gene encodes a membrane protein (ectodysplasin) that exists in multiple isoforms, and several forms characterized by the NIA group and other groups show a collagen-repeat domain and a TNF-alpha domain. The Tabby mouse, which has a phenotype similar to EDA in humans, has the same gene disrupted. In the last year, investigators have shown that: 1) the gene is itself regulated by the LEF-1/beta-catenin pathway, a major regulator of the development of skin appendages; and 2) a single one of the longest isoforms, provided to Tabby mice as a transgene, can partially restore the missing hair and essentially completely restore sweat glands. Thus, various isoforms of the protein are likely to have overlapping or cooperative functions in appendage formation.

Rare Diseases Research Initiatives

Ongoing NIA rare diseases research projects include studies of connective tissue metabolism in Hutchinson-Gilford progeria syndrome; genetic studies of Cowden syndrome, including an investigation of the role of retinoic acid and its nuclear receptors in the disease; and several studies of the WS gene and gene product.

Investigators in the neurobiology of aging are studying the molecular biology of prion diseases, including Creutzfeldt-Jakob disease; the neurologic, cognitive, and motor performance of patients with Parkinson's disease; the dementia associated with older Down syndrome patients; and the molecular and biological basis of amyotrophic lateral sclerosis (ALS).

NIA intramural scientists are continuing their study of premature aging disorders, including studies of DNA repair and transcription in BS, Cockayne syndrome, and WS. In addition, a study has been initiated of glypican 3 action in overgrowth syndromes such as Simpson-Golabi-Behmel syndrome.

National Institute on Alcohol Abuse and Alcoholism (NIAAA)

Overview of NIAAA Rare Diseases Research Activities

NIAAA's mission is to conduct and support research activities on the causes, consequences, and treatment of alcoholism and alcohol abuse. In addition to the well-known consequences of a variety of liver diseases associated with alcoholism, neurological diseases may also be part of the sequelae of alcoholism. Because of the widespread consequences of alcoholism, knowledge gained in related research programs have wide applications in other areas of human health and disease.

Recent Scientific Advances in Rare Diseases Research

Alzheimer's Disease

Approximately one-fifth of Alzheimer's disease can be related to five different genes, all of which lead in one way or another to the accumulation of $A\beta_{1-42}$. The remaining Alzheimer's cases have no apparent genetic association. The development of metabolic treatments, therefore, offers novel alternatives to what appears to be a difficult problem for genetic manipulations. Several recently published articles give a rationale for the use of mild ketosis as a treatment. A major effect of the metabolism of ketone bodies is to bypass a blockade of the pyruvate dehydrogenase multienzyme complex. It is also recognized that accumulation of amyloid peptides, both intra- and extracellularly, is a hallmark of Alzheimer's disease. Hoshi et al. have shown that a fragment of the beta chain of amyloid stimulates the activity of glycogen synthase kinase 3, leading to the phosphorylation and thus the inhibition of pyruvate dehydrogenase in primary cultures of hippocampal neurons. Hoshi et al. also reported that the 1-42 fragment of the beta chain of amyloid, $A\beta_{1-42}$, can inhibit acetyl choline synthesis in cultures of septal neurons. A block in pyruvate dehydrogenase decreases citrate concentrations, the precursor of acetyl choline, through the action of citrate cleavage enzyme and choline acetyl transferase. Metabolism of ketones overcomes the decrease in citrate content. It has now been shown that cultured hippocampal neurons die when exposed to $A\beta_{1-42}$. Addition of 4 mM D- β -hydroxybutyrate protected these neurons from $A\beta_{1-42}$ -induced death. This finding and the recent identification of the aspartate protease responsible for the cleavage of the 1-42 fragment and the possibility of immunization against amyloid give hope that new therapies may soon be available for this currently untreatable disease.

Parkinson's Disease

Except for rare exceptions such as maternal mitochondrial inheritance, Parkinson's disease appears to result from an acquired defect in the mitochondrial NADH multienzyme complex of the dopaminergic cells of the mesencephalon. The search for non-genetic therapies is therefore essential. Fortunately, Parkinson's disease is treatable for a period by administration of dopamine, although continued free radical damage eventually lessens the effectiveness of this therapy. When taken by humans, the meperidine analogue MPP+ causes increased oxygen radical formation and inhibition of NADH dehydrogenase. Recent reports show that primary cultures of mesencephalic neurons exposed to MPP+ could be protected from death by addition of 4 mM D- β -hydroxybutyrate. Although the mode of ketone body action has not been thoroughly investigated, it would be reasonable to suppose that they act by decreasing the source of mitochondrial oxygen radical formation by oxidizing the co-enzyme Q couple while at the same time reducing the redox potential of the NADP couple which, through glutathione, is

the final detoxification step for H₂O₂. Trials of dopamine therapy in combination with ketone bodies might be expected to prolong the useful therapeutic life of dopamine.

Xeroderma Pigmentosum (XP)

XP is a rare autosomal recessive genetic disorder that affects 1 in 250,000 people in the United States and Europe, but 1 in 40,000 in Japan. This disease results from a defect in a specific type of DNA repair called nucleotide excision repair (NER). NER is known to be crucially involved in the repair of DNA damage resulting from solar UV radiation. Consequently, XP patients have a very high rate of skin cancer on sun-exposed areas of the body.

In addition to skin cancer, some XP patients develop a severe atrophic neurodegeneration, which is believed to result from the formation of some type of endogenous DNA damage. Dr. P.J. Brooks at NIAAA, in collaboration with Dr. Jay Robbins at the National Cancer Institute (NCI), are investigating the nature of this endogenous DNA damage that has the capacity to cause neuronal death. These investigators have identified an oxidative DNA lesion called cyclo-deoxyadenosine (cyclo-dA) that has the characteristics of a neurodegenerative DNA lesion in XP patients. Because cyclo-dA is an endogenous oxidative DNA lesion, it may also be involved in other types of pathological conditions associated with oxidative stress, including alcohol-related brain damage and fetal alcohol syndrome (FAS).

Role of Oxidant Stress in Alcoholic Liver Disease (ALD)

ALD is a major cause of illness and mortality in the United States and around the world. ALD is characterized by fatty liver, hepatitis, fibrosis, and at the end stage, cirrhosis, which is irreversible and fatal. Although oxidant stress (free radicals) has been implicated for a long time in the pathogenesis of alcohol-induced liver injury, researchers were not sure whether it was a cause or an effect of the disease. Recently, by using animal models (rats and mice) of alcohol-induced liver injury, NIAAA-supported researchers have presented more convincing evidence for a central role of oxidant stress in pathogenesis of the disease process. In these models, alcohol is continuously administered into the stomach of animals for approximately four weeks. Alcohol makes the intestine leaky, which results in increased levels of bacterial endotoxin in the plasma, which in turn activates Kupffer cells of the liver. Kupffer cell activation initiates a cascade of events leading to increased generation of free radicals, activation of nuclear transcription factor-kB (NF-kB), and increased transcription of tumor necrosis factor-alpha (TNF), an inflammatory cytokine. These changes are associated with fatty liver, necrosis, and mild inflammation of the liver.

Three approaches were employed to confirm the role of oxidant stress. In the first approach, researchers used either an enzyme inhibitor or gene knockout animals to block the release of free radicals from free radical-generating enzymes. The first study of this approach was designed to attenuate alcohol-induced liver injury by inhibiting the activity of xanthine oxidase, an important source of free radicals in the livers of rats exposed to chronic ethanol administration. This was accomplished by using allopurinol, a xanthine oxidase inhibitor and scavenger of free radicals. Chronic ethanol administration for 4 weeks increased free radical generation, promoted NF-kB activation, and caused fatty liver, mild inflammation, and necrosis. Simultaneous administration of allopurinol blunted all of these effects significantly, suggesting that allopurinol prevents early alcohol-induced liver injury by scavenging free radicals and blunting oxidant-dependent activation of NF-kB.

In the second study of this approach, researchers tried to attenuate alcohol-induced liver injury by destroying the gene of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is a major free radical-generating enzyme in the activated hepatic Kupffer cells. For this purpose, NADPH oxidase gene knockout mice were used in the study. In wild-type mice with intact NADPH oxidase activity, chronic ethanol administration for four weeks caused fatty liver, necrosis, and inflammation. These pathological changes were associated with increased free radical generation, NF- κ B activation, and increased release of TNF from the Kupffer cells. Ethanol failed to produce these changes in NADPH oxidase-deficient mice, suggesting an important role of oxidant stress in ALD.

The second approach used was to overexpress antioxidant enzymes through gene manipulations. Superoxide dismutase (SOD) is an antioxidant enzyme that can neutralize superoxide free radicals. In order to determine whether SOD can attenuate ethanol-induced liver injury, researchers administered the adenovirus-associated SOD gene in rats. After three weeks, SOD was overexpressed in hepatocytes and Kupffer cells of the infected rats. The SOD overexpression attenuated ethanol-induced free radical generation, NF- κ B activation, TNF mRNA levels, and liver injury, further supporting the role of oxidant stress in ALD. In another study, *in vitro* infection of Kupffer cells with adenovirus-containing SOD blunted LPS-induced superoxide production, NF- κ B activation, and TNF production. These results demonstrate that in Kupffer cells, oxidant stress is required for the activation of NF- κ B and production of TNF, which is known to mediate tissue injury in ALD.

In the third approach, researchers confirmed the role of oxidant stress in ethanol-induced liver injury by administering L-2-oxothiazolidine-4-carboxylic acid (OTC), a cysteine prodrug that can replenish the levels of glutathione, a free radical scavenger. In this study, administration of OTC to rats blunted ethanol-induced oxidant stress, NF- κ B activation, TNF mRNA levels, and liver injury.

Thus, using three approaches, NIAAA researchers have clearly demonstrated the pivotal role of oxidant stress in pathogenesis of ALD. It is hoped that these findings will provide a basis for the development of antioxidant therapies for ALD.

Rare Diseases Research Initiatives

Ketosis Research

Research in ketosis for neurodegenerative and other diseases is ongoing within NIAAA, and attempts to obtain industrial collaboration in this effort are under way.

Rare Disease-Specific Workshops, Symposia, and Meetings

Cellular and Molecular Mechanisms of Alcoholic Hepatitis

Alcohol intake is an important cause of hepatitis that may lead to alcoholic cirrhosis, a major cause of mortality and morbidity in this country. Approximately 10% to 35% of heavy drinkers develop alcoholic hepatitis, though the condition is often unrecognized and poorly diagnosed. The clinical features of alcoholic hepatitis vary from none to abdominal pain, fever, jaundice, liver failure, and death. Histopathologically, alcoholic hepatitis is characterized by liver cell necrosis and infiltration of leukocytes in hepatic parenchyma. Although leukocytes have been implicated in the pathogenesis of

alcoholic hepatitis, the underlying cellular and molecular mechanisms by which leukocytes migrate to hepatic parenchyma and initiate tissue injury are not clear.

An upcoming symposium will invite about 15 speakers to address the following issues:

- Types of leukocytes involved in the pathogenesis of alcoholic hepatitis (neutrophils, lymphocytes, and monocytes).
- Chemokines that are responsible for the attraction of leukocytes.
- Adhesion molecules that promote the attachment of leukocytes to the endothelial cells and hepatocytes. Mechanisms of leukocyte transmigration to hepatic parenchyma.
- Mechanisms by which leukocytes initiate tissue injury (free radicals versus proteases).
- Potential targets of interventions for the disease.

National Institute of Allergy and Infectious Diseases (NIAID)

Overview of NIAID Rare Diseases Research Activities

NIAID research activities on rare diseases are classified into four areas: infectious diseases, primary immunodeficiency diseases, autoimmune diseases, and allergic diseases. NIAID's section of this report highlights the Institute's rare diseases advances and activities.

- Infectious diseases include diseases caused by bacteria, parasites, viruses, and fungi. Research on rare infectious diseases is aimed at delineating mechanisms of disease pathogenesis and developing more effective diagnostic, treatment, and prevention strategies.
- Primary immunodeficiency diseases are hereditary disorders caused by intrinsic defects in the cells of the immune system and are characterized by unusual susceptibility to infection. NIAID research is focused on the development of technology to make gene transfer an effective and curative therapy, and on the identification of gene defects and immunologic abnormalities that lead to defective function.
- Autoimmune diseases are diseases in which the immune system mistakenly attacks and damages the body's own cells and tissues. NIAID research is focused on the identification of mechanisms of pathogenesis and the development of new approaches to prevention and treatment.
- Allergies are inappropriate or exaggerated reactions of the immune system to substances that cause no symptoms in the majority of people. NIAID research is focused on the development of new approaches for the diagnosis, prevention, and treatment of allergic diseases.

Recent Scientific Advances in Rare Diseases Research

Rare Infectious Diseases

Blastomycosis

Blastomycosis, a systemic fungal infection caused by *Blastomyces dermatitidis*, is capable of causing disease in both immunocompetent and immunosuppressed hosts. Scientists found a genetically proven virulence determinant of *B. dermatitidis*, a protein responsible for the ability of invading *B. dermatitidis* cells to adhere to host cells (WI-1).

Cryptococcosis

Acquired immune deficiency syndrome (AIDS) patients with cryptococcal meningitis lack or have a greatly diminished inflammatory response, but still develop increased cerebrospinal fluid pressure due to mechanisms other than inflammation. Recent studies have shown that AIDS patients with cryptococcal meningitis experience improved outcome following mechanical drainage to reduce elevated cerebrospinal fluid pressure.

E. coli O157:H7 and associated hemolytic uremic syndrome (HUS)

E. coli O157:H7 is a life-threatening intestinal pathogen most commonly associated with consumption of undercooked beef. Infections in humans can lead to bloody diarrhea and even renal failure in young children and in the elderly. Recent studies indicate that antibiotics should not be used to treat children presenting with bloody diarrhea, as antibiotic use could lead to the release of more bacterial toxins and breakdown of the kidneys, evidenced by bloody urine, and possibly kidney failure from subsequent HUS. Other researchers determined that intiminO157, an *E. coli* O157:H7 outer membrane protein, causes attachment of the *E. coli* to intestinal epithelial cells.

Haemophilus influenzae type b (Hib)

Hib was the leading cause of bacterial meningitis and other invasive bacterial disease (meningitis, epiglottitis, septic arthritis, osteomyelitis, and pericarditis) among children younger than 5 years before the introduction of effective vaccines. New *Haemophilus* strains constantly emerge through the process of transformation; strains mutate and acquire new genetic information. Studies have linked the presence of a 26-base-pair sequence to several other known transformation genes. A better understanding of the mechanism of acquisition of new genes by *H. influenzae* may aid in the development of better strategies for the suppression of antibiotic-resistant strains and the identification of new cellular targets for the action of new antimicrobial agents.

Hantavirus Pulmonary Syndrome (HPS)

HPS is a newly emerging, rodent-borne viral disease first identified in the 1993 “Four Corners” epidemic in the southwestern United States. It has a case-fatality rate of about 50%. New findings suggest immune plasma from survivors might be a useful approach for treating HPS patients and preventing the development of severe disease in persons who may have been exposed. Researchers have found an association between rapid and high-titered neutralizing antibody at medical presentation and increased survival.

Herpesvirus-associated Neonatal Herpes Simplex Virus (HSV) Infection and Congenital Cytomegalovirus (CMV)

Neonatal HSV infections can present as a local infection of the skin, eyes, mouth, and central nervous system, or as a disseminated infection involving multiple organs. The introduction of antiviral therapy has significantly reduced the mortality rate for neonatal HSV. Acyclovir has been approved for congenital HSV infection. CMV, another herpes-associated virus, causes devastating consequences in congenitally infected children. Most infected infants who survive suffer from profound deafness and/or mental retardation. Scientists completed a phase III trial of ganciclovir for the treatment of congenital CMV infections. Symptomatic babies treated with ganciclovir showed improvement or maintenance of their hearing.

Lyme Disease

Lyme disease, caused by *Borrelia burgdorferi*, is the most prevalent tick-borne infectious disease in the United States. A new sensitive and specific laboratory test has been devised by researchers to diagnose Lyme disease in vaccinated individuals. Other researchers found that the presence of a soluble CD14 (sCD14) in the blood of patients with early or late Lyme disease may be an indicator of active infection by *B. burgdorferi*. In Lyme disease, damage resulting from autoimmune reactivity to host tissue may add

to the symptoms of infection. Scientists have developed a new method to differentiate between cell surface components of *B. burgdorferi* and cell surface components of the host that may be involved in the autoimmune reaction.

Pertussis

Bordetella pertussis is the primary etiologic agent of pertussis or whooping cough. Before immunization, pertussis was widespread, with high morbidity and mortality in infants and young children. Exposure of the host to *B. pertussis* results in an increased production of chemicals by human respiratory epithelial cells that cause white blood cells to be attracted to the area, increased expression of mucin (mucus), altered mucus consistency, and respiratory cilia paralysis. After effecting these changes, the organism binds to mucus. Damage to host tissue ensues due to a toxin produced by *B. pertussis*. Toxin secretion can be altered by agents that alter membranes. These discoveries may lead to new strategies for the treatment of whooping cough.

Acute Rheumatic Fever (ARF)

ARF is a disease characterized by inflammatory lesions involving primarily the heart, joints, subcutaneous tissues, and central nervous system. Cases of ARF follow a group A streptococcal upper respiratory tract infection, although the exact mechanisms mediating the development of disease remain speculative. A baboon model has been developed to study the molecular events necessary for group A streptococci (GAS) to colonize the throat and to examine aspects of the immune response to GAS in the throat that may be involved in the development of acute rheumatic fever.

GAS Invasive Disease

GAS can cause invasive disease such as necrotizing fasciitis (flesh-eating bacteria) and streptococcal toxic shock syndrome. Recently, the crystal three-dimensional structure of streptococcal pyrogenic exotoxin (SpeB), a major virulence factor of GAS, has been determined. The three-dimensional structure will provide information that will be useful for the design of inhibitors with therapeutic potential and vaccine candidates for prevention of severe invasive disease.

Group B Streptococci (GBS)

GBS cause serious illness in newborns, including sepsis, pneumonia, and meningitis. Infant and maternal GBS infections may be preventable by maternal immunization. A GBS Type II capsular polysaccharide-tetanus toxoid (Type II-TT) vaccine in which GBS capsular polysaccharide was coupled to tetanus toxoid was recently compared to an uncoupled GBS Type II capsular polysaccharide vaccine. The immune response in the recipients of the coupled vaccine was significantly higher than that in the recipients of uncoupled vaccine. This study supports the inclusion of capsular polysaccharide type II coupled to tetanus toxoid in the formulation of a multivalent GBS vaccine.

Rare Primary Immunodeficiency Diseases

Autoimmune Lymphoproliferative Syndrome (ALPS)

ALPS is a disease in which a genetic defect in programmed cell death, or apoptosis, leads to the breakdown of lymphocyte regulation, causing a proliferation of lymphocytes (blood cells critical to immune function). Recent studies have determined that the risk of becoming ill with ALPS is significantly greater in people who have an abnormality at a specific location of the *fas* gene (a gene that codes for a protein that triggers lymphocytes to die at the completion of their normal life cycle). Individuals with *fas* gene mutations are at greater risk for development of B and T cell lymphomas. Researchers have also identified other mutations involving ALPS.

B Cell Immunodeficiencies

Patients with B cell immunodeficiencies have recurrent, potentially life-threatening infections due to a lack of mature B cells (which produce antibodies). In an immunodeficient patient, researchers identified a novel mutation in B cell linker protein (BLNK), which serves as a “molecular scaffold,” bringing together enzymes and their target molecules in the B cell. Mice with the BLNK mutation were also immunodeficient.

Chronic Granulomatous Disease (CGD)

CGD is an inherited genetic disorder characterized by a failure of white blood cells called neutrophils to make oxygen compounds that kill bacteria and fungi. Scientists recently reported a promising therapeutic approach for individuals with CGD. Patients with CGD underwent a preparative regimen that causes intense immunosuppression without destroying the bone marrow, followed by the transplant of immunologically matched sibling stem cells. This approach provided a cure for a subset of CGD patients with a fully immunologically matched sibling. In another clinical trial, investigators demonstrated functional correction of the genetic defect for an X-linked form of CGD in three of five patients treated with multiple infusions of gene-corrected cells.

Familial Hemophagocytic Lymphohistiocytosis (FHL)

FHL is a rare genetic disorder caused by mutations in at least three genes that results in uncontrolled activation of the immune system, leading to death in early infancy or childhood. Researchers demonstrated that a protein called perforin, part of the cell-destroying apparatus in killer T cells, is missing or inactive in FHL patients.

Job Syndrome

Job syndrome, also known as hyperimmunoglobulin E recurrent infection syndrome (HIE), is a rare inherited disease characterized by recurrent bacterial infections of the ears and elevated levels of the antibody immunoglobulin E. Investigators have linked a genetic defect in HIE patients to chromosome 4.

Severe Combined Immunodeficiency (SCID)

SCID is a rare congenital syndrome response. Some children with SCID have lived for years in germ-free rooms or “bubbles” necessitated by their unusual susceptibility to infectious agents that can be life-threatening. A study that may prove critical to the development of a treatment for SCID involves improving the function of the thymus by stem cell transplantation. Scientists studied 83 SCID patients who had undergone bone marrow transplantation to receive stem cells over an 18-year period. T cells that developed in each patient were identified, characterized, and followed over time with molecular and cellular markers. The number of T cells reconstituted in the thymus peaked to near-normal levels 1-2 years after transplantation, with continued thymic function for up to 14 years.

X-linked Hyper-IgM Syndrome (XHIM)

Individuals with XHIM lack, or have only trace amounts of, several functional classes of antibodies or immunoglobulins (IgG, IgA, IgE), but have normal or elevated levels of the antibody IgM, making them highly susceptible to recurrent infections. XHIM is caused by a defect in the T cell surface molecule CD40 ligand, which binds to the B cell receptor CD40. Scientists are studying the treatment of patients with XHIM with a synthetic CD40 ligand protein. A second form of XHIM may be caused by a mutation in both CD40 and a cell-signaling pathway. Patients with this mutation do not produce IL-12 (which is important in eliciting an immune response to intracellular organisms) upon signaling via CD40.

Wiskott-Aldrich Syndrome (WAS)

WAS is an inherited blood cell disorder caused by mutation in the WAS protein (WASP). WAS is characterized by low levels of platelets (cells important for blood clotting), immune deficiency caused by insufficient number and function of lymphocytes (blood cells critical to normal immune functioning), and eczema. Researchers have determined that the level of mutated WASP in platelets and lymphocytes correlates with disease severity.

Rare Autoimmune Diseases

Systemic Lupus Erythematosus (SLE)

SLE is an autoimmune disease characterized by the production of antibodies against proteins in nuclei of the body’s own cells. Antibodies form complexes with nuclear proteins and become trapped in the kidneys, leading to inflammatory kidney disease. Scientists showed that C-reactive protein (CRP) promotes clearance of nuclear proteins and may help prevent lupus-related inflammatory kidney disease. In contrast, estrogen has been found to enhance the production of antibodies against the body’s own cells in lupus in animal models.

Rare Diseases Research Initiatives

FY 2000-Funded Activities in Infectious Diseases

- NIAID’s Mycoses Study Group (MSG) supported four clinical trials examining antifungal therapy for the opportunistic and endemic mycoses.

- NIAID supported genome sequencing of etiologic agents of rare diseases, including: *Rickettsia typhi* (etiologic agent of typhus), *Burkholderia mallei* (etiologic agent of glanders), *Brucella suis* (etiologic agent of brucellosis), *Bacillus anthracis* (etiologic agent of anthrax), *Coxiella burnetii* (etiologic agent of Q fever), *Cryptococcus neoformans* (etiologic agent of cryptococcosis), *E. coli* 0157:H7 (etiologic agent of gastritis and HUS), *Ehrlichia phagocytophila* (etiologic agent of ehrlichiosis), *Haemophilus ducreyi* (etiologic agent of chancroid), *Treponema pallidum* (etiologic agent of syphilis), and *Streptococcus pyogenes* (etiologic agent of group A streptococci invasive disease).
- NIAID supported studies on: the identification and characterization of genes that influence virulence in *B. anthracis* (etiologic agent of anthrax), factors that determine host sensitivity or resistance to anthrax lethal factor, genetic factors involved in the expression of virulence of *Yersinia pestis* (etiologic agent of plague), and new animal models to assess the virulence of *Rickettsiae* (etiologic agent of Rocky Mountain spotted fever and other spotted fevers).
- NIAID collaborated with the NIH Fogarty International Center (FIC) to support the “International Training and Research on Emerging Infectious Diseases” program to address research training needs related to emerging and re-emerging infectious diseases in developing countries.
- The viral hepatitis program funded a grant for hepatitis A and three grants for hepatitis D.
- NIAID is supporting phase I/II trials for three different candidate vaccines for human CMV. These include a glycoprotein subunit, engineered live recombinant viruses, and a prime-boost strategy using a glycoprotein delivered both as a subunit and in an avian poxvirus vector (Vaccine and Treatment Evaluation Unit).
- The Collaborative Antiviral Study Group (CASG) conducted phase III studies evaluating the use of oral acyclovir following the standard of care treatment with intravenous acyclovir to limit the recurrence of neonatal herpes virus infections of the skin, eyes, mouth, or central nervous system, and conducted a phase III study evaluating long-term therapy of herpes simplex encephalitis with oral valacyclovir.
- NIAID supported 46 grants and contracts on Lyme disease. Grants also were awarded to support research on other vector-borne bacterial infections. These grants support research on: animal models of disease, microbial physiology, mechanisms of pathogenesis and host immunity, vectors and disease transmission, therapeutic approaches, and test development.
- A study titled "Study and Treatment of Post Lyme Disease" was completed.
- NIAID supported a phase I clinical trial to evaluate a broadly effective GAS vaccine at the University of Maryland’s Center for Vaccine Development.
- NIAID was the sponsor of a phase I safety and immunogenicity trial utilizing a GBS type II polysaccharide-tetanus toxoid conjugate vaccine in pregnant women.

- NIAID continues to support research on the epidemiology and basic biology of GBS disease, along with GBS vaccine research and clinical trials of GBS conjugate vaccines through a 5-year multidisciplinary contract awarded in late 1997.
- In collaboration with Los Alamos National Laboratories, NIAID has established a relational database (STD GEN) for pathogens that cause sexually transmitted diseases. The genomic sequence of HSV1 was incorporated in FY 2000.
- NIAID supported the maintenance of the “World Reference Center for Arboviruses” and has ongoing contracts to screen possible antiviral compounds for activity against West Nile virus.
- NIAID participated in the development and support of programs such as The Global Alliance for Vaccines and Immunization (GAVI) in an effort to protect health and save lives through the widespread use of safe vaccines.

FY 2000 Newly Issued Initiatives in Infectious Diseases

- Bacteriology and Mycology Study Group (BAMSG) (RFP-AI-01-11): to establish a collaborative group with expertise to construct and conduct clinical studies addressing serious fungal and resistant bacterial diseases.
- Animal Models of Human Viral Infections for Experimental Therapies (RFP-AI-01-003): to evaluate experimental therapies for potential clinical efficacy and toxicity in animal models of clinically important, emerging, and rare human infections.
- Preparedness Against Illegitimate Use of Bacterial Pathogens (RFA-AI-00-004): to support the study of virulence determinants, host resistance/susceptibility factors, novel therapeutics, and novel or improved candidate vaccines against bacterial pathogens involved in biological warfare, many of which cause rare diseases.
- Challenge Grants: Joint Ventures in Biomedicine and Biotechnology (RFA-AI-00-010): to promote joint ventures between NIH and the biotechnology, pharmaceutical, and medical device industries through one-on-one matching of federal dollars by qualified organizations.

FY 2000-Funded Activities in Immune-mediated Diseases

- NIAID, through a contract with the Immune Deficiency Foundation (IDF), established and maintains a registry of clinical information on U.S. residents affected by primary immunodeficiency diseases. For each disease, the registry collects information on incidence, clinical phenotypes and phenotype/genotype correlations, natural course of the disease (including complications), effects of therapy, causes of death, and prognosis.
- NIAID, NCI, and the National Institute of Child Health and Human Development (NICHD) co-funded a research project involving the use of a new screening device to determine if the occurrence of primary immunodeficiency diseases in large urban Hispanic and African American populations is underdiagnosed.

- NIAID supported clinical trials and test development for promising tolerance induction strategies for the treatment of multiple immune-mediated disorders through the Immune Tolerance Network, an international consortium of more than 70 basic and clinical investigators from 40 institutions in 9 countries.
- NIAID chairs the Autoimmune Disease Coordinating Committee, an effort to increase collaboration and to facilitate the development of coordinated research in autoimmune diseases among the many NIH Institutes, other Federal agencies, and private groups.
- NIAID is sponsoring clinical trials to assess the safety and efficacy of hematopoietic stem cell transplantation for treating severe autoimmune diseases, along with integrated studies of underlying mechanisms, through the Clinical Trials Network for Stem Cell Transplantation for Autoimmune Diseases, established in FY 2000.
- NIAID continued to support research projects awarded in FY 1999 in response to several trans-NIH initiatives in autoimmunity, including: Environment/Infection/Gene Interaction in Autoimmunity, Target Organ Damage in Autoimmune Diseases, Pilot Trials on Innovative Therapies for Rheumatic and Skin Diseases, and Registries for Neonatal Lupus and Juvenile Rheumatoid Arthritis.

FY 2000 Newly Issued Initiatives in Immune-mediated Diseases

- Asthma and Allergic Diseases Research Centers (RFA-AI-00-012): to support a multidisciplinary research program focused on studies of immunologic and other mechanisms underlying human asthma and allergic diseases.
- Innovative Grant on Immune Tolerance (RFA-AI-00-006): to support novel work on the molecular mechanisms and applications of antigen-specific immune tolerance, which is the selective and long-term inactivation of the immune responses.
- Hyperaccelerated Award—Mechanisms in Immunomodulation Trials (RFA-AI-00-005): to support mechanistic research studies in clinical trials of interventions that enhance immune function for immune system-mediated diseases.
- Cooperative Study Group for Autoimmune Disease Prevention (RFA-AI-00-016): to support a collaborative network of investigators focused on the development of interventions to prevent autoimmune diseases.

FY 2000 Cooperative Research and Development Agreements (CRADAs)

In FY 2000, NIAID investigators entered into six CRADAs related to rare diseases. CRADAs funded the production and evaluation of human antiherpes simplex virus monoclonal antibody as a therapeutic tool for treatment of neonatal HSV, stem cell gene therapy systems for CGD, the adoptive transfer of T-cell clones for treatment of immunologically mediated and infectious diseases, the development of a hepatitis A vaccine, and an acellular pertussis vaccine clinical trial.

Rare Disease-Related Program Workshops, Symposia, and Meetings

- In an effort to stimulate research and research collaborations on rare diseases, NIAID and the Office of Rare Diseases (ORD) co-sponsored the workshop, “Protocol Development for Autologous Stem Cell Transplantation (SCT) in Pediatric Rheumatic Disease.”
- NIAID, in collaboration with other NIH Institutes, the Jeffery Modell Foundation, and the IDF, sponsored a March 2000 symposium titled, “Advances in the Diagnosis and Treatment of Primary Immunodeficiency Diseases: Risk of Cancer,” focusing on advances in biomedical research that led to new insights into the diagnosis and treatment of primary immunodeficiency diseases and on the etiology of cancer in primary immunodeficient patients.
- The June 2000 NIH Lyme Disease Coordinating Committee meeting of focused on several NIH-supported clinical studies on Lyme disease.
- The NIAID-industry “Summit on Development of Infectious Disease Therapeutics” was held September 26-27, 2000. Issues of NIAID and industry collaboration in the development of therapeutics for addressing public health priorities in infectious disease were discussed.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Overview of NIAMS Rare Diseases Research Activities

The mission of NIAMS is to support basic and clinical research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases; the training of scientists to carry out this research; and the dissemination of information on research progress in these diseases. Basic research involves a wide variety of scientific disciplines, including immunology, genetics, molecular biology, biochemistry, physiology, virology, and pharmacology. Clinical research includes the fields of rheumatology, orthopaedics, muscle biology, bone endocrinology, sports medicine, and dermatology, plus aspects of pediatrics and gerontology related to arthritis and musculoskeletal and skin diseases.

Recent Scientific Advances in Rare Diseases Research

Juvenile Rheumatoid Arthritis (JRA)

Etanercept has been shown to be a safe and effective drug in the treatment of children and teenagers with JRA, a type of arthritis that causes joint inflammation and stiffness for more than 6 weeks and begins when the child is age 16 or younger. Etanercept belongs to a new class of drug treatments called biologic agents, which are designed to interfere with the specific biological process of a disease. The drug acts as a sponge to absorb a tumor necrosis factor, a naturally occurring protein that causes inflammation.

In a clinical trial coordinated by the NIAMS Multipurpose Arthritis and Musculoskeletal Diseases Center at the Children's Hospital Medical Center of Cincinnati, Ohio, investigators in the Pediatric Rheumatology Collaborative Study Group injected 69 children with etanercept twice a week. After 3 months, all measures of arthritis impact (symptoms, joint abnormalities, ability to perform daily functions, and laboratory tests) were dramatically improved. Furthermore, the drug was well-tolerated. The success of this clinical trial marks the culmination of many years of basic research supported by NIAMS and other NIH components.

Scleroderma

The hallmark of scleroderma, also known as systemic sclerosis, is hardening of the skin. Scleroderma is a condition that can involve tissues in the lungs, heart, kidneys, intestinal tract, muscles, and joints. In severe cases, it can be fatal. Synthesis of the structural protein collagen and its accumulation are essential for normal tissue development and wound repair; however, excessive deposition of collagen can lead to scleroderma. In recent reports from NIAMS-funded studies, investigators found that scleroderma cells are resistant to factors that normally regulate collagen production. If researchers can target these factors, it may be possible to develop new therapeutics to restore a balanced collagen synthesis in patients with scleroderma.

Inflammatory Myopathy

Inflammatory myopathy refers to a family of diseases in which muscle tissues become inflamed, causing pain, tenderness, and weakness. Some of the diseases are autoimmune diseases, which occur when the body attacks its own tissues with antibodies known as autoantibodies, because they are directed against the self. A NIAMS intramural laboratory has developed an animal model of one of these autoimmune diseases, myositis, by making a genetic change in the surface of muscle cells. Although no outside

infection was involved, the animals developed muscle damage and autoantibodies of a kind found only in myositis in humans. This suggests that the development of some kinds of autoimmunity does not require any particular outside stimulus, but may arise as a programmed consequence of the body's response to certain kinds of alterations within the tissues.

Duchenne Muscular Dystrophy (DMD)

DMD is a genetic muscle-wasting disease caused by mutations in the gene for the protein dystrophin. NIAMS-funded scientists recently reported a number of exciting advances in mouse models of DMD. These advances include the successful application of the common antibiotic gentamicin to restore the function of the dystrophin protein, and the successful use of gene replacement to restore the missing protein and thereby reduce muscle disease. Such animal studies hold promise for potential future therapies for human patients affected by DMD.

Pseudoxanthoma Elasticum (PXE)

PXE is an inherited disorder characterized by progressive calcification of elastic fibers in the skin, eyes, and cardiovascular system. Scientists studying PXE have identified the gene that causes this disease. Using a population genetics approach to narrow the location of the gene to a portion of human chromosome 16, these researchers were able to determine that one gene in this region (*MRP6*) is the gene associated with the disease. A joint patent application is expected to be filed shortly by the researchers at the University of Hawaii who isolated the gene and the patient advocacy group PXE International, which supplied the scientists with blood and tissue samples from affected patients. This novel alliance has been featured as a model for handling intellectual property emerging from collaborations between patient groups and researchers. Work is continuing to determine the function of the gene and how mutations result in clinical disease. This work could lead to the design of therapeutic interventions to treat PXE.

Rare Diseases Research Initiatives

Opening of NIH Pediatric Rheumatology Clinic

The NIAMS Intramural Research Program launched an exciting initiative in fall 2000 at the NIH research hospital, the new NIH Pediatric Rheumatology Clinic. The clinic offers diagnosis, evaluation, and treatments for children with rheumatic diseases. In addition to JRA and familial fever syndromes, pediatric rheumatic diseases include lupus, scleroderma, and dermatomyositis. The clinic provides children and researchers with a state-of-the-art facility for diagnosis, treatment, and learning more about rare rheumatic diseases in children.

Workshop on Inflammatory Myopathy

In April 2000, NIAMS, the National Institute of Neurological Disorders and Stroke (NINDS), and ORD co-sponsored a workshop on inflammatory myopathy. The meeting brought together investigators from around the world and several biomedical disciplines to discuss inflammatory muscle disease. The purpose of the workshop was to stimulate research by encouraging new cross-disciplinary collaborations.

DMD

In May 2000, NIAMS again partnered with NINDS and ORD to sponsor a scientific workshop on therapeutic approaches for DMD. The meeting provided an opportunity for DMD investigators to share new findings, identify gaps in current research, and recommend future directions for promising studies of DMD. The insights from this conference are being used to develop new NIH research solicitations in DMD and other muscular dystrophies.

Facioscapulohumeral Muscular Dystrophy (FSHD)

NIAMS has a number of initiatives under way in FSHD, a genetic disease of skeletal muscle that leads to progressive weakening of the muscles of the face, shoulders, and upper arms. NIAMS has funded several projects with potential implications for FSHD pathogenesis and therapy, including grants focused on developing safe and effective methods to perform gene therapy on skeletal muscle.

Also in May 2000, NIAMS, NINDS, ORD, the FSH Society, Inc., and the Muscular Dystrophy Association of America co-sponsored a scientific conference on the cause and treatment of FSHD. Researchers from the United States, Canada, Europe, South America, and Asia met to share their latest findings and identify directions for future investigations. The recommendations that emerged from the conference include efforts to enhance understanding of the molecular processes and tissue changes associated with FSHD, ways to explore possible therapies to treat the disorder, strategies to promote the establishment of population-based studies of the disease, and development of research resources. Implementation of the recommendations has begun with the issuance of a November 2000 RFA titled, "Exploratory Research on Facioscapulohumeral Dystrophy."

NIAMS Registries

To develop research resources for FSHD, NIAMS has joined with NINDS to fund a registry on FSHD and a form of muscular dystrophy known as myotonic dystrophy (DM). The long-term goal of the registry is to facilitate research in FSHD and DM by serving as a liaison between families affected by these diseases who are eager to participate in specific research projects and investigators interested in studying these disorders. The registry will recruit and classify patients, and store medical and family history data for individuals with clinically diagnosed FSHD and DM. Scientists will be provided with statistical analyses of the registry data, as well as access to registry members who have agreed to assist with particular research studies.

NIAMS is also sponsoring a new national registry and tissue repository for patients with antiphospholipid syndrome, an autoimmune disorder in which the body appears to recognize certain fatty molecules within its own cells as foreign substances and produces antibodies against them.

Also in FY 2000, NIAMS expanded its support for a national registry of patients affected by scleroderma.

Additional Research Efforts in Scleroderma

Other initiatives in scleroderma include the issuance of an RFA for specialized centers of research (SCORs). SCORs foster coordinated research efforts that strongly emphasize basic disciplines and involve significant interaction between basic research and clinical investigations. In addition, NIAMS issued an RFA titled, "Molecular Pathogenesis and New Interventions in Scleroderma" in September

2000 and developed a new publication to make science-based health information available to patients with scleroderma and their families.

Heritable Connective Tissue Disorders

Heritable connective tissue disorders is a term used to describe a family of more than 200 disorders that affect connective tissue, the material between the cells of the body that gives tissues form and strength. The disorders result from alterations (mutations) in genes responsible for building tissues. Many of these disorders, such as osteogenesis imperfecta (OI) and Ehlers-Danlos syndrome (EDS), are considered rare diseases.

OI

People with OI have easily broken bones and may also have hearing loss, hernias, enlarged blood vessels, loose joints, poorly developed teeth, and thin, stretchy skin. NIAMS has a long-standing interest in OI and recently funded five new grants in OI supporting research activities ranging from cutting-edge gene and cell therapies to testing drug treatments in mouse models. NIAMS, along with several other Institutes, is sponsoring an RFA titled, "New Research Strategies in OI," which was issued in December 2000. NIAMS is also participating in an RFA on "Clinical Trial Planning Grants for Pediatric Rehabilitation." This RFA was issued in November 2000 and focuses on pediatric musculoskeletal conditions, burn wounds, and genetic skin disorders.

EDS

People with EDS have some degree of joint looseness; fragile, small blood vessels; and abnormal scar formation and wound healing. NIAMS scientists are studying the molecular mechanisms by which mutations in the genes for collagen are expressed, how collagen genes and proteins contribute to the framework in which cells are embedded, and human and mouse models of collagen deficiency.

In its commitment to information dissemination for patients and families affected by diseases within the NIAMS mission, the Institute recently developed a comprehensive fact sheet on heritable connective tissue disorders. Material on both OI and EDS is included in this fact sheet.

National Cancer Institute (NCI)

Overview of NCI Rare Diseases Research Activities

Cancer is not rare. It is, in fact, the second leading cause of death in the United States. In 2001, approximately 552,200 Americans are expected to die of cancer, more than 1,500 people a day, or 1 out of 4. Cancer, however, is not one but many distinct diseases. Certain cancers, including cancers of the breast, prostate, lung, and colon, can no longer be classified as rare diseases because prevalence data indicate that these cancers have exceeded the 200,000 cases per year maximum for inclusion as a rare disease. Overall cancer incidence and death rates have continued to decrease in men and women since the early 1990s, and the decline in overall cancer mortality has been greater in recent years. Despite reductions in age-adjusted rates of cancer death, the total number of recorded cancer deaths in the United States continues to increase due to an aging and expanding population. Cancer incidence and mortality rates have risen for esophagus, liver, melanoma of the skin, kidney, brain, non-Hodgkin's lymphomas, and multiple myeloma.

NCI's mission is to develop the means to decrease the incidence, morbidity, and mortality of cancer. NCI does this through the conduct and support of research in cancer biology, causes, prevention, control, detection, diagnosis, treatment, rehabilitation, and continuing care. NCI's section of this report discusses selected major research advances, research initiatives within the NCI intramural and extramural programs, and other relevant program activities.

Recent Advances in Rare Diseases Research

Cancer Biology and Etiology

Basic research studies exploring the mysteries of how cancer develops form the foundation of cancer research. Through these studies, scientists are identifying, at the molecular level, the fundamental processes that underlie a cell's transformation from normal to malignant. Identifying the processes and pathways that lead to cancer provides attractive targets for new prevention and treatment approaches. Likewise, elucidation of the external and internal factors that cause or contribute to cancer provides avenues for developing behavioral interventions and drugs to prevent cancer.

Additional Risks for Retinoblastoma Patients

A study of 1,604 one-year survivors of retinoblastoma diagnosed from 1914 to 1984 evaluated mortality from 1925 through 1997. The study, published in the *Journal of the National Cancer Institute*, found an excess of early-onset lung cancers among these patients. Because smoking rates are not abnormally high in retinoblastoma survivors, and no lung cancer deaths were reported for the patients with nonhereditary retinoblastoma, the reported excess of early-onset lung cancers suggests that carriers of *RBI* mutations are highly susceptible to smoking-induced lung cancers. These important findings demonstrate for the first time that hereditary retinoblastoma patients are at increased risk of dying from lung cancer, and highlight the need for these patients to be specially targeted for smoking prevention and cessation efforts.

In another study, bone sarcoma incidence data from patients treated by x-ray during early childhood for bilateral retinoblastoma are being analyzed and compared to those from patients treated by injection of ²²⁴Ra for tuberculosis and other benign diseases. Preliminary analyses suggest that retinoblastoma

patients, whose baseline risk of bone sarcoma is already high, are unusually susceptible to radiation-related bone sarcomas.

Origins of von Hippel-Lindau Disease (VHL)

Two cases of VHL mosaicism were found in patients identified from the NIH-VHL Family Registry. These index cases exhibited clinical manifestations of the disease but had no family history of VHL, and they were negative for VHL germline mutations using standard testing methods. As such, these patients were thought to represent de novo cases of the disease. The detection of a germline mutation in the *VHL* gene of their offspring, however, prompted additional testing. Further analysis of the affected parents' blood using FISH and conformational sensitive gel electrophoresis detected the *VHL* gene mutation in a portion of their peripheral blood lymphocytes, thereby confirming VHL mosaicism in these patients. This finding suggests that parental mosaicism accounts for some apparently sporadic or new cases of VHL and has important implications in both the clinical management and genetic counseling of families affected by VHL.

Promising Avenue for Research on the Origins of Ovarian Cancer

NCI-funded researchers recently demonstrated the exceptional value of employing nature's conservation of function in disparate organisms (flies and mice) to provide fundamental knowledge applicable to human cancer. A group of researchers sought to identify genes that control the proliferation of cells in mutant fruit flies. They isolated *lats* (large tumor suppressor), a novel gene that physically interacts with several proteins that are key to regulation of the cell cycle. Flies that have a mutant version of *lats* develop tumors in many tissues. The protein product of the fly *lats* gene is very similar in sequence to that of the human *LATS* gene, suggesting an analogous function in flies and mammals. Indeed, mice missing the *lats* gene develop tumors, particularly soft-tissue sarcomas and ovarian stromal tumors, and are hypersensitive to carcinogens. They are also defective in mammary gland development, infertile, and growth-retarded. Although the *LATS* gene is very highly expressed in human ovaries, it now remains to confirm whether it is important in the etiology of human ovarian stromal tumors, and whether it has a role in breast cancer.

Understanding the Cause of Radiation and Drug Resistance in Melanoma Cells

Melanoma is a deadly cancer whose incidence has increased in the last decade to a greater extent than that of all other cancers. Its propensity to metastasize and resist radiotherapy are major impediments in its treatment by radiation (e.g., x-rays) or antineoplastic drugs and underscores the need to understand the basic mechanisms that determine the radiation and drug resistance phenotypes. Investigators have recently identified in human melanoma cells a UV response genetic element and some of its regulatory proteins, including the proteins that confer radiation resistance. The results strongly suggest that ATF2, a transcription factor, is a key component of a heteropolymeric protein complex that modulates both radiation and drug resistance in melanoma cells. Thus, ATF2 modulators may be useful as potential radiation sensitizers in the treatment of this cancer.

Testing the Contribution of Different Genetic Alterations to the Transformation of Neural Cells

Glioblastoma multiforme is the most malignant of the primary brain tumors and is almost always fatal. A powerful new approach for testing the contribution of different genetic alterations to the transformation of neural cells was recently developed. This approach allows combinations of oncogenes and tumor suppressors to be specifically transferred to astrocytes in cell cultures and in adult animals. This system

was used to develop a mouse model for glioblastoma that has characteristics similar to the human disease. These studies suggest that some of the mutations and alterations in gene expression found in human central nervous system tumors directly contribute to the cause of these diseases. These mouse-modeling experiments may also identify essential targets for therapy and may provide test animals for preclinical therapeutic trials.

Ovarian Cancer Risk and Hypergonadotropic Hypogonadism

In 1992, NCI-supported investigators began a population-based, case-control study of ovarian cancer in eastern Massachusetts and New Hampshire to identify factors affecting risk. Consumption and metabolism of milk sugar (galactose) were of interest, based on evidence that this sugar is toxic to oocytes. Homozygosity for a mutation in galactose transferase (GALT) known as N314D or heterozygosity for mutations that more severely affect activity such as Q188R were found to increase risk for ovarian cancer, especially for endometrioid and clear cell (E/CC) types. In addition, these investigators found that long-term or recent oral contraceptive use was protective for ovarian cancer other than mucinous types. Use of combined menopausal hormones was also protective. From their preliminary analysis, the investigators concluded that a variety of genetic, reproductive, and environmental risk factors exist for ovarian cancer, and these risk factors vary by histologic type of ovarian cancer. Ovarian cancer pathogenesis likely involves a complex interplay among germline and somatic mutations and environmental factors.

Initiation and/or Progression of AIDS-associated Cancers

Kaposi's sarcoma (KS) is the most frequent neoplastic complication in patients with AIDS. It follows a disseminated and aggressive disease course, with extensive lung, gastrointestinal tract, and lymph node involvement. Kaposi's sarcoma herpes virus (KSHV), which is etiologically linked to KS, is present in KS tissue biopsies from virtually all patients with all forms of KS. The DNA sequence of the viral genome indicates the presence of a number of KSHV-specific viral proteins that are homologs of cellular proteins involved in signal transduction, and regulation of the cell cycle and the immune system. These KSHV viral protein homologs may contribute to the initiation and/or progression of KSHV-associated neoplasms.

Detection, Diagnosis, and Prognosis

Promotion of research to improve cancer screening and early cancer detection and to develop more accurate diagnostic techniques is of major importance to NCI. NCI-supported research, conducted at multiple centers throughout the country and by intramural scientists, is leading to rapid advances in these areas.

KSHV Gene Sequences in Multiple Myeloma Patients

In a recent study, KSHV gene sequences were detected in 15% to 20% of peripheral blood cells obtained from multiple myeloma (MM) patients. Yet upon mobilization chemotherapy, more than 40% of dendritic cells obtained from MM patients became positive for KSHV gene sequences. Surprisingly, none of the MM patients produced a detectable immune response against KSHV. The lack of immune responses in these patients is the current focus of research. Because MM is untreatable, inducing immune responses to KSHV in MM patients may provide an opportunity for a more helpful prognosis.

Cancer Prevention and Control

Advances in cancer prevention and control are critical to reducing the cancer burden. NCI activities in this area often focus on translation of new basic research findings in cancer biology and cause into effective interventions. Chemoprevention, behavior modification, cancer surveillance, and health communications exemplify the issues addressed to prevent and control cancer.

Measuring DNA Repair and Preventing Skin Cancers

NCI investigators are studying molecular, cellular, and clinical abnormalities in genetic diseases that predispose individuals to cancer. One such investigation has focused on xeroderma pigmentosum (XP), a cancer-prone genetic disease with cellular hypersensitivity to environmental agents. New assays using plasmids were developed to measure DNA repair and mutagenesis at the molecular level in human cells and to assign cells to XP complementation groups. Chemoprevention of skin cancer in XP with oral 13-cis retinoic acid was found to be effective in preventing skin cancers but very toxic. The lowest effective dose varied in different patients.

Cancer Treatment

NCI supported research leading to a number of treatment-related advances in 2000.

Non-myeloablative Allogeneic Hematopoietic Stem Cell (Mini-) Transplants for Hematological Cancers

Intensive, highly toxic regimens are used in conventional stem cell transplants to eliminate hematological cancers and to overcome two of nature's biological barriers: 1) host-versus-graft reaction, where the graft is rejected by the host; and 2) graft-versus-host disease (GvHD), where the graft mounts an immune attack on the host cells/tissues/organs. Conventional transplants are not normally performed on patients older than 55 years or on patients who are unable to withstand the intensive and highly toxic therapy. The non-myeloablative transplant approach or the "mini-transplant" procedure works by using just enough radiation to suppress the patient's immune system without ablating the bone marrow. The donor cells then fight the cancer/disease via an immunological monitoring process called the graft-versus-leukemia disease effect. While tolerant of the host cells/tissues/organs, the donor cells recognize the leukemic/diseased cells as foreign and kill the cancer/diseased cells. The regimen is safe and minimally toxic. Most patients undergoing "mini-transplants" leave the outpatient clinic and go home the same day. Hair loss; severe mouth sores; severe protracted low levels of white cells, red cells and platelets; and fatal regimen-related toxicities typical of conventional transplants have not been encountered.

The first patients are nearly 2 years post-"mini-transplant" and remain disease-free even by molecular analysis. The "mini-transplant" procedure, if shown to be efficacious in phase III clinical trials, will have a major economic, social, and public health impact. It will make transplantation as a treatment strategy more available (because more mismatched transplants may be possible), less toxic, cheaper, and more effective. Furthermore, this procedure may be effective in treating other non-malignant diseases such as autoimmune diseases, sickle cell anemia, and genetic diseases.

Low-dose Suramin Works Against Non-small Cell Lung Cancer

Investigators at Ohio State University have demonstrated in animal models that prior treatment with a low dose of suramin can overcome the multidrug resistance phenotype exhibited by tumors that are refractory to chemotherapeutic agents. This observation was tested in a phase I clinical trial enrolling stage IV non-small cell lung cancer patients. The trial's preliminary data are extremely encouraging; of the nine patients treated with low-dose suramin plus taxol and carboplatin, none had progression of disease, while one showed complete regression and eight showed partial regressions.

Pharmacologic Dosing of Mitomycin C in Bladder Cancer is Better than Conventional Dosing

Investigators in the United States, Canada, and Europe performed a randomized multi-institutional phase III clinical trial comparing pharmacologic dosing to conventional dosing of intravesicle therapy with mitomycin C in superficial bladder cancer. Pharmacologic dosing was accomplished by limiting patients' fluid uptake, ultrasound measurements of urine volume in the bladder, and pH adjustment of urine remaining in the bladder. The pharmacologic arm of the trial proved superior to the conventional arm and had a higher recurrence-free rate (approximately 37% versus 21%) and a longer median time to recurrence (537 days versus 337 days).

Chronic Lymphocytic Leukemia (CLL) Patients Can Be Segregated Into Two Distinct Populations

A collaboration between investigators within the NCI-sponsored CLL Research Consortium and intramural NCI scientists has identified two populations of CLL patients by their gene expression patterns using DNA array technology. One group of patients expressing a particular gene pattern had a poor prognosis and needed to receive immediate aggressive treatment. Another group of patients expressing a different gene pattern exhibited smoldering or slow-growing disease. This latter population of patients can postpone treatment to a later time.

Immunotherapy as an Adjunct to Radiation Therapy for Gliomas

The use of tumor-reactive T lymphocytes for cancer therapy has particular theoretical appeal as an adjunct to current therapies for the treatment of gliomas. Using animal tumor models, investigators have determined that lymph nodes draining a tumor inoculum are the optimal source of T cells sensitized to specific tumor antigens, in contrast to other lymph tissues. A phase II clinical trial was conducted using tumor cell lines admixed with GM-CSF to vaccinate all patients. A week later, draining lymph nodes were surgically obtained, then activated and expanded in the presence of SEA and IL2. The activated cells were infused back into the patients, who then received standard radiation therapy. The SEA/IL2 activation procedure induced dramatic T-cell proliferation. In addition, four of eight patients showed partial regression of tumors.

Risk of Leukemia from Radiotherapy and Chemotherapy

In a study of leukemia cases and controls that was undertaken within a cohort of patients diagnosed from 1970 through 1993 with testicular cancer, radiotherapy without chemotherapy was found to be associated with a threefold elevated risk of leukemia. The estimated relative risk of leukemia at a cumulative dose of 650 mg cisplatin, commonly administered in current treatment regimens, was 3.2; larger doses (1,000 mg) were linked with sixfold increased risks. Non-significant excesses were estimated for current radiotherapy regimens limited to the abdomen and pelvis. Among 1,000 patients given a treatment dose

of 25 Gy and followed for 15 years, an excess of 9 leukemias was predicted; cisplatin-based chemotherapy (dose 650 mg) might result in 16 cases of leukemia.

Rare Diseases Research Initiatives

NCI uses Program Announcements (PAs), RFAs, and proposals to announce special initiatives. These initiatives have ranged from soliciting for specialized networks and centers, encouraging research using molecular approaches in tumor/biomarker classification and identification, to encouraging and supporting clinicians and minorities in clinical research.

Genetic Epidemiology of Medulloblastoma

A study of patients with medulloblastoma was recently initiated in collaboration with researchers from NIH and the Children's National Medical Center, Washington, D.C. The study is clinically evaluating patients with this type of brain cancer; assessing risks for all types of cancer among family members; examining tumors for mutations in the *PTCH*, *APC*, or other candidate genes; and evaluating the relationship between molecular genetic alterations, tumor characteristics, response to chemotherapy and radiation treatment, and survival in this group.

Evaluation of Methods for More Accurate Diagnosis and Prognosis for Cervical Cancer

Despite recognizable inaccuracies in staging for cervical cancer and the need for large numbers of conventional and invasive procedures, modern cross-sectional imaging has not been incorporated in routine evaluation. The American College of Radiology Imaging Network (ACRIN), NCI's cooperative group for imaging studies, has initiated a clinical trial that will compare clinical staging to pretreatment evaluation by computed tomography (CT) and magnetic resonance imaging (MRI) in 465 women in order to establish the most accurate pretreatment staging, the most accurate pretreatment assessment of morphologic tumor prognostic factors, and a pathway to decreased utilization of invasive tests; and to design diagnostic test algorithms, which consider both benefits and costs of pretreatment evaluation of cervical cancer. The standard of reference for each test performance will be surgical pathology with lymph node sampling or dissection. To date, approximately 75 women have been entered into the trial, and accrual continues. Because the trial is still in its early stages, there are no definitive results to report.

Identification and Evaluation of Markers for Early Detection and Risk Assessment

The Early Detection Research Network (EDRN) is a national network of academic and industry investigators with expertise in laboratory and clinical sciences, biostatistics, informatics, and public health issues. The goal is to identify and evaluate biomarkers and technologies for earlier detection and assessment of risk. The Biomarkers Developmental Laboratories designed to identify and develop biomarkers for earlier cancer detection and risk assessment have been funded, as have the Clinical/Epidemiology Centers for conducting clinical and epidemiological research on the wide application of biomarkers. Recently formed is the Hereditary Cancer Institute at Creighton University, one of nine Clinical and Epidemiological Centers within EDRN. This Clinical and Epidemiological Center is responsible for developing and maintaining a registry of individuals at high risk for hereditary cancer. This high-risk registry is recruiting people with known genetic mutations for hereditary cancer, a valuable population for studies of biomarkers of early cancer detection.

Clinical Evaluation of Drugs to Prevent Cancer

Currently NCI's Chemoprevention Program is sponsoring 12 phase I and more than 80 phase II and phase III trials. Several agent classes are represented in the phase II and phase III trials, such as retinoids, calcium, anti-inflammatories (e.g., aspirin, sulindac, COX-2 inhibitors, corticosteroids), antiestrogens/antiandrogens, and antimutagens (e.g., dithiolthiones). Agents in phase I trials include: indole-3-carbinol, the combination of oltipraz with N-acetyl-l-cysteine, curcumin, phenethyl-isothiocyanate, perillyl alcohol, the combination of selenomethionine with vitamin E, soy isoflavones, tea polyphenols, and lycopene.

Program expansion will include: 1) identification and evaluation of additional compounds showing biological activity in experimental systems; and 2) initiation of new phase I, II, and III clinical trials in chemoprevention. A number of short-term biochemical and biological markers (i.e., atypical cytology, dysplasia, micronuclei, precancerous conditions, and oncogene suppression tests) have continued to become available and will be utilized in conjunction with and evaluated during clinical trials. Trial designs will be strengthened by utilizing these tests to make an initial prediction of cancer risk or its modulation. New technology that allows greater precision in characterizing and earlier detection of lesions (e.g., LIFE scope, digital mammography, confocal laser microscopy) will be evaluated in several clinical studies. New contract and grant awards involving specific agents, intermediate endpoints, and risk groups will be implemented.

Management of Cervical Abnormalities

NCI's cervical cancer screening and triage study is a clinical trial designed to determine the optimal management of minor and low-grade cervical cytologic abnormalities, taking into account recent knowledge of the role of human papillomavirus (HPV) in cervical cancer. Preliminary results show that HPV testing is highly sensitive in identifying which Pap-detected abnormalities require immediate attention. In this study, HPV testing identified virtually all (96.3%) of the atypical squamous cells of undetermined significance (ASCUS) abnormalities that needed treatment. Although the HPV test proved highly sensitive, two other approaches to ASCUS remain options to consider. These are immediate colposcopy (examination with a magnifying instrument) with biopsy if indicated or follow up by repeat Pap tests every 6 months. Patients and physicians may take several factors into account when deciding what to do about ASCUS, such as cost and patient preferences regarding follow-up appointments. The ASCUS/low-grade squamous intraepithelial lesions (LSIL) Triage Study (ALTS) investigators plan to analyze the cost-effectiveness of the three options when long-term data from the study become available.

Preclinical Drug Development

NCI's Developmental Therapeutics Program continues screening new synthetic and natural compounds for antitumor activity using the automated cancer cell line screen. Approximately 77,000 defined chemical structures have been evaluated since the screen became operational in April 1990. More than 7,500 compounds have demonstrated in vitro antitumor activity, of which 3,900 agents were selected for in vivo evaluation for assessment of therapeutic activity. It is obvious that there are more compounds to test/develop than current resources would allow. In the past year, the decision-making process supporting the development of new drugs has been reformed. In the place of the Decision Network, the Drug Development Group (DDG) now incorporates extramural review of proposed activities. A complete description of this process is available on the Development Therapeutics Program Web site (see <http://dtp.nci.nih.gov>). Including vaccines and other biologicals as well as chemotherapeutic agents, 31 agents are in DDG level 2A (small animal testing), 2 agents are in DDG level 2B (large animal/primate

testing), and 22 are in DDG level 3 (ready for human testing subject to obtaining an Investigational New Drug [IND]). Table 1 provides a listing of the agents in the DDG process. As the agents move through the different levels of the decision process, the level of financial commitment by NCI increases.

To further expedite the movement of academic discoveries from the laboratory to proof of principle clinical trials, NCI initiated a program named Rapid Access to Intervention Development (RAID) in 1998. On a competitive basis, the RAID program makes available to the academic research community resources that are necessary to convert a new molecule into a drug candidate suitable for clinical testing and that are generally not otherwise available to academic investigators who lack a corporate partner. These resources include: 1) GMP synthesis, formulation, range-finding, and IND-directed toxicology and pharmacology; 2) clinical trials planning; 3) regulatory assistance so that the requirements of the Food and Drug Administration (FDA) may be satisfied by any investigator who seeks to put a new molecule into the clinic; and 4) filing of the IND and direct study sponsorship by NCI, where indicated. As of April 2001, 159 applications were received, 51 applications received NCI support, and 19 applications were awaiting review. A description of the successful applicants and the projects can be found at http://dtp.nci.nih.gov/docs/raid/raid_index.html. A listing of RAID projects pertaining to rare diseases is found in Table 4. Of 51 approved RAID projects, 21 include development of agents for rare disease indications, 6 of which will enter clinical trials in approximately 12 months.

Rare Diseases Program Activities

Hepatitis C Virus (HCV)

Chronic infections with HCV appear to be responsible for a recently noted increase in the incidence of human liver cancer. This increase is particularly noteworthy in African Americans. In response to this emerging cancer problem, NCI sponsors workshops and meetings, as well as requests for grant applications in cooperation with other NIH Institutes, to stimulate research leading to the preparation of safe and effective vaccines to prevent HCV infections. In addition, NCI is co-sponsoring a 2002 NIH Consensus Conference on HCV and liver disease that will emphasize therapeutic approaches for HCV-associated liver diseases and research needed for preventive approaches to these diseases.

Beckwith-Wiedemann Syndrome (BWS)

Current studies in NCI's Genetic Epidemiology Branch (GEB) include research on BWS, an overgrowth disorder that occurs approximately once in every 15,000 births. Children with this syndrome can be mildly to greatly affected, and are at risk for developing hypoglycemia and various types of tumors. GEB staff have recently published an informational brochure titled, "Living with Beckwith-Wiedemann Syndrome," which provides a brief overview of the disorder, explains the specialized care that these children may need, and outlines the resources that are available to help these families.

Screening Drugs to Prevent Cancer

Significant effort has been devoted to developing in vitro and animal model assays that evaluate activity against specific genetic and molecular targets associated with carcinogenesis. Some of the molecular targets are oncogenes, and others are molecules on signal transduction pathways affecting cell growth and proliferation. Recently, additional assays have been added that look more generally at cellular effects, rather than at specific molecules, such as: differentiation enhancement as shown by involucrin expression in cultured normal human epidermal cells and cytokeratin alterations (keratins 5, 8, and 18 and vimentin)

in cultured human benign prostatic hyperplasia (BPH) cells; apoptosis measured as DNA fragmentation in transformed human BPH cells; and angiogenesis inhibition in chick embryo chorioallantoic membrane.

Co-sponsorship of Workshops

In 2000, NCI co-sponsored five workshops with ORD. These workshops focused on gastrointestinal stromal tumors (GIST) markers, genetic susceptibility to prostate cancers, innovative strategies for screening women at increased genetic risk of ovarian cancer, overcoming tolerance to self-tumor antigens, and Waldenstrom's macroglobulinemia (WM).

Table 1. Compounds That Passed Drug Development Group (as of April 2001)Drug Development Group 2ANSC Number

707389 Halichondrin B Analog
684682 Saporin immunoconjugate: BU12-Saporin
684683 Saporin immunoconjugate: OKT10-Saporin
684684 Saporin immunoconjugate: 4KB128-Saporin
710305 Discreet
697726 RH1
680718 Nitidine-like Compound
710464 Discreet
701315 Anti-HER2 Immunoliposomes
703940 Angiostatin
703939 RFB4-Onconase
705701 9-Nitro-Paullone
709399 Synerlip-p53
690073 Discreet
281617 Dimethane Sulfonates
281817 Dimethane Sulfonates
694501 Discreet
713205 Halofuginone
696823 Discreet
696824 Discreet
696825 Discreet
696826 Discreet
707016 Discreet
716976 BNP7787
680410 Adaphostin
718329 Discreet
656243 Dithiophene and Derivatives
656240 Dithiophene and Derivatives
656238 Dithiophene and Derivatives
682994 Dithiophene and Derivatives
719664 2-Methoxy Antimycin A₃

Drug Development Group 2BNSC Number

712392 LB42908
371331 + 112907 Cytochlor + Tetrahydrouridine

Drug Development Group 3NSC Number

700553 Discreet
603573 HeFi-1 Anti-CD30
Monoclonal Antibody
710084 Discreet
710427 Discreet
713219 SGN-00101 (Hsp-E7)
702827 SU6668
713763 BMS-275291
714373 LY353381-HC1
659853 2-Methoxyestradiol
715969 CAMPATH-1H
716711 Epratuzumab (hLL2)
716976 BNP7787
698215 R(+)-XK469
678516 ¹⁸F-FMAU
718781 OSI-774
719850 MEDI-522
683864 Rapamycin Analog
639829 Dimethyl Benzoylphenylurea
716051 STI571
706995 MS-275
710085 IDEC-Y2B8 Radiolabeled Anti-CD20
Antibody
715055 ZD1839

**Table 2. Active Research and Development Agreements
(by Agent; Company; Type— as of May 2001)**

<i><u>Agent</u></i>	<i><u>Company</u></i>	<i><u>Type</u></i>
2-methoxyestradiol	Entremed, Inc.	CRADA
280-446	Novartis	CTA
5-azacytidine	Pharmacia	CTA
506U78	Glaxo Wellcome	CTA
Abbott's proprietary research and development strategies	Abbott Laboratories	CTA
AE-941 (shark cartilage)	Aeterna	CTA
All-trans retinoic acid	Hoffmann-La Roche	CTA
ALVAC-IL-12	Pasteur-Merieux Con FR	CTA
Angiostatin	Entremed, Inc.	CRADA-LOI
Antigen genes formulated for delivery in a dermal Powderject XR gene delivery device	Powderject	CTA
Arsenic trioxide	Cell Therapeutics, Inc.	CRADA
BeneFin	LaneLabs-USA, Inc.	CTA
Bizelesin	Pharmacia	CTA
BMS 214662 (FTI)	Bristol-Myers Squibb	CTA
BMS 247550 (Epothilone B analog)	Bristol-Myers Squibb	CTA
BMS 275291 (MMPI)	Bristol-Myers Squibb	CTA
BMS 275291 (MMPI)	Bristol-Myers Squibb	M-CRADA
BNP7787	Bionumerik Pharmaceuticals	CTA
CCI-779	Wyeth-Ayerst Research	CRADA
CD40L	Immunex	M-CRADA
Clodronate	Anthra Pharmaceuticals	M-CRADA
COL-3	Collagenex	CRADA
CpG ODN	Coley Pharmaceutical Group	CSA
CTLA4-Ig	RepliGen	CTA

Decitabine	Supergen, Inc.	CRADA
Depsipeptide (FR901228)	Fujisawa	CTA
Doxil	Alza Corporation	CSA
E7389	Eisai Research Institute	CRADA
EMD 121974	Merck KGAA	CRADA
Endostatin	Entremed, Inc.	CRADA
Epratuzumab	Immunomedics, Inc.	CTA
Exemestane	Pharmacia	M-CRADA
Fenretinide	Janssen	CTA
Flavopiridol	Aventis Pharmaceuticals	CRADA
G3139	Genta	CRADA
Gadolinium texaphyrin	Pharmacyclics	CRADA
GM-CSF	Immunex	CTA
Herceptin	Genentech	CRADA
Homoharringtonine	American Bioscience, Inc.	CRADA
HSP-E7	Stressgen Biotechnologies	CTA
HUM291 (Anti-CD AB)	Protein Design Labs, Inc.	CTA
IL-12	Genetics Institute	CRADA
IL-2	Chiron Corporation	CTA
Iododoxorubicin	Pharmacia	CTA
Iressa (ZD1839)	AstraZeneca	CTA
Irinotecan LB42908	Pharmacia	CTA
LB42908	LG Chemicals, Ltd.	CRADA-LOI
Lutetium texaphyrin	Pharmacyclics	CRADA
Mage-3 peptide vaccine	GlaxoSmithKline Biologicals	CTA
MGI 114	MGI Pharma	CTA
MS-275	Nihon Schering K.K.	CRADA
MTP-PE	Jenner Technologies	CTA

O6-BG	Procept, Inc.	CRADA
Onyx-015	Onyx	CRADA
OSI-774	OSI Pharmaceuticals, Inc.	CTA
Oxaliplatin	Sanofi-Synthelabo	CRADA
P53 adenovirus	Aventis Pharmaceuticals	CRADA
Perifosine (D-21266)	Zentaris AG	CRADA
PS-341	Millennium Pharmaceutical	CRADA
PSC-833	Novartis	CTA
PV701	Pro-Virus	CRADA
QS-21	Aquila Biopharmaceuticals	CSA
R115777	Janssen	CTA
Rebeccamycin analog	Bristol-Myers Squibb	CTA
rF-TRICOM, rF-CEA-TRICOM	Therion	Intramural
Rhumab VEGF	Genentech	CRADA
Rituximab	Idec Pharmaceuticals	CTA
rV-B7.1	Therion	CRADA
SC-55494	Searle	ICRADA
Smart 1D10 (HU1D10)	Protein Design Labs, Inc.	CTA
STI571	Novartis	CTA
STI571	Novartis	CRADA-LOI
SU5416	Sugen, Inc.	CRADA
SU6668	Sugen, Inc.	CTA
Taxol	Bristol-Myers Squibb	CTA
Taxotere	Aventis Pharmaceuticals	CTA
Thalidomide	Celgene Corporation	CTA
Tirapazamine	Sanofi-Synthelabo	CTA
Topotecan hypochloride	SmithKline Beecham	CTA
Tumor necrosis factor alpha	Boehringer Ingelheim	CTA

UCN-01	Kyowa Hakko Kogyo	CTA
XK469	DuPont Pharmaceuticals Co.	CTA
Zevalin (Y2B8)	Ilex Pharmaceuticals	CTA

Table 3a. Investigational New Anticancer Agents in Early Clinical Trials (As of May 2001)
Cytotoxic Agents

Phase I

17-AAG
 Arsenic Trioxide
 Benzylguanine
 Bizelesin
 BMS 214662 (FTI)
 BMS 247550(epothilone B)
 BPU
 CCI779
 COL-3
 Compound 506U
 Cordycepin/Pentostatin
 Depsipeptide
 EF5
 EMD 121974
 G3139
 Iododoxorubicin
 KRN5500
 Lutetium Texaphyrin
 MS275
 Perifosine
 Phenylbutyrate
 PS341
 Rebeccamycin Analog
 UCN-01

Phase II

Amino-camptothecin
 Bryostatin 1
 BSO
 CAI
 CI-980
 Compound 776C85
 Dolastatin 10
 Fenretinide
 Flavopiridol
 Iressa
 Perillyl Alcohol
 Phenylacetate
 PSC 833
 Pyrazoloacridine
 Pyrazine Diazohydroxide
 R115777
 STI571
 SU5416
 Temozolomide
 Thioguanine (IV)
 Tirapazamine

Table 3b. Investigational New Anticancer Agents in Early Clinical Trials (As of May 2001)
Biological Agents

Phase I

ALVAC-B7.1
 ALVAC-CEA-B7.1
 ALVAC-IL-12
 Anti-Tac (Fv)-PE38 Immunotoxin
 BL22 Immunotoxin
 Carcinoembryonic Antigen Peptide Vaccine
 CEA Vaccinia Vaccine
 E1B-Attenuated Adenovirus
 Endostatin
 FLT3 Ligand
 Fowlpox-PSA Vaccine
 gp100 DNA Vaccine
 gp100 Melanoma Vaccines
 HER-2/neu Peptide Vaccine
 HIV 1 Vaccine
 HPV E6 & E7 Vaccine
 HPV E7 Lipopeptide Vaccine
 HuID10
 HuM291
 IL-12
 IL-12 + IL-2
 Immunotoxin ERB-38
 Immunotoxin LMB-1
 MART-1 Melanoma Vaccines
 MoAb: Anti-VEGF
 MoAb: A27. 15 and E2.3
 MoAb: B3
 MoAb: CC49-9OY
 MoAb: Humanized Her2
 MoAb: T-cell (3A1, 95-5-49, 95-6-22)
 MOV-18 Chimeric T-Cell Receptor
 P53 Adenovirus Vector
 P53 and RAS Peptide Vaccine
 Pediatric Sarcoma Peptide Vaccines
 PR-1 Peptide
 PSA-3 Peptide
 PSA Vaccinia Vaccine
 RAS Peptide Vaccine
 Vaccinia-CEA-TRICOM + Fowlpox-CEA-TRICOM
 Vaccinia-PSA Vaccine
 Vaccinia-MUC1 Vaccine
 Vaccinia-TRICOM + Fowlpox-TRICOM
 Vaccinia Tyrosine + Fowlpox Tyrosine
 VHL Peptide Vaccine
 Y2B8

Phase II

Anti-idiotypic-KLH Lymphoma Vaccine
 Carboxypeptidase G2
 Cis-Retinoic Acid
 IFN: Rec Gamma
 IL-4
 IL-21TIL
 MoAb: CC49
 MoAb: C2B8
 MoAb: OKT3
 MoAb: 14.18 Chimeric

Table 4. Raid Compounds for the Treatment of Rare Diseases (As of April 2001)

<u>Compound NSC</u>	<u>Name</u>	<u>Disease</u>	<u>Investigator</u>	<u>Pediatric Use</u>
710296	C-myb Antisense Oligodeoxynucleotide	Acute Myelocytic Leukemia	Dr. Alan Gewirtz; University of Pennsylvania, School of Medicine	Yes
710292	Lipopeptide	Cytomegalovirus	Dr. Don Diamond; City of Hope Medical Center	Yes
354258	8-Chloro-Adenosine	Multiple Myeloma	Dr. Steven Rosen; Northwestern University, Lurie Comprehensive Cancer	
711516	Chimerized Anti-amyloidosis Mabs	AL Amyloidosis	Dr. Alan Solomon; University of Tennessee	
711517	Shed Polyvalent Antigen Vaccine	Melanoma	Dr. Jean-Claude Bystry; New York University Medical Center	
711295	Mab 216 (VH4-34) Anti-Hu B Lymphocyte Antibody	Lymphoma	Dr. Nelson N. H. Teng; Stanford University	Yes
711518	Allogenic Pancreatic Tumor Vaccine	Pancreas	Dr. Elizabeth M. Jaffee; Johns Hopkins University	
711519	IGF-1R Antisense Oligodeoxynucleotide	Glioma	Dr. Robert Aiken; Thomas Jefferson Medical College	Yes
714597	Imexon	Multiple Myeloma	Dr. Robert Dorr; University of Arizona, Arizona Cancer Center	

113090	Betulinic Acid	Multiple Myeloma	Dr. Tapas Das Gupta; University of Illinois at Chicago	
650378D	Spongistatin 1	Melanoma, Ovary	Dr. George Pettit; Arizona State University, Cancer Research Institute	
734551 714503	Fenretinide plus Safingnol	Neuroblastoma, Pancreas, Acute Leukemias	Dr. C. Patrick Reynolds; University of Southern California School of Medicine	Yes
715815	Chimeric Anti-CD54 monoclonal Antibody (UV3)	Multiple Myeloma	Dr. Ellen Vitetta; University of Texas, Southwestern Medical Center	
715816	Tropism-Modified Adenoviral Vector	Ovary	Dr. Glenn Peters; University of Alabama, Comprehensive Cancer Center	
717904	Immucillin-H	T Cell Lymphoma	Dr. Vern Schramm; Albert Einstein College of Medicine	
7365	6-Diazo-5-Oxo-L-Norleucine	Neuroendocrine	Dr. Håkan Örlfors; Uppsala University Hospital, Sweden	
71887	Psuedomonas Exotoxin Construct	Glioblastoma Multiforme, Neoplastic Meningitis	Dr. Darrell Bigner; Duke University Comprehensive Cancer Center	Yes
719277	Nonpathogenic Oncolytic Poliovirus Chimeras	Glioma	Dr. Matthias Gromeier; Duke University Medical Center	Yes

720454	vac-mTag Recombinant Vaccinia Construct	Mesothelioma	Dr. Harvey Pass; Barbara Ann Karmanos Cancer Institute
720833	EBV Supernatant	Lympho- proliferative Disease	Dr. Richard Ambinder; Johns Hopkins University
720836	IL-6 plus Interferon- α	Multiple Myeloma	Dr. Richard Jones; Johns Hopkins University

National Institute of Child Health and Human Development (NICHD)

Overview of NICHD Rare Diseases Research Activities

The mission of NICHD is to conduct and support research on the reproductive, physiologic, and behavioral processes that determine the health of individuals and populations. NICHD's programs are based on the concept that adult health and well-being are determined in part by episodes early in life and that human development continues throughout the life span. Diseases or conditions that interfere with healthy development are of concern to the Institute, and NICHD supports research in the prevention, diagnosis, evaluation, and treatment of many rare diseases and disorders.

Recent Scientific Advances in Rare Diseases Research

Polycystic Ovarian Syndrome (PCOS)

PCOS is a complex disorder with a constellation of symptoms, including problems with ovulation and fertility, elevated androgen and insulin, excess body hair, obesity, and multiple ovarian cysts. Despite the significant health consequences of PCOS, the etiology remains uncertain. A common feature in women with PCOS is an increase in the frequency and amplitude of the pulses of luteinizing hormone (LH) secreted by the anterior pituitary gland. Research had shown that the ability of estrogen and progesterone to regulate pulsatile LH secretion is diminished in women with PCOS. Recently, evidence has shown that the high levels of androgen in PCOS may be important for the diminished ability of estrogen and progesterone to suppress pulsatile LH secretion. Administration of the antiandrogen flutamide to women with PCOS resulted in an increased ability of estrogen and progesterone to suppress pulsatile LH secretion. This finding has potential significance in treatments to normalize LH secretion resulting in restoration of normal ovulatory function.

Clomiphene citrate (CC) is a drug successfully used to induce ovulation in women with PCOS; however, many women are resistant to the drug. To determine if hyperinsulinemia may be involved in the resistance to CC, metformin, an insulin-sensitizer, has been administered to CC-resistant women while they are being treated with CC. Seventy-five percent of the participants given metformin ovulated, compared to 27% given placebo. Furthermore, 55% given metformin conceived, compared to less than 10% given placebo. This study provides compelling evidence that hyperinsulinemia of PCOS is associated with resistance to CC and suggests a therapy for ovulation induction.

PCOS symptoms have been noted to vary with racial and ethnic groups. Because the metabolic abnormalities may increase the risk for cardiovascular diseases, these differences are a health disparity issue that is worth pursuing. Due to higher levels of low-density lipoproteins (LDLs) in their blood, Hispanic women have a higher risk of developing blocked arteries than Caucasian women. Differences in serum lipids of Hispanic women with PCOS and weight-matched Hispanic controls have been demonstrated. The elevated levels of androgen of PCOS along with the higher level of lipids in Hispanic women may have an adverse effect on cardiovascular risk.

Disorders of the Adrenal Cortex

Genetic and molecular mechanisms of rare disorders that affect the adrenal cortex are being investigated, especially those that are hereditary and associated with multiple tumors and abnormalities in other endocrine glands. Studies of families with Carney complex (CNC), a combination of benign tumors of

connective tissue, spotty skin pigmentation, endocrine overactivity, and other related syndromes, have revealed two loci harboring genes for CNC on regions of chromosomes 2 and 17. A comprehensive genetic and physical map of the chromosome 2 region was constructed for the cloning of the CNC-causing gene. Studies in cultured primary tumor cell lines revealed a region of amplification in the center of this map. Other tumors, however, show loss of genetic material. On chromosome 17, studies have shown that a novel tumor-suppressor gene, abbreviated *PRKARIA*, is responsible for 40% of CNC cases. Studies of the functional consequences of *PRKARIA* mutations in cell lines and the developmental effects of mutant genes in transgenic animal models are under way. In collaboration with the Mayo Clinic, researchers are identifying the genetic defects in patients with CNC-related syndromes such as Peutz-Jeghers syndrome.

Primary Immunodeficiencies

Primary or genetic immunodeficiencies are a heterogeneous group of inherited developmental disorders of the immune system that increase susceptibility to infection. There are more than 80 types of primary immunodeficiencies. Depending on the specific genetic defect, the immunological symptoms of primary immunodeficiencies can be inapparent, mild, or severe. Although more types of primary immunodeficiencies are being described, they nevertheless remain rare. Most recent research advances have focused on identifying the genes, genetic mutations, and expressed proteins characterizing the molecular mechanisms and on elucidating the biochemical pathways that result in primary immunodeficiencies. For example, recent studies on Wiskott-Aldrich syndrome (WAS) indicate that the *WAS* protein plays a major role in cytoskeleton formation, cell signaling, development, and programmed death of platelets and hematopoietic cells in WAS patients. Moreover, these basic studies have led to the development of a prenatal genetic test for *WAS* mutations.

Immunodeficiency, centromeric decondensation, and facial anomalies (ICF) syndrome is a rare autosomal recessive disease characterized by variable suppression of the immune system, mild facial anomalies, and instability of areas in chromosomes 1, 9, and 16. At the molecular level the condition is characterized by a lower than normal number of simple carbon groups, called methyl groups, that bind to regions of the chromosomes. A gene responsible for this syndrome has been identified as *DNMT3B*, which encodes for an enzyme responsible for binding methyl groups to DNA. Chromosomal methylation patterns play important roles in gene expression, but the processes that determine these complex patterns are only partially understood. The finding that the methylation system is involved in other diseases such as Rett syndrome further highlights that human genetic diseases can be caused by perturbations altering the activity of genes without altering gene structure.

Alstrom Syndrome

Alstrom syndrome is a rare autosomal recessive disorder. Fewer than 100 cases have been diagnosed since the disorder was first reported in 1959. The syndrome presents in early childhood with involuntary rapid eye movements, elevated insulin, and obesity. Progressive retinal degeneration and hearing loss develop later in childhood, followed by type 2 diabetes and kidney failure in early adulthood. Although Alstrom syndrome is a rare genetic disorder, the characteristics of obesity, hyperinsulinemia, and retinal and cochlear degeneration are prevalent in the general population. The genes responsible for these common conditions remain elusive. The identification of the Alstrom gene may shed light on the pathogenesis of obesity and hyperinsulinemia, as well as retinal problems and neural deafness. Twelve Alstrom families with diverse ancestral origins have been recently ascertained. Linkage analyses in these families confirm the localization of the Alstrom gene to chromosome 2. Although it is unlikely that

mutations within the gene play a major role in common diseases, the value of identifying this gene lies in the access it may provide to metabolic and regulatory pathways involved in the etiology of neurosensory disease, obesity, type 2 diabetes, and related disorders.

Necrotizing Enterocolitis (NEC)

NEC is a mysterious gastrointestinal disease among premature infants in neonatal intensive care units, almost always developing at the time when intravenous feeding ends and stomach feeding begins. Affected infants may have abdominal tenderness and distension, gastrointestinal obstruction, hemorrhage and perforation, vomiting, and peritonitis. From 20% to 40% of affected infants die, and survivors suffer sequelae such as chronic malabsorption. A recent study of preterm infants fed human milk or preterm formula showed that those infants who began feeding on day 4 after birth demonstrated lower intestinal permeability than those whose milk or formula feeding was withheld until day 14. Antenatal steroid use was also associated with a decrease in permeability. At 28 days of age, intestinal permeability was lower in infants who were exclusively fed human milk, compared with those fed formula. The relationships between permeability and early feeding, antenatal steroid use, and human milk feeding were significant and have important clinical implications for the prevention of NEC.

Animal Models for Rare Diseases

Velo-Cardio-Facial Syndrome/Di George Syndrome (VCFS/DGS)

VCFS and the closely related, if not identical, DGS are disorders of embryonic clefting defects leading to cleft palate, other problems with mouth and throat structure, septal defects of the heart, and immunological abnormalities. As affected children grow, they exhibit learning disabilities, and older affected children develop psychiatric illness. The hypothesis presented in current studies is that VCFS/DGS is primarily caused by mutations in one gene that affects neural crest cell migration and that mutations in other genes contribute to the additional manifestations of VCFS/DGS. The region on human chromosome 22 that is responsible for producing the syndromes is equivalent to regions found on three mouse chromosomes, 6, 10, and 16. Potential mouse models are being generated for VCFS/DGS through mutations, deletions, and duplications of genes within specified regions of these mouse chromosomes. Gene deletions produce models that develop heart and parathyroid defects and perinatal lethality. Rescue of the lethal state can be achieved by insertion of a human transcription factor gene called *TBX1*. The *TBX1* gene is expressed in the pharynx, the inner ear, and the tooth buds during the embryonic and fetal periods. Its expression is essential for proper development of the fourth pharyngeal arch, which contributes to the development of the aortic arch and the conotruncal region of the heart. Overexpression of *TBX1* leads to circling behavior in mice due to inner ear defects. Underexpression of *TBX1* leads to conotruncal defects. Thus, mutations of the *TBX1* gene appear to be responsible for the cardiovascular defects common to VCFS/DGS and may also be involved in the sensorineural hearing loss often observed in VCFS.

Osteogenesis Imperfecta (OI)

A mouse model for the human non-lethal, moderately severe OI type IV has been created. Histology of the “Brittle Mouse” (Brtl) demonstrates that it reproduces the crucial characteristics of the human disease. Bones surrounding the spinal cord are disorganized and less maturely mineralized than in normal mice. Osteoporosis and disorganized areas of bone growth are seen in the jaw and nasal passages. The skull cap is thin and undermineralized. The Brtl mouse also reproduces the variability of pathophysiological

features that are frequently seen in human OI patients. The features of the mouse model range from moderately severe to lethal. The surviving mice are 50% to 80% smaller than normal litter mates, and have deformed rib cages with slender ribs, bowed legs, and generalized undermineralization. At the more severe end of the spectrum, 40% to 60% of the mutant mice die from respiratory distress a few hours after birth. Differences in mutant collagen between the mice that lived and the lethal phenotype were compared. No differences were found. Several studies are being conducted on this mouse model to better understand the pathology of OI. These studies include cellular and molecular biology to determine the basis for the low number of bone-forming cells, biomechanics to determine the basis of the bone susceptibility to fracture, and pharmacology to determine the effect of the drug alendronate on bone strength and structure.

Rare Diseases Research Initiatives

An RFA titled, “Pathophysiology, Epidemiology and Treatment of Vulvodynia” was issued in FY 2000. Vulvodynia is an elusive pain syndrome characterized by chronic vulvar pain. The solicitation of applications was intended to further the understanding of the etiology, prevalence, diagnosis, pathophysiology, pain mechanisms, and treatment strategies for the disorder. Three awards were made to investigators responding to the RFA. One study surveys women to estimate the age-specific prevalence of vulvodynia and determine the association of microorganisms and inflammatory agents in vaginal specimens with the occurrence of vulvodynia. The second study seeks to examine the neurologic and immunologic factors associated with the disorder. The third project integrates the prevalence of vulvodynia, its risk factors, and clinical correlates with current treatments to determine their validity.

A new research initiative on the association between the fragile X mental retardation gene (*FMRI*) and premature ovarian failure (POF) is being planned for support through the Specialized Cooperative Centers Program in Reproduction Research. The initiative proposes to characterize cell-specific expression of the *FMRI* gene in the human ovary to define the endocrine profile during the menstrual cycle of female fragile X premutation carriers, and to create a bank of DNA samples from well-characterized POF patients to assist investigators in the evaluation of candidate genes for POF.

Conferences, Workshops, and Other Activities Concerning Rare Diseases

A meeting co-sponsored by NICHD, the March of Dimes, and Klinefelter Syndrome and Associates to address gaps in information on Klinefelter syndrome was held in August 2000. The meeting was designed to summarize the spectrum of clinical features in 47,XXY and variant (48,XXXY) Klinefelter syndrome concerning reproductive, structural, and neurologic/cognitive/behavioral aspects and to clarify the incidence of Klinefelter syndrome and its genetic basis, including the role X-inactivation plays in the varied pathology. Other aims were to enumerate the most frequent genetic counseling dilemmas that arise from detection in utero; to update current management options for hormone deficiency, infertility, and neurologic perturbations; and to prioritize basic and clinical research initiatives.

A workshop titled, “Polycystic Ovary Syndrome: Basic Biology and Clinical Intervention” was held September 17-20, 2000. The workshop was sponsored by the National Institute of Environmental Health Sciences (NIEHS), NICHD, ORD, and the Office of Research on Women’s Health (ORWH). The meeting was designed to bring together a multidisciplinary group of scientists—including cellular and

molecular biologists, endocrinologists, toxicologists, and clinicians—in order to disseminate current research on the etiology, pathophysiology, and treatment of PCOS.

A conference titled, “Disorders of Intracellular Vesicles: The Hermansky-Pudlak Syndrome/Chediak-Higashi Syndrome Spectrum” was held in July 2000 to discuss the clinical, biochemical, molecular, and cell biological aspects of these disorders.

NICHD/National Eye Institute (NEI) intramural investigators have been working with Sigma-Tau Pharmaceuticals, Inc., in an effort to bring cysteamine eyedrops to New Drug Application (NDA). Orphan drug designation has been obtained for this product through the efforts of these investigators. The eyedrops dissolve the cystine crystals in the corneas of patients with nephropathic cystinosis.

In studies for the treatment of hypoparathyroidism with synthetic human parathyroid hormone, Rare Disease Pharmaceuticals has become interested in using these studies to obtain FDA approval of the parathyroid hormone as an orphan drug for hypoparathyroidism.

Several intramural investigators are involved with patient groups/family organizations, including the Cystinosis Foundation, the Hermansky-Pudlak Syndrome Network, the Premature Ovarian Support Group, and the Genetic Alliance.

Warren Grant Magnuson Clinical Center (CC)

Overview of CC Rare Diseases Research Activities

Clinical Rare Diseases Research Support

The NIH CC, which opened in 1953, is the clinical research facility that provides the venue for translating the basic science discoveries and scientific advances made at the benches and laboratories of the NIH ICs into clinical medicine. The CC is a hospital entirely dedicated to clinical research. As a national resource, the mission of the CC is to provide the protocol-specific patient care, services, training, and environment needed to initiate and support the clinical research sponsored by the individual NIH Institutes.

Of the 19 NIH Institutes, 15 have clinical programs that involve clinical research activities in the CC. The scope of care provided in the approximately 1,000 active clinical protocols ranges from acute, intensive medical care to studies of patients who have bipolar disorder, schizophrenia, depression, or other behavioral illnesses. General areas in which ongoing studies are active include the following:

- Medical, surgical, and pediatric oncology
- Medical genetics
- Endocrinology
- Rheumatology
- Nephrology
- Infectious diseases
- Hematology
- Cardiology
- Ophthalmology
- Otolaryngology
- Immunology
- Allergy
- Gastroenterology
- Neurology
- Neurosurgery
- Dentistry and oral surgery
- Alcohol dependence
- Gerontology
- Pulmonology
- Psychiatry and psychology
- Rehabilitation medicine
- Imaging sciences

In collaboration with Institute investigators and staff, the CC provides comprehensive support for patients with rare diseases admitted to participate in clinical research protocols. Patients with rare diseases often require specialized care due to their unique physical and/or psychosocial needs. The CC provides an integrated protocol-centered approach to meeting the needs of these patient populations. CC staff are provided with education and training opportunities related to these rare diseases to ensure that comprehensive and safe patient care and clinical research support will be provided to all patients.

The CC works closely with Institute staff to ensure that the appropriate diagnostic tests and therapeutic modalities are available to manage patients with rare diseases.

Sampling of "Rare Diseases" (Source: Active CC Protocols)

CC

- Hereditary hemochromatosis
- Transfusion dependent 5q minus syndrome
- Factor V Leiden
- Hyperhomocysteinemia

NCI

- Tac-Expressing adult T-cell leukemia
- Metastatic androgen independent prostate cancer
- Peritoneal carcinomatosis
- Mantle cell lymphoma
- Plexiform neurofibromatosis type 1
- Lymphomatoid granulomatosis

NEI

- Pigment dispersion syndrome
- Usher syndrome
- Behcet's disease

NHGRI

- Lowe syndrome
- Smith-Magenis syndrome
- Pallister-Hall syndrome

NHLBI

- Myelodysplastic syndrome
- Lymphangioleiomyomatosis
- Neuroacanthocytosis
- Diamond-Blackfan anemia
- Homozygous sitosterolemia

NIAID

- Wegener's granulomatosis
- Helminth infections
- Hyperimmunoglobulin E recurrent infection (Job) syndrome
- Autoimmune lymphoproliferative syndrome (ALPS)
- Cysticercosis

- Chronic granulomatous disease (CGD)
- Familial hypereosinophilic syndrome

NIAMS

- TNF receptor-associated periodic syndrome
- Dermatomyositis
- Systemic lupus erythematosus (SLE)

NICHD

- Pheochromocytoma
- Chediak-Higashi syndrome
- Infantile neuronal ceroid lipofuscinosis
- Turner's syndrome
- Familial isosexual precocious puberty
- Osteogenesis imperfecta (OI)
- Cushing's syndrome
- Primary pigmented nodular adrenocortical disease (PPNAD)
- Carney complex
- Hermansky-Pudlak syndrome
- Smith-Lemli-Opitz (SLO) syndrome

NIDCR

- Sjögren's syndrome
- McCune-Albright syndrome

NIDDK

- Focal segmental glomerulosclerosis
- Idiopathic membranous glomerulopathy
- Acromegaly
- Membranous lupus nephropathy
- Zollinger-Ellison syndrome

NIMH

- Progestin-induced dysphoria

NINDS

- Lafora disease
- Primary spinal syringomyelia
- Mucopolipidosis type IV

- Friedreich's ataxia (FRDA)
- Menkes disease
- Fabry's disease
- Gaucher disease

National Center for Complementary and Alternative Medicine (NCCAM)

Overview of NCCAM Rare Diseases Research Activities

NCCAM is dedicated to exploring complementary and alternative medicine (CAM) healing practices in the context of rigorous science. To achieve this goal NCCAM:

- Identifies and evaluates CAM treatment, diagnostic, and prevention modalities in each of the broad domains of CAM.
- Conducts or supports clinical trials, basic science research, epidemiological studies, health services research, and other appropriate research and investigational activities.
- Studies CAM treatment, diagnostic, and prevention systems, modalities, and disciplines and their potential for integration into the health care delivery system.

All diseases or conditions for which CAM is used are of concern to NCCAM; thus, NCCAM supports research in the prevention, diagnosis, evaluation, and treatment of several rare diseases and disorders.

Rare Diseases Research Initiatives

Spastic Cerebral Palsy

NCCAM currently supports a phase II clinical trial (FY 1998 through FY 2003) at the University of Arizona to evaluate two CAM modalities (osteopathic manipulation and acupuncture) that have been used in children to reduce complications associated with cerebral palsy versus a control group (therapeutic play). Of particular interest will be evaluations of the incorporation and compliance with the two modalities within the participating groups already providing clinical services.

Glioblastoma

NCCAM currently supports two studies on alternative approaches to glioblastoma at the California Pacific Medical Center Research Center. In the first study (FY 2000 through FY 2003), investigators are examining the efficacy of berberine, a compound isolated from Chinese herbs, to enhance the radiation sensitivity of glioma cells in vitro. In the second study (FY 2000 through FY 2004), glioma patients beginning radiation therapy are also enrolled in a double-blinded, randomized, controlled study of distant healing intentionality. Distant healing is a mental intention on behalf of one person to benefit another at a distance.

National Institute on Deafness and Other Communication Disorders (NIDCD)

Overview of NIDCD Rare Diseases Research Activities

NIDCD conducts and supports research and research training on normal mechanisms and diseases and disorders of hearing, balance, smell, taste, voice, speech, and language. NIDCD achieves its mission through a wide range of research performed in its own laboratories, a program of research grants, individual and institutional research training awards, career development awards, center grants, cooperative clinical trials, and contracts to public and private research institutions and organizations. The Institute also conducts and supports research and research training that is related to disease prevention and health promotion. NIDCD addresses special biomedical and behavioral problems associated with people who have communication impairments or disorders. NIDCD also supports efforts to create devices that substitute for lost and impaired sensory and communication functions.

Recent Scientific Advances in Rare Diseases Research

Mitochondrial Genes and Deafness

Mitochondria are specialized structures within cells that play a crucial role in metabolism and energy production. Mitochondria contain their own genes, which act to replicate the mitochondria during cell division. All of the mitochondria present in individuals are derived from the mother's egg. Therefore, diseases that appear to be passed exclusively through the maternal lineage are often linked to defective mitochondrial genes.

NIDCD-supported investigators have identified several specific mitochondrial mutations that predispose an individual to hearing damage from aminoglycoside ototoxicity. Most recently, these investigators have identified a genetic locus in the nucleus of the cell that acts to modify the effects of the mitochondrial mutations. These findings could be used to develop genetic tests to determine whether an individual has an increased risk for aminoglycoside-induced hearing damage.

Usher Syndrome

Usher syndrome is characterized by hearing loss, retinitis pigmentosa, and/or vestibular areflexia. The frequency of this syndrome has been estimated at 5% of the deaf population, with more than half of the deaf and blind individuals (>10,000) in the United States affected by this disease. The severity of the hearing loss and the presence of vestibular dysfunction distinguishes two clinical subtypes of Usher syndrome, types I and II. A third form of Usher syndrome (type III), which has a late onset, has recently been described. These three phenotypes are genetically distinct. NIDCD's Intramural Research Program is continuing to support the Hereditary Hearing Impairment Consortium, the members of which are working to identify and characterize all the genes responsible for Usher syndrome. A major advance in this area of research was the finding that the gene for Usher type Ib codes for an unconventional myosin protein; information that led to the realization that a known animal model of deafness was a homolog for this type of Usher syndrome.

Recently, several NIDCD-supported scientists reported the cloning of the gene for Usher syndrome type IIa. The *USH2A* gene encodes a protein, "Usherin," that has structures similar to other proteins involved in assembling cells and tissues into functional organs. Within the last 6 months NIDCD-supported scientists have identified the genes responsible for Usher type Ic and Usher type Id. The cloning of these

genes and analysis of the proteins they produce are critical steps towards developing strategies to treat this devastating disease.

Waardenburg Syndrome (WS)

WS is an autosomal dominant disorder characterized by pigmentary disturbances and cochlear deafness in some individuals. There are at least three distinct forms of this syndrome (types I, II, and III). A transcription factor gene, microphthalmia-associated transcription factor (*MITF*), was cloned by NIDCD intramural scientists and was assigned to chromosome 3p14.1-p12.3. This gene is a human homolog of the mouse *mi* gene. Phenotypes of mice with mutations at *mi* alleles are closely related to those of WS type II, and mutations of *MITF* have been found in some WS type II families. Mutation of a second gene, *PAX3*, also encoding a transcription factor, causes WS type I and WS type III. Current evidence suggests that variable disease expression is dependent on the genetic background provided by both parents. Importantly, recent characterization of the *PAX3* gene structure and alternative transcripts by NIDCD intramural scientists will enable a comprehensive mutation screen for individuals with WS.

Stickler Syndrome

Stickler syndrome is a rare autosomal dominant disorder causing progressive sensorineural hearing loss, myopia, retinal detachments, arthropathy, and craniofacial abnormalities in affected individuals. Stickler syndrome may be caused by mutations in any of the genes encoding the three polypeptide subunits of type XI collagen: *COL2A1*, *COL11A1*, and *COL11A2*. In conjunction with NHGRI scientists, NIDCD intramural scientists are characterizing the otolaryngologic and auditory phenotypes of individuals affected with Stickler syndrome.

Auditory Neuropathy

A small but substantial number of patients with bilateral hearing loss that was assumed to be sensory in etiology, have, in fact, normal cochlear function. These patients have severely abnormal neural function as evidenced by poor or absent auditory brainstem responses. Standard remediation strategies for bilateral hearing loss, such as hearing aids, are of little use to these patients. When this disorder strikes young children or infants, it can cause severe disruption of normal language and speech development. The most likely etiology is a neuropathy of the auditory nerve, hence the term "auditory neuropathy." This disorder is rare but more common than previously expected. Investigation of the physiologic mechanisms, the genetic basis, and possible treatments for this disorder is ongoing.

Endolymphatic Sac Tumors (ELSTs) in von Hippel-Lindau (VHL) Disease

Studies are being conducted in the NIDCD intramural division on a group of individuals affected by VHL disease and tumors of the inner ear. These ELSTs have been found to develop in approximately 10% of individuals carrying mutations of the *VHL* gene. Symptoms of hearing loss, balance disturbances, and tinnitus represent the primary clinical manifestations. Recent molecular genetic studies have confirmed the phenotypic association of ELST with VHL disease by demonstrating loss of heterozygosity at the *VHL* locus in tumor cells obtained from surgical specimens. Preliminary results from a clinical trial of hearing preservation surgery in individuals with early stage ELST suggest that these tumors can be safely resected while preserving hearing at preoperative levels and maintaining or improving vestibular function.

Prospective studies of this population of individuals should provide insight into the natural history of hearing and balance disturbances associated with ELST, while basic investigations will focus on the mechanisms by which ELSTs cause auditory and vestibular dysfunction.

Large Vestibular Aqueduct Syndrome (LVAS)

LVAS is characterized by progressive childhood sensorineural hearing loss in association with enlarged vestibular aqueducts. Recent data indicate that at least some cases are associated with mutations in the Pendred syndrome gene (*PDS*). NIDCD intramural scientists are working to identify the genetic basis of LVAS, including several cases where it is clearly not caused by mutations in *PDS*. Whether congenital cytomegalovirus infection is involved in this form of hearing loss is currently being investigated.

Hereditary Cerebellar Ataxia Syndrome of Early Onset

Several abnormal genes that are associated with inherited cerebellar syndromes of imbalance and incoordination have been identified. Relatively little is known about how different mutations lead to specific phenotypes. There is typically great heterogeneity in the clinical signs and symptoms within families with the same mutation and across families with mutations in the same gene. An NIDCD-funded group of investigators at the University of California at Los Angeles has previously demonstrated linkage to chromosome 19p in four families with episodic vertigo and ataxia. This research group has identified a missense mutation in the calcium channel gene on chromosome 19p in a family with severe progressive cerebellar ataxia of early onset involving the trunk, the extremities, and speech.

More recent work has identified a number of related ataxias associated with different mutations in the same calcium channel gene. In addition, mutations in other calcium channel genes have been found to be associated with inherited ataxias. Calcium channelopathies have thus emerged as important model systems to study the role of calcium channels in neuronal function.

Olfactory Function

NIDCD-supported scientists are investigating relationships between decreased olfactory function and a number of rare diseases. NIDCD-supported studies have shown that olfactory loss appears to be among the first signs of such common neurodegenerative diseases such as Alzheimer's disease and idiopathic Parkinson's disease. Recent psychophysical studies have evaluated the prevalence and magnitude of olfactory loss in Parkinson's disease subtypes, Down syndrome, schizophrenia, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and the rare amyotrophic lateral sclerosis/Parkinsonism/dementia complex of Guam. It is hoped that better understanding of the associations between olfactory function and rare diseases will lead to earlier diagnosis and improvement in monitoring of these rare diseases.

Kallmann Syndrome

Kallmann syndrome is a rare genetic disorder with two main symptoms: an absence of the sense of smell and failure of the gonads to mature. There is a 5- to 7-fold preponderance of affected males compared to females, suggesting that the X-linked form of the disease is the most frequent. NIDCD-supported research has led to the identification of a common developmental defect in neuronal migration, which links the two major disease symptoms. A unique family of proteins and their receptors that regulate neuronal migration and direction during development are under investigation by NIDCD-funded

scientists. Additional research is focused on the isolation and cloning of an X-linked gene responsible for Kallmann syndrome.

Papilloma and Carcinoma of the Vocal Tract

Papillomas and carcinomas are the most important neoplasms affecting the human vocal and speech tract. Carcinomas of the upper airway and vocal tract have affected the lives of more than 320,000 Americans and lead to more than 12,000 deaths annually in the United States. A major cause of these recurrent tumors is human papillomaviruses, which infect the whole airway in these patients. NIDCD intramural scientists and an NIDCD-funded team of extramural molecular biologists/virologists and clinicians are addressing the molecular basis for the disease and possible new treatments. A recent finding is that these tumors show an accentuated response to growth factors when compared to other cells. The specific intracellular signaling molecules that mediate this effect have been identified. Research has also shown that these tumors produce factors that stimulate the blood supply and immune cells in ways that help promote tumor growth and spread. Drugs that block the effects of these factors may provide a new approach for prevention and therapy of these cancers.

The multiple recurrences of these respiratory papillomas and the importance of immune function in controlling viral infection has led to studies demonstrating that patients have a normal immune response to most infections, but a suppressed immune response to papillomaviruses. Recently, NIDCD-supported investigators identified the mechanism by which papillomavirus evades the immune system and subsequently developed a strategy to block this immune evasion. In parallel preclinical studies, NIDCD intramural scientists are testing the possibility of interleukin therapy to enhance the cytotoxic T cell response to these papillomas. Additional approaches being tested include photodynamic therapy with Foscan as an adjunct to surgery. These exciting findings, which have stimulated multiple new therapeutic approaches, could ultimately result in nonsurgical treatment of laryngeal papillomas.

Velo-Cardio-Facial Syndrome (VCFS)

VCFS is a disorder that has been associated with more than 30 different features, the most common being cleft palate, heart defects, characteristic facial features, minor learning problems, and speech and feeding problems. VCFS is also known as Shprintzen, DiGeorge, cardiofacial, or conotruncal anomaly unusual face syndrome. These syndromes have a missing chromosomal segment at 22q11. VCFS is inherited in only about 10% to 15% of cases, however. In most instances, neither parent has the syndrome or carries the defective gene, and the cause of the deletion in the affected child is unknown. An NIDCD-supported team of researchers has completed a detailed sequence analysis of the DiGeorge chromosomal region (DGCR) of chromosome 22q11. The 22q11.2 deletion occurs more frequently than originally anticipated, and the endpoints of the deletions occur in clusters. There is considerable variability in the abnormalities associated with deletions of similar size. The presence of a deletion is not always sufficient to cause a palatal defect, strongly suggesting that modifier genes interact with the genes of the deletion region. Due to the heterogeneity of chromosomal deletions, it has been difficult to identify a single gene responsible for any of the observed phenotypes. Recent work now suggests that the Clathrin heavy chain-like gene is a strong candidate gene for VCFS.

Cluttering

Cluttering is a rare developmental speech disorder that is usually familial and may co-occur with stuttering. NIDCD intramural scientists are studying risk factors for the development of these disorders in

families. The purpose is to identify speech-learning mechanisms that may be associated with the emergence of these disorders during the critical period of speech development.

Spasmodic Dysphonia

A focal laryngeal dystonia, spasmodic dysphonia has two forms. The most common form is adductor spasmodic dysphonia, while the less frequent form, abductor spasmodic dysphonia, is often misdiagnosed. NIDCD intramural investigators have identified families with abductor spasmodic dysphonia. All the affected family members are women and none has mutations in the *DYT1* gene, as typically observed for idiopathic torsion dystonia. Familial abductor spasmodic dysphonia may be an independent disorder.

Williams Syndrome (WMS)

WMS is a rare (estimated incidence: 1 in 25,000 live births) genetic disorder in children characterized by a constellation of distinctive facial features; cardiac and dental anomalies; hypercalcemia; mental retardation; and a unique behavioral profile of linguistic abilities selectively preserved in the face of severe general cognitive deficits. NIDCD-supported studies of young children with WMS have documented extreme retardation early on in all developmental milestones, including language. Results suggest that different cognitive domains in WMS (language, spatial cognition, affect) have different starting points and different trajectories, unlike patterns discerned in normal controls, and that some aspects of brain organization (e.g., cerebellar abnormalities) are present from a very early age.

Rare Diseases Research Initiatives

Research Initiatives and Program Activities (FY 2000)

NIH Workshop, September 12, 2000, Supported by the NIH Office of Rare Diseases and NIDCD: "The Olfactory Model System and Rett and Kallmann Syndromes: Sniffing Out Insights into Brain Development."

Several of the less common neurologic developmental disorders, such as Rett and Kallmann syndromes, are associated with severe anomalies of the olfactory sensory system. There has been recent progress in characterizing the impact of these developmental disorders on the olfactory system and in characterizing molecular and genetic defects associated with these syndromes. The conference focused on recent findings in these syndromes and the feasibility of using the olfactory system as an accessible sensorineural model for exploring primary molecular genetic defects at the level of the developing central nervous system.

National Institute of Dental and Craniofacial Research (NIDCR)

Overview of NIDCR Rare Diseases Research Activities

The mission of NIDCR is to improve and promote dental, oral, and craniofacial health through research and research training. NIDCR's programs encompass basic and clinical studies of the broad range of diseases, disorders, and syndromes involving the oral cavity and craniofacial structures; related developmental biology studies; and applied research on biologically compatible and biomimetic materials to re-engineer damaged or dysfunctional tissues. Furthermore, NIDCR supports behavioral and epidemiological studies to better assess the scope of the problem, identify risk factors and biomarkers for disease, understand health disparities, and provide the knowledge base for improved preventive and health care. Research on rare diseases and syndromes such as cancers of the head and neck, orofacial clefting syndromes, early-onset periodontitis, ectodermal dysplasias, craniosynostosis, noma, and disorders of tooth and bone formation such as osteogenesis, amelogenesis, and dentinogenesis imperfecta, compose a significant portion of NIDCR's program activities.

Recent Scientific Advances in Rare Diseases Research

Papillon-Lefevre Syndrome (PLS)

An NIDCR-supported researcher has identified mutations in the gene known as "cathepsin C" as the primary cause of PLS. PLS is a rare and devastating condition that affects the skin and teeth, causing an early-onset periodontitis that is unresponsive to traditional treatment. Affected individuals lose their primary teeth during their preschool years and all their permanent teeth by the time they are young adults. The periodontitis infection results in severe destruction of bone tissue in the jaws that support the teeth. Discovery of the gene for PLS may provide the means for early diagnosis and future therapies to prevent or slow the associated tooth loss.

Gingival Fibromatosis

Gingival fibromatosis is characterized by an enlargement of the gingiva. Gingival fibromatosis may be passed down as an inherited condition, or may occur after exposure to certain pharmacological agents, including calcium channel blockers, phenytoin, or cyclosporin. In one large Brazilian family, the cause of hereditary gingival fibromatosis has been mapped to chromosome 2p21-p22. In an unrelated individual with no history of exposure to drugs, another defect located on chromosome 2p21-p22 was identified. These two studies show that there can be more than one type of genetic defect able to precipitate gingival fibromatosis.

Cleft Lip and Palate Syndrome

NIDCR-sponsored researchers have now identified the gene responsible for one form of cleft lip/palate syndrome, called *CLPEDI*. Victims of this syndrome suffer not only from cleft lip and palate, but also from ectodermal dysplasia, a congenital defect of the ectodermal tissues involving the skin, hair, teeth, nails, and sweat glands; developmental defects of the hands; and in some cases, mental retardation. NIDCR-supported investigators have identified the gene responsible for this syndrome as *PVRL1*, which encodes for a protein important in cell-cell adhesion. Mutations in the gene lead to abnormal proteins that prevent normal development. The identification of this gene and its role advance our understanding

of the processes involved in orofacial development and the mechanisms underlying cleft lip/palate. In turn, this knowledge may be used to develop new approaches for repair and replacement of oral tissues.

Polymorphisms and Risk for Oral Cancers in African Americans

To begin to explain the differences in survival rates between whites and African Americans with oral cancer, investigators have been studying genetic differences in smokers with and without (primary) oral cancer. They identified polymorphisms (variations) in genes that code for enzymes involved in the metabolic detoxification of certain tobacco carcinogens termed GSTs. A strong association was found between the absence of one form of GST in African Americans with oral cancer and heavy tobacco use; however, no significant associations were observed between these particular variations and oral cancer risk in whites. Thus, the results indicate that this particular gene variation plays an important role in risk for oral cancer among African Americans and implicate GSTs as important tobacco carcinogen detoxifying enzymes in this population. Consequently, these genetic variations may be used in the future to screen and identify those African Americans at highest risk of oral cancers.

Ectodermal Dysplasia

Hypohidrotic ectodermal dysplasia results in abnormal development of the teeth, hair, and sweat glands. Affected children have limited ability to maintain safe body temperature levels and have sparse hair, misshapen or missing teeth, and dry skin. Scientists recently identified the gene responsible for one form of hypohidrotic ectodermal dysplasia. The discovery of the gene was accelerated by the identification of a mutation in a mouse gene called "downless," which resulted in animals with sparse hair and other features that mimic the human disorder.

Detection of Genetic Mutations in Head and Neck Cancers in Saliva

Mitochondrial DNA is present in extremely high levels in almost every cell and may be of great value for detection of molecular markers for cancers. For example, mitochondrial DNA mutations were identified in 46% of head and neck cancer patients, and mutations identical to those observed in tumor tissue were detected in 67% of saliva samples from these patients. Current studies are evaluating the potential of saliva for its use as a diagnostic fluid for the noninvasive detection of head and neck cancers.

Saliva as a Diagnostic or Prognostic Fluid for Breast Cancers

C-erbB-2 has served as a prognostic (predictor of disease progression) breast cancer marker, and has now been detected in saliva from three groups of individuals (healthy women, women with benign breast lesions, and women diagnosed with breast cancer). The salivary levels of *c-erbB-2* in the cancer patients, however, were significantly higher than the salivary levels in the healthy controls and the individuals with benign lesions. Salivary *c-erbB-2* may therefore have potential use as a diagnostic or prognostic marker (in combination with mammography and physical examination) of breast cancer.

Tooth Agenesis

Nearly 20% of the U.S. population has congenitally missing teeth. The missing teeth are often third molars, but may be any of the other teeth found in the human dentition. The forms of missing teeth, ranging from least to most severe, are called hypodontia, oligodontia, and anodontia, respectively. Researchers have identified multiple gene networks that control the formation of teeth. A recently

published study showed that a mutation in the *PAX9* gene resulted in congenitally missing molar teeth in three generations of a particular family. Interestingly, *PAX9* is a member of a family of genes involved in formation of the eyes, teeth, palate, and thyroid gland. Discoveries such as this may form the basis for the development of gene-based diagnostics for dental anomalies, and also could provide a biological basis for the future design and fabrication of tooth replacements.

Rare Diseases Research Initiatives

Global Network for Women's and Children's Health Research

Jointly sponsored by NIDCR and six other NIH Institutes, an RFA published in March 2000 invited applications to establish a flexible research network focused on critical global health problems of women and children. International multidisciplinary teams of investigators will work collaboratively on research questions aimed at improving health and preventing premature disease and death among women and children in developing countries. In FY 2001, NIDCR plans to co-support a project focused on evaluating treatment interventions to reduce the prevalence of oral clefts in South America.

State Models for Oral Cancer Prevention and Early Detection

In the United States, 30,000 new cases of oral and pharyngeal cancers are diagnosed each year, and 8,000 deaths occur annually from these cancers. While oral and pharyngeal cancers represent 3% of all cancers in the United States, the overall 5-year survival rate for victims of the disease is 53%, one of the lowest of any of the major cancers. The survival rate for African American men is much lower than the rate for whites.

Awards are planned for FY 2001 in response to a joint NIDCR/NCI initiative titled, "State Models for Oral Cancer Prevention and Early Detection." Researchers supported through this initiative will develop statewide assessments of:

- The level of oral cancer within the state.
- The level of knowledge of oral cancer risk factors among health professions (e.g., dentistry, medicine, pharmacy, nursing, dental hygiene, physician assistants, and health educators) and the public.
- Practices in diagnosing oral cancers in health professions.
- Whether the public receives annual oral cancer examinations from health care providers.

A statewide assessment, as opposed to any other approach, is key because each state has unique demographics, oral cancer epidemiology, and practice acts governing health professions. Findings from these assessments will help in the development of ethnically and culturally sensitive health promotion activities to reduce morbidity and mortality from oral and pharyngeal cancers.

Mouse Mutagenesis and Phenotyping: Developmental Defects

NIDCR is a participant in this multi-Institute-sponsored initiative that established a facility in FY 2000 to produce various laboratory mouse models for the study of developmental defects. The mouse models produced in this facility are expected to help NIDCR-supported researchers discover the many cellular, molecular, and genetic mechanisms that direct embryonic and post-embryonic growth and function and

thus advance our understanding of the mechanisms of rare diseases involving the oral cavity and craniofacial structures.

Construction of an Artificial Salivary Gland

Saliva is a remarkable, multipurpose fluid often taken for granted. More than one million people suffer a loss of salivary gland function as a result of Sjögren's syndrome, an autoimmune disease, or from radiation treatment for head or neck cancer. The resulting loss of saliva flow markedly impairs quality of life. Without adequate saliva, patients may experience difficulty speaking, chewing, and swallowing. These patients may also experience rampant tooth decay, infections, loss of taste, and considerable discomfort. No effective treatments currently exist to help these patients. NIDCR scientists are focused on creating a small tube that can be placed into the cheek a patient whose salivary gland cells have been destroyed. The tube would be lined with cells engineered to secrete a saliva-like substance. Scientists believe that this artificial salivary gland will be ready for clinical testing within 5 to 7 years.

Temporomandibular Disorder (TMD)

The multiplicity of factors that may cause or contribute to TMD has unfortunately led to an even greater number of treatments that may not have been validated. NIDCR is conducting clinical trials examining the effects of conservative versus surgical interventions, with preliminary findings indicating that surgical interventions offer no increased benefits. To address the needs of patients who require joint replacement, NIDCR is conducting basic research on engineering more biocompatible implants. Given the complexity of TMD and the need to approach this condition in a multidisciplinary manner, NIDCR has established the TMD Interagency Working Group to facilitate progress in dealing with these disorders through cooperation, communication, and collaboration among the many Federal agencies that conduct or support TMD-related research.

World Health Organization (WHO) Conference on International Collaborative Research on Craniofacial Anomalies

NIDCR supported a November 2000 international consensus conference on craniofacial anomalies organized by WHO. The meeting brought together clinicians, epidemiologists, statisticians, and molecular and developmental biologists from around the world. Sessions focused on clinical treatment, genetics, gene/environment interactions, epidemiology, and bioethical issues in medical genetics; and addressed guidelines for standardizing criteria, protocols, and methodologies that are needed to facilitate future global collaborative research efforts in the prevention and treatment of craniofacial anomalies. A future WHO Conference on the Prevention of Craniofacial Anomalies was planned for May 2001. The conference attendees focused on recent evidence that specific maternal vitamin deficiencies are associated with an increased risk of cleft lip and palate and worked towards developing recommendations for the design of intervention trials to determine whether maternal vitamin supplementation will reduce the birth prevalence of clefts.

Workshop on Strategies for Tooth Structure Regeneration

NIDCR and ORD co-sponsored a workshop on May 12, 2000 on "Strategies for Tooth Structure Regeneration." A variety of rare disorders that affect children are characterized by tooth abnormalities such as missing dentition, early tooth loss, and deformities in tooth structure. These disorders include the ectodermal dysplasias, van der Woude syndrome, Williams syndrome (WMS), osteogenesis imperfecta

(OI) type I, osteopetrosis, and many others. The specific purpose of the workshop was to provide a critical assessment of the state of knowledge in molecular genetics and bioengineering approaches that are essential for developing therapeutic strategies for tooth regeneration. The workshop provided a forum for biomedical researchers, clinicians, and engineers to discuss their vision and develop direction for overcoming obstacles facing the development of therapeutic strategies.

Workgroup on Genetics and Craniofacial and Dental Anomalies

In November 1999, NIDCR held a workshop to assess the current status of dental, oral, and craniofacial genetics research. A committee of 60 scientists met to identify opportunities and obstacles for genetics research in the NIDCR research portfolio. The workgroup identified 47 heritable disease categories involving craniofacial, oral, and dental manifestations, the majority of which are rare disorders affecting children. The workgroup developed a set of prioritized recommendations for genetic and genomic research activities and resources to be developed during the next 5 years that will accelerate discoveries of causal gene products and facilitate prenatal diagnoses of these disorders.

Office of International Health Activities on Noma

NIDCR is a Collaborating Center of the International Action Network Against Noma that was launched by WHO in 1994. Noma is a devastating infection of severely protein-malnourished children in which extensive amounts of facial tissues are destroyed. The fatality rate exceeds 80%. The etiology and pathogenesis of noma remain unknown. In June 1999, NIDCR sponsored a seminar on international collaborative research on noma to enhance awareness of the disease and discuss research directions. NIDCR is funding research on noma involving the use of new techniques to identify bacteria in the lesions that currently cannot be cultured. Through a supplement to a Fogarty International Training and Research in Emerging Infections grant, NIDCR is supporting clinical studies to characterize the earliest lesions in noma patients. The results will help identify ways to manage the infection while addressing the nutritional deprivation.

Activities with Voluntary Rare Diseases Organizations to Stimulate Research

Skeletal Disorders and Diseases

NIDCR is a participant in an NIH consortium that provides support for the NIH Osteoporosis and Related Bone Diseases National Resource Center. The Center is mandated to increase awareness and knowledge about osteoporosis and related bone diseases and is a partnership between NIH and the leading national nonprofit organizations in the field (the National Osteoporosis Foundation, the Paget Foundation, and the Osteogenesis Imperfecta Foundation). Adolescent females represent a major at-risk population for future osteoporosis, and they are a key target of several of the Center's programs. Tasks of the Center include expanding the acquisition of research information, promoting the Center to physician and public audiences through exhibits and public service announcements, and disseminating information via electronic methods and print publications to medical and managed care organizations and at-risk populations. The Center also develops partnerships to evaluate model education programs to enhance bone health and reduce future risk of osteoporosis among at-risk populations.

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Overview of NIDDK Rare Diseases Research Activities

NIDDK supports research on many rare diseases. Although diseases such as type 1 diabetes and type 2 diabetes are not rare, there are rare single-gene defects that cause diabetes such as maturity-onset diabetes of the young (MODY) and lipodystrophy. Many of the genes causing these disorders were recently identified. NIDDK also supports research on kidney and liver diseases. Each year 20,000 babies are born with kidney problems, 2,000; of these children will die, and 1,000 will begin treatment for renal failure. The most common childhood renal problems are genetic renal diseases, kidney and urinary tract malformations, focal segmental glomerulosclerosis (FSGS), and primary glomerulonephritis.

NIDDK also supports research on genetic metabolic and hematologic diseases such as cystic fibrosis (CF), lysosomal storage diseases (LSDs), congenital disorders of glycosylation, Cooley's anemia, and sickle cell disease (SCD).

Recent Scientific Advances in Rare Diseases Research

Lipodystrophies

Lipodystrophies are a group of conditions caused by abnormal lipid metabolism resulting in the reduction or absence of fatty (adipose) tissue. The lipodystrophies may be acquired or inherited, and both the anatomic location and degree of fat loss vary among the different disorders. These conditions are often accompanied by insulin resistance and/or diabetes, elevated levels of blood lipids, and vascular disease. There is a congenital form of the disorder in which the patients have no subcutaneous fat. This year the gene for this form of lipodystrophy was localized to 9q. At puberty, patients with familial partial lipodystrophy (FPLD), Dunnigan variety, lose subcutaneous fat from the extremities, trunk, and gluteal regions of the body, but excess fat becomes deposited in the face, neck, and back.

Last year, investigators found that mutations in the lamin *A/C* gene, which was known to cause a form of muscular dystrophy, also causes FPLD. The lamin *A/C* protein is a component of the cell's nuclear envelope. NIDDK-supported researchers detected four independent mutations in this gene in members of 14 families. All of the alterations resulting in FPLD occur within a particular region of the lamin *A/C* protein. Mice with a targeted deletion of this gene not only develop a form of muscular dystrophy, but also lack distinguishable white fat, which serves as a valuable source of energy. Collectively, these data imply that mutations within this region of the lamin *A/C* protein are involved in one or more activities required by fat cells in specific tissue beds. It has been hypothesized that in FPLD, loss of fat cells affects insulin sensitivity through reduced levels of "adipocyte-derived circulating factors," such as leptin, a hormone produced by fat cells that regulates food intake and energy metabolism. A clinical trial has been initiated to determine if the symptoms of lipoatrophy improve with leptin treatment.

Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI)

NIDDK supports several studies aimed at understanding the etiology and mechanism of PHHI. Mutations in several different genes have been shown to cause this disorder. Children with PHHI can have a defect in *SURI*, the high-affinity sulfonylurea receptor, or the potassium pore (*KIR6.2*), which together form the potassium channel in the pancreatic beta cell. A mutation in glucokinase, the primary "glucose sensor" of the pancreatic beta cell that lowers its affinity for glucose, can also result in PHHI, as can mutations in

glutamate dehydrogenase. Another mechanism described for congenital hyperinsulinism is focal adenomatous lesions of the pancreas, which involves loss of heterozygosity with retention of a *SURI* or *KIR6.2* mutation. It was recently shown that patients with defects in the potassium channel can be identified by loss of an insulin response to tolbutamide. This test could identify patients that have PHHI due to mutations in *SURI* or *KIR6.2*. Many of these patients respond to surgical interventions.

Polycystic Kidney Disease (PKD)

PKD is a genetic disorder characterized by the progressive development and subsequent growth of numerous cysts in the kidneys. It can be inherited as autosomal dominant (ADPKD) or as autosomal recessive (ARPKD) types. In humans, ADPKD has a later onset and slower development than ARPKD, which usually affects newborns and young children. Most affected children who survive the neonatal period die from renal insufficiency associated with ARPKD. NIDDK supports a comprehensive research portfolio on PKD, which includes investigator-initiated research programs, more complex Program Project Grants, Specialized Centers of Research (SCORs) in PKD, and a new research program (CRISP Study) that includes a consortium established with the purpose of developing and testing new or improved radio-imaging techniques to ascertain renal disease progression in patients affected with PKD.

IgA Nephropathy

IgA nephropathy is one of the most common primary forms of glomerulonephritis in children worldwide. IgA nephropathy was initially considered a benign condition, but in the light of more recent studies and extended follow-up of patients, the overall prognosis remains unclear. Most adult patients continue to have hematuria, and 20% to 30% have been found to progress to end-stage renal failure one or two decades after the initial diagnosis. Four research projects currently receive support, the focus being on determinants of the autoimmune process and studies of mechanisms of fibrosis leading to progression to end-stage renal disease (ESRD). An ongoing clinical trial comparing different treatment approaches to halt progression in children and young adults affected with IgA nephropathy should reach completion within the next 12 to 18 months.

Focal Segmental Glomerulosclerosis (FSGS)

FSGS is a common irreversible glomerular process with steroid-resistant nephritic syndrome. Complications including frequently relapsing nephrotic syndrome, generalized edema, cardiovascular problems, thromboembolisms, and progression to ESRD are commonly found and make disease management difficult, especially in pediatric patients. The risk of disease progression is even greater in African American children. FSGS is one of the most common recurrent renal diseases in children, resulting in new injury to the transplanted kidneys in 20% to 30% of cases, and in graft loss in 40% to 50% of transplant cases. Three projects supported under the Pediatric Nephrology program are studying the molecular genetic mechanisms of nephritic syndrome in FSGS.

Cystic Fibrosis (CF)

CF is the most common fatal genetic disease in Caucasians, affecting approximately 1 in 2,500 newborns. Patients are diagnosed in early childhood due to symptoms of the disease such as failure to thrive. With management of the nutritional problems and infections, the life expectancy for CF patients has been increased to 30 years. Since the cloning of the *CF* gene and identification of its protein product, CFTR, as a cAMP-regulated chloride channel, there has been impressive progress in the molecular understanding

of this disorder. NIDDK supports a research portfolio directed at further defining the molecular mechanisms underlying CF and translating information about the molecular basis of the disease into new treatments. This year NIDDK-supported researchers defined new interactions between CFTR and other cellular proteins. CFTR has been shown to interact with a membrane-bound protein, syntaxin 1A, which binds to the amino-terminal of CFTR and regulates the ability of the tail of CFTR to interact with the regulatory domain to control channel activity. CFTR has also been shown to interact with other membrane proteins such as ezrin and EBP50 through its PDZ domain. These adaptor molecules may control the phosphorylation of CFTR. In addition, CAP70 may enhance dimerization of CFTR by bridging two CFTR molecules. These molecular interactions may also explain how CFTR regulates the activity of the epithelial Na⁺ channel (ENaC). In sweat glands, ENaC cannot be activated when CFTR is defective. This new understanding of the interactions of CFTR with other cellular proteins should help to explain the complex pathophysiology of cystic fibrosis.

Niemann-Pick Type C (NP-C) Disease

NP-C, a lysosomal storage disease, is an autosomal recessive lipid storage disorder characterized by progressive deterioration of the central nervous system resulting in death in early childhood. Biochemical characterization of cells from NP-C patients reveals that they accumulate large amounts of unesterified cholesterol resulting in downstream effects on cholesterol homeostasis. The frequency of the disease is estimated at about 1 in 100,000 live births. Defects in the gene for *NP-C1* account for about 95% of the cases of NP-C, while defects in the gene for *NP-C2* account for the remaining cases. NIDDK-funded researchers have made two important findings in the past year. First, they have established that *NP-C1* can function to transport fatty acids, but not cholesterol, out of endosomes and lysosomes. Second, they found that the protein defective in *NP-C2* is a widely expressed lysosomal protein that binds cholesterol. These results provide new avenues for investigating the functions of *NP-C1* and *NP-C2* in cholesterol transport and homeostasis, which are greatly impaired in children with NP-C.

Congenital Disorders of Glycosylation (CDGs)

Defects in the modification of proteins by carbohydrates cause a syndrome of mental and psychomotor retardation, dysmorphism, and blood coagulation defects. Defects in the numerous enzymes that are in the pathway for N-glycosylation have been shown to cause this group of disorders. Last year, a new form of this disorder was described. These patients have a defect in *DPM1*, the gene coding for the catalytic subunit of dolichol-phosphate-mannose synthetase. Supplementation with mannose has been shown to be successful in treating some CDG patients with defects in phosphomannose isomerase. Patients with the newly identified *DPM1* mutations are being supplemented with mannose to see if this therapy will be effective in these patients.

Cooley's Anemia

Patients with Cooley's anemia (also known as beta-thalassemia) continue to suffer from the sequelae of transfusion-induced iron overload due to the inadequacies of current iron-chelation therapy. Most of the patients are children and young adults. Compliance with the use of subcutaneous desferrioxamine (DFO) continues to be a major problem despite convincing evidence that it markedly reduces morbidity and prolongs life. The full potential of iron-chelation therapy will not be realized until a more effective and more easily administered drug is available. NIDDK is supporting two new clinical studies: one examining tissue damage potentially arising from free iron appearing in the blood immediately after chelator treatment, and a second assessing oral and subcutaneous iron chelation in combination, which is

proving to be a more effective therapy than use of individual chelating drugs alone. Based on recommendations from a 1998 NIDDK workshop on "Iron: From Current Biochemistry to New Chelator Development Strategies," a Request for Grant Applications was issued to improve the control of iron transport and metabolism, develop a better understanding of the biological consequences of iron overload, and improve therapy. As a result, several new projects were funded that will increase our understanding of how chelating drugs act, and how to use them more effectively. NIDDK currently is conducting preclinical testing of a new iron-chelating drug that will go into clinical studies late in 2001, sponsored by a pharmaceutical company.

Alpha-1-Antitrypsin (AAT) Deficiency

AAT deficiency causes an inherited form of lung and liver disease. In this disease, an abnormal alpha-1-antitrypsin molecule (alpha-1-ATZ) is produced in patients, and as a result of its accumulation in hepatocytes, creates liver damage leading to cirrhosis and in some cases to cancer. This year chemical chaperones were shown to increase the amount of alpha-1-ATZ that can be correctly folded and exported into the circulation. In particular, 4-phenylbutyrate was able to increase alpha-1-antitrypsin levels in transgenic mice with the Z mutation to between 20% to 50% of normal. Because phenylbutyrate has been used in humans to treat other disorders, these studies may lead to a pharmacological intervention for this disorder.

Biliary Atresia (BA)

NIDDK supports a clinical study designed to determine the basis of poor growth for children with BA, a neonatal liver disorder, and to ascertain if these patients are likely to benefit from the anabolic and growth-promoting effects of supplemental growth hormone and/or supplemental nutrition. Although surgical approaches have attempted to correct the anatomic problem, these children typically fail to grow adequately. Ultimately, 70% of affected children require liver transplantation, with the most common indication for transplantation being poor growth. BA patients have a disturbance of the growth hormone-insulin-like growth factor (GH-IGF) axis. They have increased insulin, increased growth hormone, increased IGFBP-1, depressed IGF-I, and depressed IGFBP-3. This pattern of disturbance can be seen with either malnutrition or growth hormone (GH) resistance. An interventional study will determine if treatment of children with biliary atresia with either recombinant human growth hormone (rhGH) or supplemental nutrition early in the course of the liver disease will correct the alterations of the GH-IGF axis and improve outcome measures.

Rare Diseases Research Initiatives

Last year NIDDK and NHLBI issued RFA DK-99-009, "Biology of Iron Overload, and New Approaches to Therapy." Twelve grants were awarded from this RFA in FY 2000. These grants studied iron transport and chelation in rare diseases such as hemochromatosis and beta-thalassemia.

NIDDK issued RFA DK-00-013 for a Cystic Fibrosis Core Center. Applications were received in FY 2000, and one Center will be funded in 2001.

NIDDK has joined the National Heart, Lung, and Blood Institute (NHLBI) in an initiative titled, "Genetic Modifiers of Single Gene Defect Diseases," issued August 25, 2000. This initiative will fund studies to identify and characterize the modifier genes responsible for variation in clinical progression and outcome of heart, lung, and blood disease due to single-gene defects. It is anticipated that 18 applications will be funded in FY 2001.

This year NIDDK issued two Program Announcements to study rare diseases. PA-00-055, "Hemochromatosis and Diabetes Mellitus," was issued on February 9, 2000. This announcement solicits grant applications studying the molecular mechanisms underlying the pathogenesis of diabetes mellitus in hemochromatosis and other forms of iron overload. PA-00-091, "Research Studies on the Hereditary Calcium Oxalate Stone Diseases," was issued on April 27, 2000. This announcement solicits grant applications to study primary hyperoxaluria and other genetic causes of oxalate stone disease. Current active PAs on rare diseases include PA-99-040, "Molecular and Genetic Mechanisms in Pancreatitis"; PA-94-036, "Characterization and Treatment of Genetic Metabolic Diseases"; and PA-98-002, "Polycystic Kidney Disease."

Rare Diseases-Related Program Activities

Conferences on Rare Diseases

NIDDK and ORD supported the "First Annual Shwachmann-Diamond Syndrome International Meeting" held April 4, 2000 in Boston, Massachusetts. This meeting provided a forum for clinical and basic researchers to present their most recent data on Schwachmann-Diamond syndrome. The meeting brought together gastroenterologists, hematologists, geneticists, and patients for their perspectives on this disease.

NIDDK, NHLBI, and ORD organized a workshop titled, "Design Issues for Clinical Trials in Acute Renal Failure," held September 10-12, 2000. The workshop focused on designing clinical trials to prevent acute renal failure, to treat the condition using drugs and dialysis, and to prevent acute renal failure after transplantation. NIDDK will use the advice from this meeting to plan a multicenter Acute Renal Failure Trial Network.

NIDDK and ORD supported a workshop titled, "Congenital Disorders of Glycosylation," held on November 8, 2000. This satellite meeting was held in conjunction with the Society for Glycobiology to raise awareness for this group of disorders. It brought together researchers studying this group of newly discovered genetic diseases to present their findings.

NIDDK and the Oxalosis and Hyperoxaluria Foundation organized a workshop on "Oxalosis and Calcium Oxalate Stone Disease," held November 16-17, 2000. This workshop brought together experts in the field, introduced new investigators to the field, and discussed the development of a network of investigators to facilitate sharing of data.

National Institute on Drug Abuse (NIDA)

Overview of NIDA Rare Diseases Research Activities

With respect to the prevention of drug abuse and treatment of drug abusers, NIDA provides national leadership, conducting and supporting biomedical and behavioral research, health services research, research training, and health information dissemination. NIDA plans, conducts, fosters, and supports a comprehensive program of research and research training relating to the causes, prevention, treatment, patterns, and consequences of drug abuse and addiction, through research performed in its own laboratories and through contracts and grants to scientific institutions and individuals. NIDA supports training in fundamental sciences and clinical disciplines relating to drug abuse by making individual and institutional research training awards, and coordinates with other research institutes and Federal agencies on activities relevant to drug abuse and addiction. NIDA also conducts and fosters health information dissemination activities, including the collection and dissemination of research findings and related educational materials for health professionals, educators, and the lay public. In addition, NIDA coordinates with institutions and professional associations and with international, national, state, and voluntary agencies working in these areas, including collaboration with the Substance Abuse and Mental Health Services Administration (SAMHSA) on services research issues and other programmatic issues.

History of NIDA Rare Diseases Research

Four drug abuse treatment medications have received orphan product designation: levomethadyl acetate hydrochloride, naltrexone, buprenorphine, and naloxone. Levomethadyl acetate hydrochloride (trade name ORLAAM), an alternative to methadone used for opiate maintenance therapy, received New Drug Application (NDA) approval in 1993. Naltrexone, an opiate antagonist for use in detoxified patients, was approved in 1985 and no longer enjoys orphan exclusivity. The opiate partial agonist buprenorphine and a combination of buprenorphine plus naloxone have also received orphan designation but do not currently have approved NDAs.

Incidence and prevalence figures for dependence on controlled substances (other than alcohol or nicotine) are always difficult to estimate, as they vary from type of drug, community, and supply availability (generally a function of supply interdiction/law enforcement). Unlike other disease conditions, illicit marketers have a reason (profit) to introduce and infect the population with abusable and/or dependence-producing substances. Illicit drugs utilized in some communities are not always available or in vogue in other communities. Thus, there may be various drug dependence indications that, in and of themselves, may affect fewer than 200,000 persons in the United States. It is very clear, however, that abuse of opiates (heroin and other narcotics) and stimulants (such as cocaine and “crack cocaine”) are endemic in the United States. Even the lowest estimates put dependence levels of these substances at figures well above the 200,000 threshold generally used for defining orphan products. The total disease burden of drug abuse in the United States has been estimated to exceed \$110 billion per year.

Injection drug use and sexual contact among users is a highly correlated vector in the spread of HIV, hepatitis, and tuberculosis, creating a public health problem of enormous magnitude while at the same time receiving treatment as an orphan disease by the pharmaceutical industry.

Despite the enormous public health burden of this disease state, there exists little or no incentive for pharmaceutical companies to pursue research and development of new treatment medications for drug abusers. Although the total numbers of persons afflicted may seem sufficient in the aggregate, unlike other disease states, many drug abusers are not seeking treatment at the same time. Therefore, the actual population who may be a potential market for medications is only a fraction of the total number who could benefit. Additionally, many of these persons will be treated in publicly funded clinics where reimbursement is perceived by companies as modest or inadequate and subject to artificial control. Some treatment agents may themselves be abusable and will be strictly controlled. An example of these agents is methadone, classified as a Schedule II controlled substance for use in opiate maintenance therapy. Some 900 U.S. clinics are licensed to dispense methadone and serve approximately 190,000 persons per year with a pharmaceutical market value of approximately \$30 million per year. This is simply not an attractive market to most manufacturers based on projected return on investment when compared to nearly any other indication they could pursue. Each of these points is well-documented in the recent Institute of Medicine *Report on the Development of Medications for the Treatment of Opiate and Cocaine Addiction*, 1995, and are well-known to the pharmaceutical and market research industries.

Pharmacological treatment of drug-dependent populations is not the dominant treatment modality in the United States. Most therapeutic regimens are non-pharmacologically based. Because no medications have as yet demonstrated efficacy for the treatment of cocaine dependence, NIDA's orphan product experience to date has focused on medications to treat opiate dependence.

While opiate and cocaine dependence do not fit the "numerical" definition of orphan products, de facto they certainly do. As an instructive example, consider the development and approval of the methadone alternative levomethadyl acetate hydrochloride (trade name ORLAAM). Despite the facts that human data on 6,000 subjects from government-sponsored studies were available for levomethadyl acetate hydrochloride, the compound was off-patent, and that the government had a large supply of the compound available for anyone interested in obtaining an NDA, no private-sector entity attempted to finish the development of this compound until NIDA paid a contractor to do so. Similarly, the development of naltrexone was largely a NIDA-funded effort. Therefore, these products should be viewed as entirely "orphan-like" insofar as their ability to attract private-sector sponsors; until recently, this was also persuasive to FDA.

In the case of pharmacological treatment for opiate dependence, the population in treatment cannot at present exceed 190,000 per year. Given these facts, historically orphan designation was permitted for two products substantially developed by NIDA (naltrexone, 1985, and ORLAAM, 1993) as treatments for opiate dependence. ORLAAM received orphan designation on the basis that it could be used to transfer patients from methadone (and there were fewer than 200,000 receiving methadone), and naltrexone received designation on the basis that there were fewer than 200,000 detoxified addicts at any given time.

In the more recent designation of buprenorphine (1994), FDA appeared to be taking a more restrictive view via application of their more recently promulgated regulations. Although the issue was not definitively answered in the case of buprenorphine, FDA expressed the view that orphan designation where the population in question exceeded 200,000 would be difficult unless there was a medical/biological reason why 200,000 persons could not utilize the product. In other words, treatment capacity or the number of persons seeking treatment might not suffice for orphan designation.

In the case of buprenorphine, this did not prove to be an insurmountable burden because that product's sponsor could prove to FDA that based on historic, current, and projected expenditures, it would not recoup its investment during 7 years of exclusive marketing in the United States. Thus, a unique situation exists where the preclinical efforts are completed, NIDA is supporting clinical trials, and the sponsor has the expertise to manufacture new formulations. Buprenorphine became the first product to receive an orphan designation based on an economic, rather than a population-based, rationale. The sponsor selected this route because there was less certainty that FDA would continue to allow orphan designation based on the capacity of the treatment system as opposed to the actual incidence and prevalence of opiate dependence.

Recent Scientific Advances in Rare Diseases Research

The discovery of opiate receptors by NIDA-funded scientists in the 1970s opened a new era of neurobiological research that continues today. Scientists continue to map brain receptor system types and subtypes, continuously gaining understanding of their structure and function. This information will allow the design of interventions (behavioral, chemical, and genetic) that may be useful in the treatment of a huge number of human disorders, all of which are mediated in the brain.

A generation of research has shown that drug addiction is a complex biomedical and behavioral disease that has its roots in those parts of the brain that underlie, mediate, and allow us to have the emotions that make us human. Just as we have learned that depression is a brain disease that can be treated with medicine, so too have we learned that drug addiction is a brain disease that can and should be treated with medicine.

There is a critical distinction between drug abuse and drug addiction. Drug abuse is a voluntary behavior; the casual user makes a free and conscious decision to break the law and use an illicit, mind-altering substance. Drug addiction is a disease of the brain, resulting from repeated and prolonged self-administration of such a substance. Addiction is brought on by drug-taking behavior in much the same sense that lung cancer is brought on by cigarette smoking and heart disease is brought on by excessive fat intake. Once the disease is established, however—be it in the brain, the lung, or the heart—the physiological dysfunction must be corrected to restore health.

The role of a medication is to re-establish normality to brain function and behavior so that the addicted patient has the opportunity for rehabilitation through counseling, psychotherapy, vocational training, and other therapeutic services. While the mechanisms of many central nervous system disorders have not been elucidated, scientists working in the field of drug abuse have now identified and cloned the putative site of action in the brain for every major drug of abuse. Thus, the potential to develop new treatments is enormous. For example, having cloned the dopamine transporter mechanism where cocaine exerts its action, NIDA scientists are now designing molecules that will block cocaine's effects at this site without disrupting the essential neurotransmitter functions of dopamine.

NIDA and other scientists have developed pharmacological agents for the treatment of opiate dependence in various functional categories. For example, methadone and ORLAAM are mu agonist medications currently approved for opiate treatment. Naltrexone is an opiate antagonist approved for treatment, and naloxone is approved for use in the treatment of opiate overdose. NIDA is currently working on a partial mu agonist (buprenorphine) that will further contribute to the arsenal of agents available for treatment.

Rare Diseases Research Initiatives

As described in the history section above, NIDA considers medications for the treatment of dependence on controlled substances to be de facto orphans. The development of medications for the treatment of these conditions may well be considered rare disease research within the context of an urgent public health need with a wholly inadequate private-sector response. Therefore, NIDA's medications development program effort may (until facts prove otherwise) be considered as part of a rare disease research initiative.

In 1990 the NIDA Medications Development Division (MDD) was established. In 1999, MDD became part of the Division of Treatment Research and Development (DTR&D). The functions of DTR&D are as follows:

- Conduct studies necessary to identify, develop, and obtain FDA marketing approval for new medications to treat drug addiction and other brain and behavior disorders.
- Develop and administer a national program of basic and clinical pharmacological research designed to develop innovative biological and pharmacological treatment approaches.
- Support training in fundamental sciences and clinical disciplines related to the pharmacotherapeutic treatment of drug abuse.
- Collaborate with the pharmaceutical and chemical industry in the United States and other nations and the federal medications development programs of other institutes and entities.
- Work closely with FDA in ensuring that research designed to show the clinical efficacy of new compounds is evaluated and approved in the most expeditious manner possible.

DTR&D operates within the larger context of a NIDA-wide Medications Development Program that incorporates basic research discoveries from other divisions (intramural and extramural) in the quest to develop new pharmacological treatments. Application of research results from the intramural and extramural community allows the Division to have access to the latest theoretical bases and an opportunity to test new hypotheses in controlled clinical settings.

Recognizing that physicians will soon have a choice of five different FDA-approved products for treating opiate addiction (methadone, ORLAAM, buprenorphine, buprenorphine/naloxone, and naltrexone) and no FDA-approved products for treating addictions to stimulants (e.g., cocaine or methamphetamine), NIDA's efforts are currently shifting toward a greater emphasis on discovery and development of medications for treating stimulant dependence. While initial clinical trials in this area have focused on medications that are already marketed for other indications, substantial efforts are also being devoted to the discovery and development of novel compounds that may specifically address the problem of stimulant dependence.

It is worth noting that within the pharmaceutical industry approximately, \$75 million are devoted to biological screening and pharmacological testing for each successful new medication that reaches the market. NIDA is willing to make such an investment in medications discovery and is working to establish collaborations with pharmaceutical companies that will allow NIDA to screen chemical libraries at biochemical targets (e.g., dopamine D-1 receptors) implicated as potential sites of action for effective medications. Efforts are also directed toward supporting synthesis of novel compounds for screening and pharmacological testing through grants and contracts. Significant areas of research and development are summarized below.

Opiate Addiction Treatment

Buprenorphine/Buprenorphine-Naloxone Combination

A 16-week, 735-patient, 12-center trial of buprenorphine, an opiate partial agonist medication for the treatment of heroin dependence, has been completed. Data from this study and other previous and ongoing studies have been analyzed. These data demonstrate the effectiveness of buprenorphine in reducing illicit opiate abuse and in retaining patients in treatment (Ling et al., *Addiction*, 1998).

Under development is a combination dosage of buprenorphine plus naloxone, which would be useful as a potential “non-narcotic” or “take-home” treatment medication. Testing is currently under way to determine the abuse potential of buprenorphine plus naloxone. A tablet form of buprenorphine and of buprenorphine plus naloxone is also under development, and commercial sponsorship for these products has been negotiated. A 12-site, 52-week trial of the tablet products was completed in September 1998. An NDA for buprenorphine was submitted in 1997. The NDA for buprenorphine combined with naloxone was submitted and reviewed in 1999.

FDA has deemed both submissions “approvable,” and it is hoped that actual approval may occur in the fall of 2001.

Given that buprenorphine combined with naloxone received an “approvable” status by FDA in calendar year 1999, plans are under way to enhance the introduction of this new front-line product to the American treatment system. The hope is that pharmacological treatment with buprenorphine products will enable it to be used in a wider variety of settings than is currently the case for methadone and ORLAAM.

To test the viability of this approach, NIDA and the Department of Veterans Affairs (VA) began a study in 1999 designed to assess the use of a buprenorphine/naloxone sublingual tablet formulation in non-clinic-based settings for the treatment of opiate addiction. The study will also evaluate the administration of buprenorphine to adolescents (ages 15-21 years). A total of 583 patients have been enrolled.

Data from this study will be used to expand labeling and will be considered in developing guidelines for the administration of the buprenorphine/naloxone sublingual tablet in office-based settings.

As an immediate goal, this protocol will be implemented in multiple settings (e.g., university-based or affiliated clinics, community mental health clinics, or private physicians’ offices), with buprenorphine prescribed and supplemented with relapse prevention therapy delivered by an appropriately trained medical assistant. A collaborative arrangement of this protocol allows physicians practicing in one of the above settings to participate in this study provided they agree to adhere to the requirements of the protocol. The study will be specifically aimed at answering the following critical treatment expansion issues:

- To determine and document the safety of extending buprenorphine treatment to a younger population (ages 15-21).
- To document physicians’ patterns of preferred prescribing practices, including induction, dose adjustment, maintenance, and take-home dosing.

- To document the ease or difficulty physicians encounter in delivering buprenorphine treatment in the various treatment settings, the accommodations they must make, and the advantages/disadvantages of this treatment strategy (from the physicians' perspectives).
- To document the acceptance, compliance, preferences, and necessary adjustments from the patients' perspectives.
- To document treatment education issues regarding consideration of physician guidance materials for future course work by professional societies following anticipated FDA approval.

Depot Naltrexone

Naltrexone, a marketed, long-acting, orally effective opioid antagonist, was approved in 1985 for the indication of blocking the pharmacological effects of exogenously administered opiates. It is an adjunct to the maintenance of the opioid-free state in detoxified, formerly opioid-dependent individuals.

One of the major obstacles to the success of naltrexone has been patient compliance with therapy. Naltrexone must be taken at least three times per week and has no effect other than to block the effects of heroin, a drug that the patient is not supposed to use. Because of this, many patients forget to take or stop taking their medication. Therefore, the greatest success with naltrexone has been in the limited population of highly motivated formerly opiate-dependent patients.

Via a Small Business Innovative Research (SBIR) contract, NIDA completed the production and preclinical testing of a batch of 120 doses of depot naltrexone in 1999. These doses were designed to provide a sustained level of naltrexone for 30 days when administered subcutaneously in humans and to produce a blood level of about 2-3 ng/ml. This product has been shown to block challenges in primates, and a similar preparation has been shown to block some of the effects of morphine challenges in humans.

This preparation was tested in an inpatient clinical study to assess its ability to block specific responses to heroin challenges between 12 and 25 mg. Based on the promising results of this study, an outpatient double-blind study was designed to test the product. The outpatient study began in the summer of 2000. Approximately 8 of 60 subjects planned have been completed. Additionally, a small positron emission tomography (PET) study to assess the receptor occupancy of naltrexone during a challenge protocol is planned. Naltrexone was approved in 1994 for the treatment of alcohol abuse. The depot preparation may also be of value for the treatment of that disease.

Dextromethorphan

A revolutionary advance in our understanding of one of the basic neurochemical mechanisms underlying opiate addiction has resulted from recent animal investigations of the role of N-methyl-D-aspartate (NMDA) receptor antagonists in opiate addiction. Drugs that antagonize the NMDA receptor complex are capable of inhibiting opiate withdrawal and tolerance and, in some cases, of reversing tolerance.

As a first step, NIDA plans to assess the toxicity of NMDA receptor antagonists and to evaluate their effects on conditioned cues and drug abuse. It is hypothesized that this class of drugs has a unique pharmacological effect in decreasing the conditioned cues that appear to perpetuate opiate-seeking behavior. Furthermore, we anticipate that these compounds will not have the abuse liability associated with other opiate treatments, such as methadone and buprenorphine.

Medication Systems for Neonatal Treatment

NIDA has expressed an interest in the development of medications and formulations to treat withdrawal symptoms in babies born to opiate-dependent mothers. Although many scientists are interested in novel drug-delivery systems, very few have considered the treatment of neonates. NIDA plans to focus specifically on narcotic skin patches for the treatment of neonates born to addicted mothers.

Infants born to mothers who abuse opiates or who are on methadone maintenance can go through withdrawal periods lasting for 3 or more weeks. Currently, there are no FDA-approved medications for the treatment of these neonates, although paregoric (opium solution) has been used.

A transdermal delivery system (patches) for opiate agonists such as fentanyl or buprenorphine offers advantages over oral or injectable dosage forms because of its convenience for use in neonates. This technology is available, is currently in use, and could be readily adapted for use in neonates undergoing withdrawal. Clinical trials will require careful planning, and both FDA approval and regulations concerning use in treatment may present unique problems not anticipated by the Narcotic Addict Treatment Act.

Opioid Peptides as Medications

The three major types of opioid receptors in the brain are mu, delta, and kappa. Morphine, heroin, methadone, and ORLAAM bind to the mu receptor with high affinity. The naturally occurring ligand for the kappa opioid receptor is dynorphin A.

Animal studies indicate that dynorphin A alleviates opiate withdrawal and that this peptide also decreases tolerance to chronically administered mu opioid receptor agonists (e.g., morphine). Indeed, kappa opioid abnormalities in the central nervous system may underlie the drug-seeking behavior of heroin addicts. The effects of dynorphin on opiate withdrawal are currently being investigated by NIDA-funded scientists in opiate-dependent subjects.

Cocaine Addiction Treatment

Compounds in Advanced Clinical Testing

Several small studies of potential cocaine addiction treatment agents have been completed and are in various stages of data analysis. Clinically significant findings will be followed up in larger controlled trials as warranted. The development of anticocaine medications has proven to be a daunting task, and to date only one potential medication has been identified that justifies a phase III trial. A retrospective analysis of a phase II trial of selegiline in cocaine addicts indicated a differential outcome in favor of selegiline versus placebo. This finding needs to be replicated in a larger and more statistically powerful clinical trial. The DTR&D will conduct a phase III multicenter trial of selegiline beginning in the spring of 2001.

Additionally, there is a growing body of evidence generated by NIDA grantees concerning the potential use of disulfiram in the treatment of cocaine dependence. Disulfiram (Antabuse), marketed as aversive therapy for treating alcoholism, is also showing promise in the treatment of cocaine dependence. Several NIDA-sponsored studies conducted at Yale University documented interaction of disulfiram with cocaine in humans. Pharmacokinetics studies showed that disulfiram increases plasma concentrations of cocaine

and potentiates physiological-cardiovascular responses to cocaine. Three efficacy trials conducted with different populations of cocaine-dependent individuals suggest that disulfiram in combination with each of three different therapeutic interventions (cognitive behavioral treatment, 12- step facilitation, or clinical management) is effective in treating cocaine dependence. In cocaine-alcohol abusers, disulfiram treatment showed sustained effect on reduced cocaine and alcohol use 1 year after cessation of the therapy. Disulfiram treatment of cocaine-abusing opioid-dependent patients maintained on methadone resulted in significant decrease of the amount and frequency of cocaine use. A preliminary study showed that disulfiram also decreases cocaine use in cocaine-opioid-dependent addicts maintained on buprenorphine.

NIDA is currently sponsoring three large outpatient clinical trials with disulfiram as the treatment for cocaine dependence:

- A study on 160 opioid-cocaine-dependent patients maintained on methadone, conducted at Yale University.
- A study on 180 opioid-cocaine-dependent patients maintained on buprenorphine, conducted at Yale University.
- A study evaluating disulfiram and naltrexone alone and in combination in the treatment of 208 alcohol-cocaine-dependent individuals, conducted at University of Pennsylvania.

All these studies include some form of behavioral or cognitive therapy and drug counseling. They are monitoring not only cocaine use, but also opiate or alcohol use. Finally, NIDA is planning a clinical pharmacology/safety study of the interactions between disulfiram and IV-administered cocaine prior to launching a large-scale phase III multicenter trial with this medication.

CRADA with NeuroSearch, AG

In late 1997, NIDA entered a CRADA with Danish company NeuroSearch, AG, to perform research on NeuroSearch's proprietary compound NS2359, which is targeted exclusively as a potential treatment for cocaine dependence. As part of this agreement, NeuroSearch has conducted the first of many safety trials on this product. Assuming that analysis of the data generated from this trial is supportive of its safety, NIDA will undertake the necessary safety and efficacy trials required by FDA to further develop NS2359.

GBR 12909

Major neurochemical effects of cocaine include release of dopamine (DA), serotonin, and noradrenaline via a transporter-mediated exchange mechanism. There is considerable evidence that the initiation and continuation of cocaine use is associated with the effects of the drug on the dopaminergic, serotonergic, and noradrenergic modulation of the central nervous system (CNS) function. Animal studies suggest that the mesocorticolimbic dopaminergic pathways are important mediators of cocaine's reinforcing and addictive properties. Cocaine binds to these transporters and blocks the removal of these neurotransmitters from the synaptic gap. The neurobiological mechanisms underlying the effects of cocaine are not well-understood. Preclinical studies indicate that cocaine's blockade of the DA transporter plays a key role in producing cocaine's addictive and reinforcing effects. Primate and non-primate studies have shown that GBR 12909 has a strong affinity for the DA transporter. GBR 12909 is a high-affinity, selective, and long-acting inhibitor of DA uptake that produces a persistent and non-competitive blockade of DA transporters and substantially reduces cocaine-induced increases in extracellular mesolimbic DA. In addition, GBR 12909 has a higher affinity than cocaine for the DA transporter. Ongoing research is searching for a dopamine sparing cocaine antagonist that might be

developed as a pharmacological treatment to block cocaine from acting at the transporter level to produce its reinforcing effects. GBR has been postulated to act by binding only to precise sites on the dopamine transporter that are required for cocaine binding and making available the sites where DA binds to the transporter.

A phase I clinical study was conducted in support of an IND application filed by NIDA. The main objectives of this study were to determine the safety, tolerance, and pharmacokinetics of multiple escalating dosages of oral GBR 12909 in healthy volunteers. In addition, PET scans measuring the occupancy of the DA transporter by GBR 12909 were obtained. The occupancy scan results will be correlated with the safety data to determine an optimal oral dose of GBR 12909. The current study has completed all four dosage levels. Unexpected adverse events included insomnia and increased libido, and disinhibition of aggressive feelings. The data are being analyzed.

Dopamine Agonists

The activation of the dopaminergic reward system in the brain appears to be the principal neurochemical mechanism involved in the addiction to stimulants such as cocaine and amphetamine. Chronic abuse of these drugs results in DA deficiency in the brain, which has been hypothesized to lead to craving for stimulants, depression, anhedonia, and dysphoria.

Several clinical reports, such as that reported for amantadine, suggest that DA agonists decrease cocaine use. NIDA proposes to clinically test several approved medications that would increase dopaminergic tone in the brain and to study novel compounds. Examples of these agents are: 1) direct dopamine agonists and partial agonists, 2) dopamine precursors, 3) reversible monoamine oxidase inhibitors, and 4) drugs that inhibit dopamine, serotonin, and noradrenaline neuronal re-uptake (mimicking cocaine but that have a slower onset of action and are presumably less addictive).

Most recently, studies in rodents, and to a lesser extent in monkeys, have differentiated the roles of D1 and D3 receptors with regard to cocaine. The D1 system may inhibit the effects of cocaine, while the D3 system may provide a cocaine substitute of lesser dependence potential. Compounds that affect both systems are under study.

Kappa Opioids

Recent studies have shown that kappa opioid compounds exhibit effects opposite to those of cocaine in terms of dopamine release and neuron-firing patterns. In animal studies, kappa opioids block drug discrimination and self-administration of cocaine and also prevent context-independent sensitization to cocaine. NIDA and NIDA grantees are testing compounds of this class in clinical studies.

Glucocorticoid Antagonists

Studies have shown that cocaine causes the release of stress hormones known as glucocorticoids in rats and humans. There is some evidence from rat studies that glucocorticoid antagonists and corticotropin-releasing factor (CRF) antagonists reduce cocaine self-administration in a dose-related manner. NIDA

will follow up on these basic research findings with additional studies aimed at developing a potential treatment for cocaine addiction. DTR&D is pursuing CRF antagonist projects with a pharmaceutical company supplier.

Immunology

Researchers funded by NIDA's DTR&D reported that they have successfully immunized rats against many of the stimulant effects of cocaine. Cocaine was prevented from entering the brain when rats were "vaccinated" with a substance that triggers the body to produce antibodies to cocaine. These antibodies then acted as biological "sponges" to which cocaine binds, thereby reducing the amount available in the blood to reach the brain. The results of this research are presented in "Suppression of Psychoactive Effects of Cocaine by Active Immunization" in the December 14, 1995, issue of *Nature*.

Researchers Kim Janda, Ph.D., Rocio Carrera, M.A., George Koob, Ph.D., and colleagues at The Scripps Research Institute demonstrated a greater than 70% reduction in cocaine uptake in the brains of rats inoculated with the antibody-producing compound as compared to a group that was not inoculated. Researchers designed the compound so that the antibodies produced would respond specifically to the cocaine molecule yet not affect normal brain chemistry.

In the study, Dr. Janda and colleagues used an "active immunization" approach by developing a substance that when administered to rats would trigger the immune system to produce antibodies that are specific for the cocaine molecule. The researchers inoculated the rats over a 35-day period and then tested their responses to cocaine. The immunized animals showed significantly lower responses to the stimulant effects of cocaine than control animals because the immunization prevented much of the cocaine from getting to the brain. Cocaine concentrations in the brain tissue of the immunized animals were found to be dramatically less than the concentrations of cocaine in brain tissue of controls.

Other immunotherapy research for drug abuse treatment has explored the use of catalytic antibodies and other external agents that can be used to treat cocaine dependence. The research reported in *Nature* differs by inducing the production of antibodies that remain in the bloodstream for an extended period of time and block cocaine's effects after it is used.

The biotechnology company ImmuLogic Pharmaceutical Corporation (Waltham, Massachusetts) also recently announced development of a cocaine vaccine. Dr. Barbara S. Fox of ImmuLogic discussed some of the company's findings in *Chemistry and Engineering News*, December 18, 1995, and at a January 18, 1996, meeting of the Maryland Bioscience Alliance at the request of NIDA. ImmuLogic had previously received phase I SBIR funding from NIDA. In 1996, NIDA awarded a \$700,000 SBIR award to ImmuLogic to complete preclinical development of a vaccine to treat cocaine dependence. Results of ImmuLogic's early vaccine work in animals have received attention in national and trade press, were presented at the College on Problems of Drug Dependence, and were published in *Nature Medicine* 2, 1129-1132: 1996.

The vaccine links a protein to cocaine, resulting in a molecule that induces antibody formulation. Once titers reach a certain level, cocaine's ability to cross the blood-brain barrier is impeded. The award expedited completion of preclinical design and early testing of the vaccine. A phase I (dosing and tolerability trial) was successfully completed in 1999. ImmuLogic is no longer in business and sold the rights to the vaccine to Cantab, Ltd., a British company specializing in vaccine development. NIDA has supported this research via a Strategic Program for Innovative Research on Cocaine Addiction Pharmacotherapy (SPIRCAP) and SBIR grant. Thirty-four subjects completed the initial phase I study in

the United States. Specific antibody titers for cocaine were developed in the vaccine-challenged subjects. Under the SPIRCAP award, two additional studies are planned to convene this year: an inpatient study examining the extent to which the antibody can block the effects of administered cocaine, and an outpatient study. To date, no adverse events have been reported, and the company plans to continue development of the vaccine.

Serotonin Antagonists

Cocaine increases extracellular levels of serotonin, dopamine, and noradrenalin in the brain. Serotonin is an extremely versatile neurotransmitter that activates numerous subreceptors. Studies of two subtypes of receptors (5HT-2 and 5HT-3) suggest that these structures are involved, directly and indirectly through an effect on dopamine, in the rewarding and, possibly, the mood-elevating effects of cocaine. Antagonists of these receptors, which decrease dopamine release, may reduce cocaine craving and use. Therefore, NIDA plans to test serotonin antagonists as potential medications for cocaine addiction.

Serotonin Re-uptake Inhibitors

A major symptom of cocaine addiction is anhedonia, which is clinically very similar to depression. It has been postulated that cocaine addiction is a form of self-medication for chronic depression. Initial results of studies of the potent serotonin re-uptake blocker fluoxetine (an antidepressant) have yielded mixed results in attempting to answer the suggestion that it may be efficacious in facilitating abstinence from cocaine use. Evaluation of serotonin re-uptake inhibitors as potential medications will continue. One such compound, the currently marketed antidepressant Venlafaxine, a serotonin and norepinephrine uptake inhibitor, is being studied by two NIDA grantees.

Nootropic Drugs and Cocaine Dependence

Abuse of stimulants such as cocaine and amphetamines is associated with some degree of neurological damage, resulting in cognitive impairments, brain perfusion deficits, strokes, intracranial hemorrhages, and development of early symptoms of movement disorders. NIDA-sponsored studies are under way to test several nootropic medications as potential treatments for cocaine-induced neurological deficits.

Cocaine “Receptor”: Imaging Studies

In addition to the categories of compounds being tested as described above, a new and potentially useful technology is being investigated as to its value for predicting efficacy of potential cocaine treatment medications. Research in the field of structure-activity relationships have revealed highly selective and potent binding ligands for the dopamine transporter. NIDA intramural researchers have identified three “generations” of such compounds, with each succeeding generation being more selective and potent than the previous one. RTI-55, the first potent compound, was shown to be an effective in vivo labeling agent in animal studies and was subsequently examined in human imaging studies by single-photon emission computed tomography (SPECT). A second compound, RTI-121, was found to be more selective for the dopamine transporter but had a higher apparent lipid solubility and exhibited lower specific to nonspecific binding in vivo. NIDA researchers are testing new compounds and are also utilizing some older compounds (e.g., WIN-35,428) in brain imaging studies. Procedures have been developed for estimating the occupancy of transporter sites in vivo. Dopamine transporter imaging studies of cocaine abusers have been completed (see section on GBR 12909). This technology may make it possible to

estimate the effectiveness of a potential treatment compound or regimen by correlating receptor occupancy (as shown in imaging studies) with actual clinical results. NIDA will continue to follow this line of research.

Methamphetamine Treatment Discovery Efforts and Program Activities

Methamphetamine is a potent psychomotor stimulant that has gone through episodic periods of widespread use and abuse in the United States. Cocaine abuse and addiction surpassed use of methamphetamine in the 1970s and 1980s, but methamphetamine abuse and addiction has been re-appearing in some regions of the United States and is widespread in western U.S. cities such as San Francisco, Denver, Phoenix, and Los Angeles. According to the National Household Survey on Drug Abuse, an estimated 3.8 million people had tried methamphetamine by 1994, and by 1999 the total number had increased to 9.4 million. The epidemic is spreading to rural areas, and nationwide there were approximately 11,000 acute hospital admissions related to amphetamine toxicity in 1999.

There are no accepted treatment medications for methamphetamine addiction or abuse. As a result, NIDA has developed a Medication Discovery Program for methamphetamine and is funding a number of extramural and intramural studies to develop medications to treat methamphetamine abuse.

Preclinical Methamphetamine Program

Methamphetamine abuse has become a substantial drug problem in certain parts of the American Southwest, and data suggest that its use is increasing and spreading to other parts of the United States. Methamphetamine is a powerful stimulant that shares some similarities with cocaine, but also differs from cocaine in certain ways. A January 10, 2000, Methamphetamine Think Tank meeting was held in order to gather a group of consultants to consider the direction of a methamphetamine treatment development program. Based upon recommendations of these consultants, several types of methamphetamine-specific screening assays are being developed to evaluate and characterize compounds for their potential usefulness in the treatment of methamphetamine dependence. Some of the assays recommended are similar to those used in the Cocaine Treatment Discovery Program, and there will be substantial overlap between the two programs. Existing contract protocols will be used to test compounds for their interactions with dopamine transporters, but additional assays will be utilized to measure dopamine release in vitro, which is an effect of methamphetamine that is not shared by cocaine. In addition, behavioral assays are being set up to assess a compound's ability to block the locomotor stimulant effects of methamphetamine, block the discriminative stimulus effects of methamphetamine, and determine effects on methamphetamine self-administration. In addition, assays to measure effects of potential treatment compounds on the cardiovascular system, both alone and in combination with methamphetamine, are being developed. Methods for assessing the neurotoxic effects of methamphetamine are under development, and these assays may be useful in assessment of potential treatment medications.

Clinical Methamphetamine Program

Since January 2000, NIDA has been working on choosing sites for a new methamphetamine clinical trials network and has identified four sites (Des Moines, Los Angeles, San Diego, and Honolulu) with high concentrations of methamphetamine users. Plans are under way to visit these sites and begin developing a protocol for Bupropion as the first medication trial for this network. NIDA hopes to start a multisite Bupropion study after the prerequisite interaction studies are completed. Four clinical pharmacology

studies are also being developed in Los Angeles and San Francisco to study safety interactions of methamphetamine and selegiline as a potential treatment.

Phencyclidine (PCP) Treatment

Dr. Michael Owens at the University of Arkansas for Medical Sciences in Little Rock receives NIDA funding to develop a new generation of monoclonal antibody-based medications for treating drug abuse (“Immuno-Therapy for Drug Abuse,” R01 DA07610, and “Antibody-Based Therapy for Methamphetamine Abuse,” R01 DA11560). This research is focused on treatments for methamphetamine, methylenedioxymethamphetamine (MDMA, also known as ecstasy), and PCP abuse. These medications function as pharmacokinetic antagonists and are designed to reverse the effects of drug overdose and/or help blunt the reinforcing effects of drugs of abuse. Because of the unique pharmacological profile of these new medications, they would be well-suited for use with other, more conventional, chemically based medications and treatments such as behavioral modification to aid in the long-term recovery from drug addiction.

If successful in humans, these treatments will not only provide a rapid reversal of drug effects in an emergency room setting, but also reduce or prevent the long-term medical problems associated with stimulant drugs of abuse (i.e., neurotoxicity and addiction). Dr. Owens’ studies of treating PCP effects are the most advanced and are providing the model system for development of antibody-based therapies for other classes of drugs and toxins. To this end, Dr. Owens has successfully developed an anti-PCP monoclonal antibody that removes PCP from the CNS of rodents in minutes. The group is presently focused on completing the preclinical animal studies necessary for the filing of an IND application and on refining previously developed scale-up methodology for the production of the monoclonal antibody fragments. In other studies, Dr. Owens has shown that a single administration of an anti-PCP monoclonal IgG antibody can significantly reduce PCP’s behavioral effects and CNS concentrations for at least 2 weeks, even when PCP is repeatedly administered in large doses to the animals over the entire 2-week period. This is important because a 2-week time period in animals would be equivalent to about 1 to 2 months of protection in humans. Dr. Owens’ preliminary studies of antibody-based treatments for methamphetamine are showing the same type of long-term neuroprotective effects.

In collaboration with Dr. Brooks Gentry, a clinician/scientist at the University of Arkansas (“Mechanisms of Onset and Offset of Rapid Stimulant Effects,” K08 DA00339), Dr. Owens is studying the fundamental medical consequences of rapid input of drugs of abuse into the CNS. These experimental data from animal models of human drug abuse will help in understanding why drug abusers usually prefer rapid routes of drug administration (intravenous and smoking) over slower modes of administration (oral). Finally, these pharmacokinetic and pharmacodynamic studies are being used to aid in the development of novel therapeutic approaches to the treatment of stimulant drugs of abuse.

National Institute of Environmental Health Sciences (NIEHS)

Overview of NIEHS Rare Diseases Research Activities

NIEHS supports basic research into the fundamental mechanisms of how environmental exposures interact with the human body to produce disease and dysfunction. This research on molecular pathways and environmental interaction has also yielded insights into the basic mechanisms involved in the pathogenesis of rare diseases and conditions.

Recent Scientific Advances in Rare Disease Research

***TTP* and Related Proteins in Inflammatory Diseases**

One major area of study in the laboratory began with the cloning of a gene that was rapidly induced by insulin. The protein encoded by this gene, known as *TTP*, is the prototype of a novel class of CCCH zinc finger proteins. *TTP* is rapidly induced, translocated from the nucleus to the cytosol, and phosphorylated on serine residues by insulin and by many other mitogens and growth factors. Mice deficient in this protein develop a complex syndrome consisting of arthritis, wasting, dermatitis, and early death.

During the past year, NIEHS scientists demonstrated that *TTP* deficiency in mice also led to increased stability of the mRNA-encoding granulocyte-macrophage colony stimulating factor (GM-CSF), a cytokine important for maintenance of the normal white blood cell count. Studies in cell-free systems and in cultured cells are under way to identify inhibitors of this interaction, which might be useful therapies for neutropenic states. Concerning the mechanism of action of *TTP* and its relatives, NIEHS reported that these proteins can destabilize certain mRNAs, even when those mRNAs do not contain polyA tails, indicating that initial deadenylation is not required for subsequent mRNA degradation. NIEHS began to establish the "rules" that govern the binding of this novel class of RNA-binding proteins to its target sequences, and identified a number of protein-coding polymorphisms and one non-expressing mutation in the human genes-encoding members of this family of proteins. Studies are under way to determine the biochemical and clinical significance of some of these variants.

Effect of Diet on Occurrence of Chronic Disease

Diet may affect the risk of several chronic human diseases, although additional research is needed. This project has two main thrusts: 1) the study of diet-cancer relations and 2) the study of diet in relation to risk of amyotrophic lateral sclerosis (ALS). Defects in antioxidant defenses (e.g., superoxide dismutase 1 [SOD1]) are a cause of ALS, and thus it is reasonable to suspect that antioxidant intake may also affect the incidence or progression of this disease.

One research focus on diet and ALS has been an analysis of dietary data from a case-control study of ALS. NIEHS examined the dietary intake of calcium, magnesium, and antioxidants among 107 ALS cases and 262 community controls. Overall, these dietary factors were not related to risk of ALS, though modestly protective associations were suggested for magnesium and lycopene.

A second study of the same relationship is an add-on to a large cohort study under way at NCI. The cohort consists of members of the American Association of Retired Persons (AARP) who have

completed a dietary questionnaire (approximately 600,000 people). Researchers expect approximately 150 cases of ALS to develop in this cohort by 2002.

Systemic Lupus Erythematosus (SLE)

SLE is a severe, disabling autoimmune disease. Approximately 90% of lupus patients are women. Although few studies provide detailed data pertaining to the prevalence of this disease, conservative estimates indicate that 100,000 women in the United States are living with SLE. Researchers recently finished data collection in the Carolina Lupus Study, the largest population-based case-control study of hormonal and environmental risk factors for the development of SLE conducted to date. Four specific analyses based on these data were presented at the annual meeting of the American College of Rheumatology in 1999 and 2000. These analyses include hormonal and reproductive risk factors, medical history risk factors (i.e., allergies, infections), occupational silica dust exposure, and demographic differences in the clinical and immunologic presentation of the disease. Manuscripts for these and other analyses have been submitted for publication.

Friedrich's Ataxia (FRDA)

FRDA is the most common cause of recessive ataxia and occurs at an incidence of 1 in 30,000 Caucasians. The yeast homologue of the *FRDA* gene, *YFHI*, which codes for the protein frataxin, is responsible for regulating the amount of iron in the mitochondria. The absence of frataxin leads to iron accumulation and the production of radicals. NIEHS scientists have established that frataxin limitation leads to nuclear as well as mitochondrial DNA damage. This novel finding has implications for the pathological symptoms associated with the disease and potential treatment strategies. This research also has many implications for possible origins of aging and cancer.

Lung Hemorrhage in Cleveland, Ohio, Infants

Over the past 5 years, a relatively rare disorder, acute pulmonary hemosiderosis, or hemorrhage, has been found in a large number of infants in inner-city Cleveland. There have been 37 cases in a limited area of Cleveland resulting in 12 deaths, including 7 deaths originally thought to be due to sudden infant death syndrome (SIDS). The environmental mold *Stachybotrus chartarum* has been identified as the causative agent, with the young child's developing lung being particularly vulnerable. NIEHS is supporting a pilot study that will allow a local physician to further define the environmental components for a condition that has a 30% mortality rate and is disproportionately affecting inner-city children. The researchers have identified environmental tobacco smoke as a possible trigger for the acute bleeding. Additionally, the research has led to the development of a home remediation program that completely eliminates the mold spores from contaminated homes.

Diethylstilbestrol (DES)

Once used by pregnant women to prevent miscarriage, the potent synthetic estrogen DES was shown to cause health problems in women exposed to DES in utero ("DES daughters"). These women were at risk of developing clear cell adenocarcinoma, a rare vaginal cancer, as well as having reproductive abnormalities. To date, "DES sons" have shown increased reproductive tract abnormalities but not an increased cancer risk, although this is a possibility as the population ages. A recent NIEHS study shows the unexpected: that the environmental exposures of one's parents and grandparents can have adverse effects on our own health, even if we have never been directly exposed to a particular compound.

Although this multigenerational effect has only been demonstrated with DES, it suggests new avenues of investigation for assessing the many “environmental estrogens” that have been developed. Although these compounds have a far weaker estrogenic effect than DES, the possibility exists that subtle adverse effects could show up in our sons, daughters, grandsons, and granddaughters. More immediately, this research proves that the sons of “DES daughters” need to be closely monitored by their physicians.

Incontinentia Pigmenti (IP)

IP is a genetic disorder characterized by unusual patterns of discolored skin. Males with this disorder usually die before birth, so females are the major patient group. In rare cases, IP can cause developmental abnormalities such as dwarfism and club foot. NIEHS studies have definitively linked IP with deficiency of IKK γ /NEMO expression. This connection provides additional evidence for the importance of the IKK complex and NF κ B for prevention of programmed cell death in mice and in humans. IKK γ /NEMO-deficient mice can be used as a model for studying IP, which will help women with IP to make more informed reproductive decisions.

Dioxin's Effects on Gender Ratios in Offspring of Exposed Men

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is considered to be one of the most toxic synthetic substances, if not the most toxic. TCDD causes cancer and disrupts multiple hormonal functions. TCDD is a by-product of several manufacturing processes such as paper production and pesticide formulation. NIEHS conducted a follow-up study on previous work that demonstrated a significant increase in the number of female births after TCDD exposure accidents. Serum samples taken at the time of the exposure accident were analyzed for TCDD. The data indicate a positive correlation between increased probability of female births with increasing TCDD concentration in the sera of the fathers. The effect starts at concentrations less than 20 ng/kg body weight, a level about 20 times higher than the normal TCDD concentration in humans. This level of contamination is similar to doses that cause epididymal impairments in rats.

Dioxin is a ubiquitous toxin in that all human beings have some exposure. The Seveso incident occurred more than 20 years ago and the effects on offspring gender ratios are still present. This demonstrates the persistence of dioxin and its adverse effects following exposure. Also, the level of exposure compared to “unexposed” populations is relatively low, suggesting that these effects may also be seen in the general population or susceptible individuals. These observations could have profound public-health implications.

Rare Diseases Research Initiatives

Refsum's Disease

NIEHS scientists are studying phytol metabolites, which are activators for the nuclear receptor RXR. Patients with Refsum's disease accumulate the metabolite phytanic acid to levels that are about 100 times higher than normal values. Because it is alleged that phytanic acid has its sole origins from the diet, NIEHS researchers are examining the effects of its removal from the diets of rodents.

SGD Syndrome

Lactoferrin is an antibacterial and antiviral protein. It is the major protein in the specific granules of the neutrophils. The only genetic disease linked to lactoferrin is SGD syndrome, in which patients lack the specific granules in neutrophils. SGD is characterized by lactoferrin deficiency with recurrent infections.

Friedreich's Ataxia (FRDA)

FRDA is an autosomal recessive neurological disease that affects mitochondrial iron homeostasis. Deletion of the yeast homolog of this gene causes mitochondrial iron accumulation and a petite phenotype. Experiments have indicated nuclear DNA damage in yeast by reactive oxygen species. These experiments therefore have implications in the treatment of this disease and establish a novel paradigm of mitochondrial proteins having a nuclear-protective role.

Ataxia Telangiectasia (AT) Cancer

This research effort investigates the molecular mechanisms involved in cell cycle checkpoint responses to exposures to ionizing radiation (IR) and other environmental agents in both normal human fibroblasts and fibroblasts that lack normal function of the AT cancer susceptibility gene products. In particular, researchers are interested in the role of the AT mutated (ATM) gene product in cell cycle checkpoint responses to exposures to environmental carcinogens and the signaling pathways that are generated from broken DNA to the inactivation of cyclin/CDK protein kinase complexes. In addition to aiding the understanding of the process of carcinogenesis, these studies hold great potential for providing insight into the mechanism of action of non-genotoxic environmental carcinogens.

Nijmegen Breakage Syndrome (NBS)

NBS a rare autosomal recessive disorder characterized by increased sensitivity to IR, defective cell cycle checkpoint responses, and elevated cancer incidence. Both NBS and a related ataxia telangiectasia-like disorder are caused by mutations in the chromosomal DNA repair genes *hNBS1* and *hMRE11*, respectively. Functional homologs of the human genes, referred to as *XRS2* and *MRE11*, are present in the genetically tractable budding yeast *Saccharomyces cerevisiae*. NIEHS scientists and others have demonstrated that these genes perform similar functions in both yeast and human cells, leading to the view that the *MRE11* and *XRS2* proteins are components of a complex with DNA exonuclease and endonuclease activities. The nuclease function was recently found to be critical for repair of broken chromosomal DNA by homologous recombination, but not for recombination-independent mechanisms of repair performed by the complex. In addition, these proteins may be important in dealing with categories of double-strand breaks that differ from those induced by IR. This information will be useful in understanding consequences of the genetic defect.

DNA Triplet-repeat-based Diseases

There are more than 14 rare neurological and neuromuscular diseases (including Haw River syndrome, affecting a small group of African American families in North Carolina) that result from the expansion of triplet-repeat DNA sequences. NIEHS scientists are investigating the underlying systems responsible for triplet-repeat expansion and have proposed a molecular model in which triplet expansion is due to a deficiency of 5'-flap cleavage during DNA replication. In particular, the interaction of the human enzyme responsible for 5'-flap cleavage (*FEN1*) with other components of DNA metabolism (such as proliferating

cell nuclear antigen [PCNA] and DNA polymerases delta and epsilon) is being addressed genetically. Researchers have now established that the nuclease function of the replication protein DNA polymerase delta may also play an important role in processing replication intermediates. During lagging strand synthesis, a replication intermediate is created that must be processed by either DNA polymerase delta or the *FEN1* nuclease. The lack of processing is proposed to lead to a double-strand break that may be instrumental in triplet-repeat expansion. This research will provide further understanding of how the disease might arise and the possible consequences of variations in the relevant DNA metabolic proteins.

National Eye Institute (NEI)

Overview of NEI Rare Diseases Research Activities

NEI was created on August 16, 1968, by Public Law No. 90-489 for the purpose of supporting and conducting research for improving the prevention, diagnosis, and treatment of diseases that affect the eyes and vision. Eye diseases and blindness cost the nation an estimated \$38.4 billion per year. More than 12 million people in the United States suffer some significant impairment of vision. Over the years, NEI-supported vision researchers have conducted many pioneering studies that have greatly advanced our understanding of eye diseases, including those classified as rare, and provided eye-care professionals with new tools and methods to prevent or cure many sight-threatening conditions.

In June 1998, NEI published *Vision Research: A National Plan: 1999 - 2003*. This plan is the sixth in a series that dates back to the publication of *Vision Research Program Planning* in 1975. The development and publication of the aforementioned plans address the Nation's visual health needs, including rare diseases of the eyes and visual pathways.

Recent Scientific Advances in Rare Diseases Research

Retinitis Pigmentosa (RP) and Related Disorders

RP is a group of blinding hereditary retinal degenerative diseases characterized by a progressive loss of vision due to the degeneration of photoreceptor cells. The incidence of RP in the United States is about 1 in 3,500 births, and RP affects more than 100,000 people. RP patients frequently report night blindness and loss of mid-peripheral vision during adolescence, and are usually legally blind by age 40. Photoreceptor cells of the retina (the rods and cones) are responsible for the capture of light and the initiation of an electrical signal to the brain in the process of vision. The study of signaling in photoreceptor cells, termed the visual phototransduction cascade, has provided a detailed molecular description of this pathway. Gene therapy is one of the more promising interventions to slow or stop the progression of this blinding eye disease and other inherited retinal degenerative diseases.

Dominantly inherited diseases such as RP are an especially challenging problem for development of gene-based therapies. Ideally, the defective gene has to be replaced to eliminate the protein product responsible for causing the photoreceptor degeneration and loss. For this to happen, the gene must be inactivated, repaired, or replaced.

Two different strategies have been developed by NEI-funded investigators to attack this problem. In the first case, triplex technology has been applied to the human rhodopsin gene that is involved in autosomal dominant RP (ADRP). The most common form of ADRP results from mutations in rhodopsin. Several so-called triplex-forming sites have been identified within the rhodopsin gene, and seven of these bind small DNA oligonucleotides. Such triplex-forming oligonucleotides (TFOs) bind to native DNA and offer a therapeutic potential by interrupting the expression of a disease-causing protein.

In the second case, another group of investigators has developed a ribozyme-based approach to photoreceptor cell rescue. Ribozymes are small RNA molecules that cleave a complimentary mRNA sequence, blocking production of its protein. Results are encouraging: two different and biologically active ribozymes slow the rate of retinal degeneration in a transgenic rat model of the disease.

Leber's Congenital Amaurosis (LCA)

In 1869, Theodor Leber first described this recessively inherited retinal degeneration with RP-like pathology, which causes incurable childhood blindness. No treatment is currently available for many childhood genetic diseases like LCA, as the necessary gene is either missing or defective. With recent advances in our understanding of the basis for genetic diseases, scientists have been able to identify defective genes that are associated with specific diseases. Once the gene is identified, however, the patient is still faced with the prospect of no immediate cure. Such was the case for LCA, when in 1997 the disease-causing mutations in a gene known as *RPE-65* were linked to an estimated 10% of LCA cases. NEI scientists recently produced mice lacking the *RPE-65* gene. The absence of the *RPE-65* gene produces a defect in the visual cycle, a series of biochemical events in the light-sensing retina that initiate vision. The defect eventually results in impairment of photoreceptor cell function and retinal degeneration.

In order to better understand the function of *RPE-65*, scientists studied the individual components of the visual cycle pathway and found that *RPE-65* is involved in a biochemical reaction called an isomerization. Thus, the mice lacking *RPE-65* allowed scientists to focus on the possible function of this molecule. Next, a way was found to bypass the defect in the visual cycle. For this, *RPE-65*-deficient mice were fed a form of vitamin A called 9-cis-retinal. This chemical is not normally found in photoreceptor cells, but it forms part of the functional visual pigment isorhodopsin. The resulting improved photoreceptor physiology and function was dramatic and showed that a potential pharmacologic intervention may help to restore vision to children with LCA.

Dramatic progress toward finding a cure for LCA was recently reported by NEI-supported scientists. These scientists conducted experiments in which they successfully restored vision in a naturally occurring large animal model (dog) of LCA that suffers from visual impairment typical of that seen in children with LCA. These researchers inserted a wild-type *RPE-65* gene into the retina of a dog using recombinant adeno-associated virus (AAV) as a vector. While this research shows great promise, there is still much work to be done before gene therapy can be used to treat human patients with LCA.

Ocular Melanoma

Although rare, choroidal melanoma is the most common primary eye cancer in adults. Many choroidal melanomas enlarge over time, lead to loss of vision, and spread to other parts of the body to eventually cause death. There had been uncertainty in the medical community about the value of giving radiation treatments prior to removal of the eye of patients with large ocular melanoma. In cancers occurring elsewhere in the body, prior radiation has reduced the rate of tumor recurrence after surgery. The Collaborative Ocular Melanoma Study (COMS) is the first controlled, randomized, multicenter clinical trial large enough to measure the survival rate of patients who received radiation treatment prior to eye removal. Patients with tumors large enough to require removal of the eye were randomized to either receive radiation treatment to the affected eye before it was removed or to have the eye removed without radiation treatment. After 5 years of follow-up, both groups had similar survival rates. About 60% of the patients are alive after 5 years of follow-up and these patients will be followed to determine the risks and long-term effects of both treatments. Radiation therapy is costly and has the potential for side effects. Unless a survival benefit is shown with further follow-up, it is unlikely doctors will advise radiation therapy use.

Leber's Hereditary Optic Neuropathy (LHON)

LHON is a maternally inherited genetic disease that results in substantial loss of central vision in affected patients. Most genetic diseases are caused by mutations in chromosomal DNA inside the cell nucleus. LHON, however, is the first disease to be associated with mutations of the small amounts of DNA that reside inside the mitochondria. This DNA encodes for subunits of complex 1 of the respiratory chain, the key biochemical cascade that manufactures the cell's supply of the high-energy molecule adenosine triphosphate. The three most common mutations causing LHON have now been identified, providing a useful diagnostic test for LHON and new insight into the pathogenesis of the disease.

Hereditary Hyperferritinemia Cataract Syndrome (HHCS)

HHCS is a genetic disorder in which L-ferritin is overexpressed, resulting in the formation of cataracts. HHCS was initially identified in a single family, but has since been found in other families. Through the use of expressed sequence tag (EST) analysis, gene arrays, and other state-of-the-art molecular biology techniques, NEI intramural scientists have sought to gain a greater understanding of this very rare disorder. Because L-ferritin was found to exist in such large amounts in affected HHCS lenses, these scientists have hypothesized that L-ferritin may destabilize other lens proteins, causing formation of cataracts; or that oxidative damage to the lens is the result of a disruption in the normal control of iron levels in the lens, causing the formation of cataract. NEI extramural researchers are studying potential preventive and therapeutic strategies by further studying the pathobiology of HHCS cataractogenesis. The efforts of these research activities may result not only in preventive strategies for individuals at risk for HHCS, but also in a greater understanding of the pathogenesis of cataracts in general. Ferritin is a major iron storage protein in cells, and it is a critical regulator of oxidative stress. Oxidative stress has been implicated as a major factor in the development of all cataracts. NEI-supported research on HHCS not only will benefit those individuals who are afflicted with this rare blinding disorder, but also may provide increased understanding and ultimately prevention of cataracts in general. Cataract remains the leading cause of blindness worldwide.

Sjögren's Syndrome (Dry Eye)

The hypothesis that lacrimal gland secretory cells actively provoke Sjögren's syndrome autoimmune responses has gained support on the basis of analyses of the intracellular traffic of histocompatibility molecules and autoantigens. The hypothesis is gaining further support due to new experiments based on autologous mixed cell reactions that may re-create autoimmune responses under defined cell culture conditions. A new autoantigen (the cytoskeletal component α -fodrin) implicated in Sjögren's syndrome autoimmunity has been identified. The autoantigen appears to have considerable specificity, since antibodies to it were found in the serum of 95% of patients with Sjögren's syndrome. No antibodies were found in normal individuals or in patients with other autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. Thus, α -fodrin may have considerable diagnostic potential. Moreover, neonatal vaccination with α -fodrin prevented development of the disease in mice, opening the possibility of new therapeutic approaches for Sjögren's syndrome.

Corneal Dystrophies

Corneal dystrophies are a heterogeneous group of conditions that involve abnormal corneal development and result in defects in structure or clarity. Though relatively rare in the United States, the most common corneal dystrophy is keratoconus, which is characterized by a progressive thinning process of the cornea

that may be accompanied by scarring. Keratoconus leads to progressive nearsightedness, astigmatism, and a cone-shaped cornea. Clinical care for keratoconus is time-consuming for patients and physicians because of its chronic progression and the difficulty of achieving a stable contact lens fit for visual rehabilitation.

Linkage analysis has shown that four clinical types of corneal dystrophy result from mutations in a single gene. Granular, Reis Bucklers', lattice type 1, and Avellino corneal dystrophies all map to the *βig-h3* gene, which encodes the keratoepithelin adhesion protein. It appears that in these four corneal dystrophies, the mutated keratoepithelin forms amyloidogenic intermediates that precipitate in the cornea, causing a progressive opacification. Three other corneal diseases also involve amyloid-like deposits: polymorphic amyloid degeneration, lattice corneal dystrophy type IIIA, and gelatinous drop-like dystrophy. Keratoepithelin is a good candidate gene for further investigation in these families. Because of the accessibility of the cornea, these disorders represent excellent model systems for study of the molecular details of amyloid depositions in devastating diseases such as Alzheimer's disease.

Motor Neural-Ophthalmic Disorders

Blepharospasm, a motor neuro-ophthalmic disorder, is characterized by a forcible involuntary closure of the eyelids that may last as long as several minutes and usually occurs in people at midlife. While the etiology of this condition is not well-understood, researchers believe that in some people the condition may be a form of Parkinson's disease. NEI-supported researchers have recently reported the development of a rat model to study blepharospasm. The researchers depleted a small amount of striatal dopamine and slightly weakened the eyelid-closure muscle (orbicularis oculi) in these animals. The results of this research showed that by themselves, neither of these procedures produced the characteristic uncontrollable blinking spasm of blepharospasm, but when the two procedures were combined they produced forceful blinking and spastic lid closure.

NEI-supported scientists have reported that injecting the anticancer drug doxorubicin (DXN) directly into the eyelids may offer permanent relief from blepharospasm. DXN acts by reducing the number of muscle fibers in the orbicularis oculi muscle by as much as 70%, offering alleviation from the spasms that are characteristic of this disease. To fully alleviate the spasms, however, patients must often undergo a series of painful DXN injections. NEI-supported researchers are trying to find a way of increasing the myotoxic effects of the DXN injection so that patients can have permanent relief of spasms with a minimum of treatments. Researchers injected bupivacaine and hyaluronidase 18 hours apart into the eyelids of rabbits. Two days later, the researchers injected the injured eyelids with DXN. The results of these experiments showed that the myotoxic effects of DXN were increased when the drug was injected 2 days after the animals' eyelids were injured with bupivacaine. The researchers also observed that the skin irritation that normally occurs as a result of DXN injection was no worse in the dual drug regimen than when DXN is administered alone. In May 2001, NEI-funded researchers reported the results of experiments in which Doxil, a liposome-encapsulated form of DXN, was injected into monkey eyes that had been preinjured with the bupivacaine/hyaluronidase protocol. The researchers showed that Doxil administered after preinjury of the eye with bupivacaine/hyaluronidase is an effective and safe treatment to remove orbicularis muscle fibers from monkey eyelid and thus offer permanent relief from blepharospasm. Additionally, these scientists also reported that this treatment regimen did not produce any bruising, ulceration, or other skin injuries at the injection site and may be a much less painful therapy for blepharospasm in human patients.

Rare Diseases Research Initiatives

NEI will continue to fund high-quality, investigator-initiated research on the prevention, etiology, pathology, and clinical intervention of rare diseases that cause visual impairment and disability.

Rare Diseases Program Activities

The National Advisory Eye Council and NEI have established the following goals for rare diseases research in *Vision Research: A National Plan 1999-2003*:

- Identify novel causes of inherited retinal degenerations: further examine the cellular and molecular mechanisms whereby identified gene defects cause retinal degenerations.
- Further develop and critically evaluate therapies involving gene delivery, growth factors, and transplantation for the treatment of retinal disease.
- Improve the understanding of ocular surface physiology.

National Institute of General Medical Sciences (NIGMS)

Overview of NIGMS Rare Diseases Research Activities

NIGMS supports broad-based fundamental research that is not targeted to any specific organ system or disease. Examples of this research include:

- Studies on the structure and function of organelles and membranes at the cellular and molecular levels.
- Investigations into the organization and function of the genome in organisms ranging from bacteria to humans.
- Development of new and improved instrumentation and technology for application to biological problems.
- Studies on basic bio-related organic chemistry for the elucidation of biosynthetic pathways and the development of new synthetic strategies for molecules of biological interest.
- Investigations of basic pharmacological mechanisms at levels ranging from the receptor to the molecular.

In general, support of investigations related to specific diseases, unless of wide applicability across disease or organ system lines, is not the responsibility of NIGMS, but rather would be assigned to one of the categorical NIH Institutes.

Human Genetic Cell Repository

The NIGMS Human Genetic Cell Repository provides a valuable resource for investigators studying genetic disorders. Located at the Coriell Institute for Medical Research in Camden, New Jersey, the Repository collects, characterizes, maintains, and distributes cell lines from patients and families with a wide variety of genetic disorders, as well as from normal persons whose tissues serve as controls. More than 6,600 cell lines, representing more than 500 different diseases, are available to qualified investigators. The Repository stimulates research on rare diseases by providing access to cell lines and DNA samples derived from these cell lines that are not readily available. Among the cell lines requested most frequently in the last year are those from patients with rare diseases such as ataxia telangiectasia, xeroderma pigmentosum (XP), cystic fibrosis (CF), Bloom syndrome, fragile X-linked mental retardation, Nijmegen breakage syndrome (NBS), Cockayne syndrome, and glycogen storage disease.

Recent acquisitions for the collection include samples from patients with the following rare disorders: neuronal ceroid lipofuscinosis, Rett syndrome, Friedreich's ataxia (FRDA), fascioscapulohumeral dystrophy, glutaric acidemia, atransferrinemia, factor X deficiency, immuno-osseous dysplasia, and Letterer-Siwe disease. These cell lines, as well as those previously acquired, are used for biochemical, cellular, and molecular studies to help elucidate the causes of genetic defects.

The Repository has a growing collection of cell lines in which the mutation has been characterized at the molecular level. These include samples with characterized trinucleotide expansions from patients with Huntington's disease, dentatorubral-pallidoluysian atrophy, myotonic dystrophy, and fragile X, and characterized mutations in Bloom syndrome, hemochromatosis, CF, adenomatous polyposis of the colon, and myotonic dystrophy.

In addition, the Repository supplies DNA isolated from two complete panels of well-characterized human-rodent somatic cell hybrids and from chromosome-specific somatic cell hybrid panels for nearly every human chromosome. The hybrids are a valuable resource to investigators interested in mapping the location of disease-related genes, frequently the first step in characterizing the etiology of the disease.

In cooperation with the National Human Genome Research Institute (NHGRI), the Repository also houses the 450 cell lines (and DNA derived from them) that constitute the DNA Polymorphism Discovery Resource. These samples, which represent the genetic diversity of humans, will help researchers identify genes that are involved in the etiology of complex genetic diseases, such as many cardiovascular disorders and cancers.

Recent Scientific Advances in Rare Diseases Research

Tracking RNAs in Osteogenesis Imperfecta (OI)

OI type 1 is an inherited bone disease, which results from mutations in the collagen type 1 gene (*COL1A1*). OI type 1 can be a relatively mild disorder characterized by recurrent bone fractures. The severity of the disease, however, appears to correlate with the ability of defective *COL1A1* RNA to leave the nucleus. In cases in which mutant RNAs are able to leave the nucleus and enter the cytoplasm of the cell, a more severe disease or death can result. An NIGMS-supported researcher has characterized the processing of normal and mutant *COL1A1* RNAs in the nucleus and has defined a novel RNA transport step, which is impeded for mutated *COL1A1* RNAs in a milder form of OI. This work offers new insight into the relationship of nuclear structure and RNA metabolism and advances our understanding of a process that is critical to the clinical outcome of OI patients.

The Drosophila Tuberous Sclerosis Complex Gene Restricts Cell Growth and Cell Proliferation

In the human genetic disease tuberous sclerosis, tumorous masses of cells known as hamartomas grow in many of the body's tissues, including the brain, heart, kidneys, and skin. Mental retardation and seizure disorders also occur in patients with this condition. About one-third of the cases are familial and are associated with mutations at either the *TSC1* or *TSC2* gene locus. The mechanisms by which the proteins encoded by these genes (hamaritin and tuberin) function are not known.

Several NIGMS-supported investigators have now shown that normally these two proteins act together to limit cell size and to prevent differentiated cells from undergoing cycles of cell division and proliferation when it is appropriate. They have characterized the Drosophila homologues of *TSC1* and *TSC2* and have shown that cells with disabling mutations in both of these genes grow at about the same rate as normal cells but have longer cell-doubling times and thus reach a larger size. Cells that express extra copies of normal versions of these two genes are smaller than normal. Cells with the mutated genes also do not respond to the usual signals that prevent them from initiating new cycles of cell division, causing more of the larger cells to accumulate. This latter observation correlates with the elevated levels of Cyclin A and Cyclin E found in these cells. In humans, the lack of the normal restraints on cell growth result in hamartomas containing huge masses of cells that arise in organs such as heart and brain where the number of fully differentiated cells need to be tightly regulated. Understanding how *TSC1* and *TSC2* function may provide guidance in the design of a specific therapeutic strategy to halt the inappropriate cell growth seen in this condition.

Identification of a Gene Duplication Associated with Trisomy 2p Syndrome

People who have three copies of part of chromosome 2 have a constellation of serious developmental defects, including facial, skeletal, and genital abnormalities and growth retardation. These defects are collectively referred to as trisomy 2p syndrome. An NIGMS-supported investigator recently identified a gene called prolactin regulatory element binding (*PREB*), which appears to be responsible for most or all of the developmental defects that are characteristic of trisomy 2p syndrome. The *PREB* gene maps to a small segment of chromosome 2, designated as 2p23. Most people who have trisomy 2p syndrome have three copies of the 2p23 segment. The investigator who examined cells from four individuals with partial trisomy 2p syndrome saw three copies of the *PREB* gene in the cells of each individual. Previously, this researcher had shown that the protein encoded by the *PREB* gene is a transcription factor that regulates expression of many genes. He observed that in mice, the *PREB* protein is synthesized in fetal tissues that will differentiate into the head, skeleton, genitals, and pituitary gland. Assuming that the *PREB* protein is synthesized in the same tissues in human fetuses, these observations suggest that people with three copies of the 2p23 chromosomal segment have the developmental defects characteristic of trisomy 2p syndrome because they have an extra copy of the *PREB* gene and thus synthesize higher-than-normal levels of the *PREB* protein. The consequence of synthesizing higher-than-normal levels of the *PREB* protein in the fetus, according to the investigator's theory, is abnormal transcription of genes whose products are critical for head, skeletal, genital, and pituitary development. If the theory is correct, then trisomy 2p syndrome is the first chromosomal abnormality for which all of the associated developmental defects are caused by an extra copy of a single gene.

Xeroderma Pigmentosum (XP) and Cancer: Not Just a Problem with DNA Repair

XP is a rare genetic disease that is associated with a variety of symptoms, the most serious of which is a 1,000-fold increase in the incidence of melanomas and other skin cancers. People with XP have mutations that affect one of two proteins, *XPB* or *XPD*, both of which are involved in DNA repair. Regardless of which protein is affected, individuals with XP are severely deficient in their ability to repair damaged DNA. Because accumulation of mutations has long been associated with carcinogenesis, it is generally thought that the increased incidence of skin cancer in people with XP is caused by their inability to repair DNA damage caused by sun exposure. People with trichothiodystrophy (TTD) or Cockayne syndrome (CS) who are unable to repair DNA damage have symptoms that are strikingly similar to those of people with XP, except that people with TTD or CS are not unusually susceptible to skin cancer. These observations suggest that in people with XP, the inability to repair sun-damaged DNA is not solely responsible for their increased incidence of skin cancer.

Two NIGMS-supported investigators have shown that in cells from unaffected people, *XPB* and *XPD* interact with other proteins that regulate transcription of the *c-myc* gene. In contrast, in cells from people with XP, *XPB* and *XPD* do not interact with those proteins, so *c-myc* is not regulated correctly. Because overexpression of *c-myc* is associated with tumorigenesis, these investigators suggest that the XP mutations' effect on transcription of *c-myc* and perhaps other genes involved in tumorigenesis is responsible for the increased incidence of skin cancer in people with XP. These observations provide insight into the genesis of skin cancer in the vast majority of people who do not have XP, and they suggest that the proteins with which *XPB* and *XPD* interact could be targets for therapeutic intervention to reduce the incidence of skin cancer in XP patients.

Structural Analysis of the Plant Homeodomain (PHD) Protein Domain

More than 400 proteins in eukaryotes have something in common: a sequence of approximately 60 amino acids that is very similar in every protein. This sequence is called the PHD domain. Mutations that affect PHD domains are associated with several diseases, including ATR-X syndrome, autoimmune polyglandular syndrome type 1, squamous cell carcinomas, myeloid leukemias, and Williams syndrome (WMS). Each of these diseases is associated with a defect in a single protein. The only thing that all of the proteins have in common is their PHD domains. These observations suggest that PHD domains play an essential role in maintaining normal function in human cells. To gain insight into how the PHD domain functions, an NIGMS-funded investigator has determined the three-dimensional structure of the PHD domain in a protein called KAP-1, which associates with other proteins to repress transcription of many genes. The investigator has shown that the PHD domain coils up to form a looped structure, which has two zinc-binding sites. Mutations that disrupt one of the zinc-binding sites or create a third site change the structure of the *KAP-1* protein and affect its ability to repress transcription. The investigator then determined the structure of mutant *ING1* and *ATR-X* proteins, which are associated with carcinomas and with alpha thalassemia mental retardation X-linked (ATR-X) syndrome, respectively. In every mutant protein, the structure of the PHD domain was abnormal: either one of the normal zinc-binding sites was disrupted or an extra zinc-binding site had been created. These observations suggest that the disparate human diseases that are associated with mutations in PHD domains may be caused by the mutations' effects on zinc binding, which is critical for the proteins to function normally.

National Heart, Lung, and Blood Institute (NHLBI)

Overview of NHLBI Rare Diseases Research Activities

NHLBI provides leadership for a national program in the causes, diagnosis, treatment, and prevention of diseases of the heart, blood vessels, lungs, and blood, and sleep disorders; and in the uses of blood and the management of blood resources. Through research in its own laboratories and extramural research grants and contracts, NHLBI conducts and supports an integrated and coordinated program that includes basic investigations, clinical trials, epidemiological studies, and demonstration and education projects.

While the major part of the research supported by NHLBI addresses common conditions such as hypertension, coronary heart disease, and chronic obstructive pulmonary disease, a significant amount of research is devoted to rare diseases in children and adults. NHLBI activities related to rare disease research in FY 2000 are described below.

Recent Scientific Advances in Rare Diseases Research

Heart and Vascular Diseases Program

Abetalipoproteinemia

Abetalipoproteinemia is a rare congenital disorder that prevents the body from producing low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and chylomicrons—lipoprotein complexes that carry dietary lipids (including cholesterol) from the lymph through the bloodstream. Individuals with abetalipoproteinemia are unable to digest fats properly. Other clinical findings include ataxia (lack of coordination), peripheral neuropathy, and several forms of nerve dysfunction. Genetic, biochemical, and metabolic approaches to studying this disease are supported by NHLBI at four institutions. In FY 2000, studies were directed at understanding abnormal synthesis of apolipoprotein B (*apoB*), the major protein in LDL, due to the absence of microsomal triglyceride transfer protein (MTP). Investigators have found that the amount of MTP may determine *apoB* levels. The region of *apoB* necessary for binding to MTP has been examined and a potential site identified. Inhibiting MTP-binding to *apoB* is associated with a marked decrease in the amount of *apoB* secreted from cells.

Antiphospholipid Syndrome (APS)

Patients with APS have circulating autoantibodies to certain phospholipids (lipids containing phosphorus), chiefly cardiolipin, as well as the lupus anticoagulant. APS manifests itself clinically by recurrent venous and arterial thrombosis, fetal deaths, and autoimmune thrombocytopenia. Many patients with APS have systemic lupus erythematosus (SLE), although patients may have APS in the absence of SLE. Further complicating the clinical picture is an increased incidence of atherosclerosis.

Functional genomics has brought new focus to the study of APS. Research that will determine whether genetic factors predispose individuals to developing this unusual class of antibodies is under way. It is known that allelic variability in the genes for certain lipid carrier proteins also results in the generation of APS. NHLBI is supporting a study on the molecular genetics of one of these proteins, apolipoprotein H, in SLE patients to further our understanding of the link between SLE and the production of antiphospholipid antibodies. Several NHLBI grantees are engaged in efforts to develop more standardized immunoassays that will reliably detect individual antiphospholipids.

Recent findings provide strong support for the involvement of antiphospholipid antibodies in atherogenesis. Autoreactive antibodies were found to form against phospholipid components of dead or dying (apoptotic) cells and were found to cross-react with normal vascular constituents that are produced in response to environmental stimuli, including bacteria. In other studies, small differences in a common lipid carrier protein appeared to be genetically linked to APS as well as to atherosclerosis.

Arrhythmogenic Right Ventricular Dysplasia (ARVD)

ARVD is a family of rare cardiomyopathies that result in sudden cardiac death and malignant heart rhythm disturbances, including fibrillation. Most forms are believed to be due to the inheritance of autosomal dominant mutations in genes with largely unknown identities, but which clearly affect myocardial integrity. ARVD is characterized by marked, selective, right ventricular dilatation, myocardial cell death, and cell replacement with fat cells and fibrous tissue. Expression in gene carriers is variable, but in those who display symptoms the outcome is frequently lethal. NHLBI is supporting work on ARVD at one of its Specialized Centers of Research (SCORs) in Sudden Cardiac Death and is providing guidance to another group on an amended application to study ARVD. The SCOR investigators have reported on the localization of a mutation to chromosome 10p12-14 in one family with a common congenital form of ARVD. Although the identity of the gene affected is not yet known, this is the first step in determining the molecular basis of this form of the disease.

Bartter Syndrome

Bartter syndrome, a rare autosomal recessive disease, typically manifests itself through salt imbalance and low blood pressure. Previous studies have shown that a single-gene mutation in the Na-K-Cl co-transporter is responsible for this disorder. One of the NHLBI SCOR programs in Molecular Genetics of Hypertension has found that the disease is genetically heterogeneous. In addition to the mutation on the Na-K-Cl co-transporter, mutations on potassium and chloride channels have been discovered, and there are indications that additional Bartter's genes remain to be found. The defect in salt homeostasis also has an impact on calcium handling, suggesting a strong linkage between salt and calcium homeostasis. The hypotensive state of Bartter syndrome suggests that these mutated genes protect against the development of high blood pressure.

β -Sitosterolemia

β -sitosterolemia is a rare genetic disease characterized by increased absorption of dietary cholesterol and plant and shellfish sterols. Patients with β -sitosterolemia have a markedly increased risk of premature cardiovascular disease. An effective treatment for β -sitosterolemia is not available, but ezetimibe, a cholesterol absorption inhibitor, is being evaluated as a new therapeutic. NHLBI's intramural Molecular Disease Branch is seeking to elucidate the genetic defect in β -sitosterolemia and to develop a new approach to the treatment of the dyslipoproteinemia present in these patients. In FY 2000 the Branch established the genetic defect in β -sitosterolemia as a defect in either the ABCG5 or ABCG8 transporters present in the enterocyte and liver.

Brugada Syndrome

Brugada syndrome is a rare inherited disorder characterized by cardiac electrophysiological abnormalities, specifically right bundle branch block and ST elevation in the precordial leads, and is associated with a high occurrence of sudden cardiac death. Brugada syndrome is currently believed to be similar in cause and potential treatment to some forms of long QT syndrome (LQTS). Both disorders appear to be caused by mutations at different locations in the *SCN5A* cardiac muscle sodium ion channel gene and resulting aberrations in depolarization of these cells. One group of NHLBI-supported investigators is studying the relationship between Brugada syndrome and another set of inherited arrhythmias known to be a major cause of sleeping deaths in young Asian and Indonesian men. A second group is studying new mutations of Brugada syndrome and potential modifiers of their variable expression in different family members. This group has identified a second site on chromosome 3 associated with Brugada syndrome and has extensively characterized cardiac electrical abnormalities in families with this mutation.

Congenital Heart Disease

Congenital heart disease affects about 8 in 1,000 live-born infants, or about 32,000 per year in the United States, making it the most common birth defect. Abnormal formation of the embryonic heart results in both structural and functional heart defects. Congenital heart disease is an important cause of infant mortality, pediatric and adult morbidity, and shortened adult life expectancy. About one-third of affected infants and children require open heart surgery or interventional cardiac catheterization to repair or improve their heart defects. Approximately the same proportion have extracardiac anomalies associated with the congenital heart defect, including chromosomal abnormalities and syndromes involving other organ systems.

NHLBI has supported research in pediatric cardiovascular medicine since the Institute first funded heart research grants in 1949. Since that time, NHLBI-supported researchers have been instrumental in developing diagnostic imaging techniques, including fetal imaging; surgical techniques, including various operations and refinements in cardiopulmonary bypass; and medical therapies now used to ensure healthy survival for most affected children. NHLBI-supported researchers have made significant contributions to the epidemiology of congenital heart disease and to understanding the molecular and genetic basis of normal and abnormal heart development.

This year, two NHLBI-supported researchers reported discovery of a genetic abnormality associated with congenital heart defects in mice. Although the defect has not yet been found in humans, understanding abnormal heart development in animal models will help to clarify the process in humans. Other researchers have been able to develop high-resolution ultrasound imaging for the characterization of hemodynamic function in the developing mouse embryo. Researchers funded through the SCOR Program in Pediatric Cardiovascular Disease have shown that mutations in a single human gene, *Nkx2.5*, are responsible for a variety of structural congenital cardiovascular malformations, some of which are also associated with arrhythmias.

DiGeorge Syndrome

DiGeorge syndrome occurs with an estimated frequency of 1 in 4,000 live births. It is characterized by many abnormalities, including cardiac outflow tract anomalies, hypoplasia of the thymus and parathyroid glands, and cleft palate and facial dysmorphogenesis. It is usually caused by a heterozygous deletion of chromosomal region 22q11.2 (*del22q11*). NHLBI supports both human and animal studies of DiGeorge

research through several grants, including two SCORs in Pediatric Cardiovascular Disease. Recent studies in mice implicate the transcription factor *Tbx1* as a key candidate gene for the cardiac outflow tract defects seen in DiGeorge syndrome. *Tbx1* is shown to be required for normal development of the pharyngeal arch arteries, parathyroid glands, and the conotruncal (outflow) region of the heart in mice. Mice missing one copy of this gene had problems analogous to those experienced in affected humans, and mice missing both copies had even more severe malformations.

Doxorubicin (DXN) Cardiomyopathy

DXN (brand name: Adriamycin) is a potent, broad-spectrum antitumor agent effective in treating a variety of cancers, including solid tumors and leukemia. Unfortunately, its clinical use is limited by dose-dependent cardiac side-effects that lead to degenerative cardiomyopathy, congestive heart failure, and death. In addition, patients treated with the drug many years ago when they were children are developing dilated cardiomyopathy. Endocardial biopsies from patients undergoing DXN therapy reveal a disruption of myofibrils, impairment of microtubule assembly, and a swelling of the endoplasmic reticulum. DXN cardiotoxicity is also characterized by a dose-dependent decline in mitochondrial oxidative phosphorylation and a decrease in high-energy phosphate pools.

Several NHLBI-supported investigators have reported research advances in the past year. One investigator has focused on molecular genetic mechanisms involved in DXN cardiotoxicity in an in vivo rat model. The studies have demonstrated that DXN selectively deregulates the expression of cardiac-specific or cardiac-restricted genes by depleting the levels of tissue-specific transcription factors and co-factors, resulting in disruption of normal cardiomyocyte function. Another investigator has found that mitochondrial changes seem to contribute to the progressive inability of cardiac tissue to tolerate metabolic stress, particularly when associated with induction of the membrane permeability transition pore by DXN. That study is testing the hypothesis that DXN interacts with mitochondrial membranes to initiate a series of reactions that lead to increased rates of free radical generation. A third investigator is examining explicit pathways through which reactive oxygen species are involved in DXN-induced cardiomyopathy.

Dysbetalipoproteinemia

Dysbetalipoproteinemia is a rare disorder with a strong heritable component characterized by the presence of beta-migrating VLDL. Dysbetalipoproteinemia leads to formation of characteristic yellow skin plaque (xanthomas) and predisposes to early ischemic heart disease and peripheral vascular disease. Research into the genetics and the biochemical events underlying the etiology and pathophysiology of the disease is taking place under two investigator-initiated NHLBI grants. A mutant form of the protein apoprotein E (*apoE2*) has been identified as the primary molecular defect. Animal models are being developed to facilitate basic research on the disease. In FY 2000 these investigators used cell studies to demonstrate that apoE2 differs metabolically from other forms (*apoE3* and *apoE4*) in that its half-life (an indicator of the time the protein spends in the cell) is significantly higher.

Familial Homozygous Hypercholesterolemia (FHH)

FHH is a genetic defect in the LDL receptor that results in very high plasma levels of cholesterol and a marked increase in the risk of early heart disease. No effective treatment has been developed to treat these patients and definitively correct the molecular defect involved. NHLBI's intramural Molecular Disease Branch has a program directed toward the development of improved techniques for diagnosis, evaluation, and treatment of patients with FHH. New approaches are being studied to determine the

extent of atherosclerosis in these patients. Both invasive and non-invasive techniques are being evaluated to establish which approach will be more effective in the selection of treatment programs to reduce the severe atherosclerosis characteristic of this disease.

Familial Hypertrophic Cardiomyopathy (FHC)

FHC is associated with myofibrillar disarray in the heart muscle, which leads to hypertrophy, or enlargement of the heart. Although patients can remain asymptomatic for some time, eventually shortness of breath, palpitations, and heart failure emerge, and sudden death ensues. Some patients die during childhood, whereas others survive for six or seven decades. FHC is associated with mutations in more than one protein, suggesting that the condition represents a heterogeneous group of disorders. During the past decade, scientists made significant progress in uncovering the genes associated with FHC. It is known, for instance, that FHC can be caused by various mutations in the contractile proteins that make up the heart wall. Understanding remains elusive, however, of who will die suddenly or whether certain factors such as high blood pressure or extreme stress will trigger sudden death. NHLBI supports research on the genetic basis and mechanisms involved through several investigator-initiated grants and in two SCORs in Heart Failure.

Using a genetically engineered animal model for FHC, one NHLBI-supported investigator has shown decreased hypertrophy and a 36% reduction in fibrous tissue by using Losartan, an angiotensin II blocker. This is the first demonstration of a therapeutic effect on collagen and fibrous tissue. Until now, FHC in humans presenting without obstruction has been treated by using beta-blockers or calcium channel blockers, which serve only to ameliorate symptoms. By contrast, treating acquired heart failure with ACE inhibitors or angiotensin II receptor blockers has now been shown to prevent and/or reverse cardiac hypertrophy. These successful results in animal models lay the groundwork for clinical trials to evaluate such therapy for FHC. The same investigator has successfully used Doppler myocardial tissue imaging on the transgenic rabbit model of human hypertrophic cardiomyopathy (HCM) to detect those afflicted with FHC before hypertrophy develops. Studies are now under way in human families with FHC. Another investigator has produced two genetically engineered mouse models of FHC. Each model has a single-site mutation comparable to mutations observed in humans with FHC. The models will prove valuable in attempts to unravel the mechanism by which sarcomere protein gene mutations cause HCM.

Familial Hypobetalipoproteinemia (FHBL)

FHBL is an apparently autosomal dominant disorder of lipid metabolism characterized by very low levels of apoprotein B-containing lipoprotein cholesterol. NHLBI is supporting an investigator-initiated grant examining the genetic, biochemical, and metabolic aspects of this disease. FHBL is related to alterations in the *apoB* gene on chromosome 2p23-24. Specific mutations associated with FHBL have been identified and characterized. In hypobetalipoproteinemic patients with no detectable plasma signs of this defect, however, fewer than 5% have this particular *apoB* gene mutation. A genome-wide search has located other genes that may be responsible for FHBL. A newly identified genetic susceptibility for FHBL has been identified on chromosome 3 (p21.1-22), which has been narrowed down to an area containing three potential candidate genes.

Infectious Myocarditis

Infectious myocarditis, which affects both children and adults, is an inflammation of the heart muscle that sometimes leads to progressive heart failure and the need for heart transplantation. Post-mortem studies suggest that approximately 20% of the cases of this insidious, usually asymptomatic, disease result in sudden unexpected death in adults younger than age 40. Although many infectious agents have been linked to myocarditis, among the most common causes in the United States are Coxsackievirus and human immunodeficiency virus (HIV). Worldwide, the most common myocarditis is Chagas' disease, caused by the parasitic protozoan *Trypanosoma cruzi*. Animal studies have shown a causal link between viral myocarditis and the development of dilated cardiomyopathy.

NHLBI supports basic studies on the cellular and molecular pathogenesis of infectious myocarditis. The studies include defining the role of the Coxsackievirus receptor in viral pathogenesis, studying if the innate immune response to Coxsackievirus influences whether the adaptive immune response leads to later development of autoimmune myocarditis, determining whether antimyosin antibodies and T cells in the disease are cross-reactive with viral or bacterial antigens, and examining the role of HIV and cocaine in infectious myocarditis.

Using a primate model infected with simian immunodeficiency virus (SIV), a virus similar to HIV, an NHLBI-supported investigator has made a critical discovery regarding the pathogenesis of acquired immune deficiency syndrome- (AIDS-) related cardiac dysfunction; namely that cardiac myocytes are not the target for SIV. Instead, the virus may be infecting cardiac dendritic cells or monocytes, both of which bear the CD4 receptor required for viral infection. Studying the effects of a soluble viral regulatory factor called Tat, another investigator has found that Tat-mediated changes may create a pro-inflammatory stimulus, thereby increasing the morbidity of HIV infections.

Liddle Syndrome

Liddle syndrome is a rare autosomal dominant disorder of severe hypertension characterized by increased renal reabsorption of sodium resulting in hyperaldosteronism, or overproduction of the hormone aldosterone from the outer portion (cortex) of the adrenal gland. Excess aldosterone results in low potassium levels (hypokalemia), under-acidity of the body (alkalosis), muscle weakness, excess thirst (polydipsia), excess urination (polyuria), and hypertension. Previous studies showed that a mutation in the gene encoding the beta-subunit of the epithelial sodium channel is responsible for this disorder. A diagnostic test for Liddle syndrome has been developed by one of the NHLBI SCOR programs on Molecular Genetics of Hypertension. In order to study the mechanism responsible for Liddle syndrome, the same SCOR has developed a mouse model that develops high blood pressure, metabolic alkalosis, and hypokalemia accompanied by cardiac and renal hypertrophy, very similar to a human form of salt-sensitive hypertension. This mouse model exhibits both sodium channel and renin locus dependency for blood pressure control, as recently described in the normal healthy human. This represents the first potential digenic (reproduced in alternate generations) model for hypertension.

LQTS

LQTS is characterized clinically by a prolonged QT segment on the cardiac electrocardiograph that is associated with syncope, ventricular arrhythmias, and frequently sudden cardiac death. This family of conditions is thought to be caused by alterations in the cardiac cell action potential induced by mutations in at least six cardiac ion channel genes. There are two principal forms of LQTS. The first occurs when cardiac symptoms spontaneously occur, as in the congenital autosomal dominant Romano-Ward

syndrome and the autosomal recessive Jervell-Lange-Nielson syndrome, where affected individuals frequently have the same cardiac symptoms and congenital deafness. The second form is “acquired LQTS,” when individuals with normally silent mutations in some of these same cardiac ion channel genes are exposed to drugs that interact with one or more of the same ion channels involved in causing the unconditional congenital forms. In acquired LQTS following drug exposure, the symptoms and outcome are much the same as they are in the purely congenital, spontaneous form.

NHLBI currently supports work through 1 SCOR on Sudden Cardiac Death grant and through at least 10 R01 grants that address the various molecular, clinical, and genetic bases of LQTS. One of these grants maintains an international registry of clinical and genetic data on 2,235 affected individuals in 936 families (104 of which were added this year). Researchers continue gene investigations worldwide to determine the numbers of affected individuals within the registry and to identify known and new mutations in registry members. LQTS researchers are also evaluating triggering factors for the malignant ventricular arrhythmias and are comparing these by genotype to ascertain if individuals with LQT1 have a different disease course than those with LQT2, LQT3, LQT4, or LQT5. In addition, another grant supports a small clinical trial of gene-directed pharmacotherapy for the LQT3 variant of the disease.

NHLBI investigators recently summarized the functional and clinical consequences of the various ion channel deficiencies that have been discovered. Their report includes suggestions that mutations in calcium, sodium, and potassium channels may all cause similar cardiac electrical abnormalities, and that sodium channel inhibitors may be useful in treating patients with one form of the disease. Studies of clinical symptoms and their respective mutations are producing data that may be useful in identifying and treating patients with different forms of the disease. Pharmacogenetic studies on mutations involved in acquired LQTS have also provided information important in identifying and removing from the U.S. drug market a number of prescription and over-the-counter drugs that increase susceptibility to sudden death by these same mechanisms.

Niemann-Pick Type C (NP-C) Disease

NP-C disease is an autosomal recessive lipid storage disorder usually characterized by enlargement of the liver and spleen (hepatosplenomegaly) and severe progressive neurological dysfunction. Biochemical analyses of NP-C cells suggest an impairment in the intracellular transport of cholesterol to post-lysosomal destinations. The gene deficiency in Niemann-Pick disease types A and B has been identified as the acid sphingomyelinase. The gene deficiency in Niemann-Pick disease types C and D has been identified as the NP-C-1 protein, but few clues regarding its potential function(s) have been derived from its predicted amino acid sequence. The accumulation of cholesterol in NP-C results from an imbalance in the flow of cholesterol among membrane compartments. A putative cholesterol sensor in the plasma membrane that affects cholesterol trafficking into and out of cells was further characterized this year. The investigators are testing whether the cholesterol pool inside the cells is regulated by the plasma membrane sensor and whether the Golgi apparatus serves as an intermediary in cholesterol transport.

Smith-Lemli-Opitz (SLO) Syndrome

SLO syndrome is an inherited disorder caused by a defect in the enzyme involved in cholesterol biosynthesis. As a result, cholesterol synthesis is inadequate to meet biological demands for building cell membranes and bile acids. Newborns with SLO have a distinctive facial dysmorphism; suffer from numerous congenital anomalies, including cleft palate, congenital heart disease, genitourinary abnormalities, and malformed limbs; and have severe developmental delays, digestive difficulties, and

behavioral problems. SLO syndrome is thought to account for many previously unexplained cases of mental retardation. Current NHLBI-supported research on SLO focuses on identification of relevant mutations, generation of animal models, development of sensitive and specific assays for screening newborns and verifying diagnoses in older individuals, clarification of aberrant biochemical pathways, and amelioration of behavioral and digestive problems through dietary and pharmacologic treatment.

Some clinicians have posited that it may be necessary to add bile acids to the baby formula of SLO infants, but recent sterol balance findings in an NHLBI-supported study indicate that this is not a useful tack because bile acid synthesis occurs at normal levels. In another study, NHLBI-supported investigators improved the commonly used diagnostic and screening tests for SLO by improving the separation of accumulated unsynthesized cholesterol, thus achieving a more accurate determination of its concentrations in blood and other biological fluids such as amniotic fluid.

Tangier Disease

Tangier disease is a rare syndrome characterized by a deficiency of high-density lipoprotein (HDL), mild hypertriglyceridemia, neurologic abnormalities, and massive cholesterol ester deposits in various tissues such as the tonsils. Tangier disease is inherited as an autosomal co-dominant trait and appears to be due to excessive breakdown of HDL. Patients with Tangier disease have a defect in intracellular lipid trafficking that prevents removal of cholesterol from cells. The identification of a defective gene on chromosome 9 as causing Tangier disease has led to the concept of its product, the protein *ABCA1*, as the gatekeeper for eliminating excess cholesterol from tissues and therefore a key determinant of the amount of cholesterol accumulating in the artery wall. NHLBI's intramural Molecular Disease Branch is systematically evaluating patients with low HDL to establish the frequency of genetic mutations in the *ABCA1* transporter. Efforts by an NHLBI-supported investigator to understand the role of the protein in intracellular cholesterol trafficking recently led to the finding that the cellular location of *ABCA1* was in areas distinct from the lipid-rich plasma membrane domains called rafts. Furthermore, the cholesterol transported out of the cell by *ABCA1* does not appear to be from these lipid-rich rafts.

Williams Syndrome (WMS)

WMS is a rare genetic disorder characterized by a constellation of features such as mental retardation, aberrant cranial shape, unusually gregarious personality, premature wrinkling of the skin, dysmorphic facial features, short stature, colon and bladder diverticuli, dental maldevelopment, early joint laxity, late joint contractures, vocal cord abnormalities, and infantile hypercalcemia. It can also include supravalvular aortic stenosis (SVAS), a congenital vascular disorder generally diagnosed during infancy or childhood. It has been estimated that SVAS occurs in 1 in 20,000 live births. Narrowing of the ascending aorta is a dominant feature of SVAS, but other arteries (including the pulmonary arteries) are often affected. If not corrected through surgery, SVAS may lead to increased intracardiac pressure, myocardial hypertrophy, and heart failure.

A NHLBI-supported study has established the elastin gene (*ELN*) as the locus for SVAS in both inherited and sporadic cases. Complete deletion of one form of the gene was found in more than 100 people with WMS. In mice missing one of the two copies of the *ELN* gene, the levels of gene products (messenger RNA and protein) were cut in half, even though their arterial functions remained near normal under usual blood pressure conditions. These mice showed increased numbers of elastic lamellae and smooth muscle cells (by 35%) as did humans (by 250%). These compensatory increases in muscular capability take place during arterial development. By contrast, the arterial pathology seen in mice missing both copies of the *ELN* gene is profoundly aberrant, with uncontrolled smooth muscle cell proliferation resulting in

blockage of the artery. These findings occur in the absence of endothelial damage, thrombosis, or inflammation. This demonstration that hemodynamic stress is not a primary driving force of the pathology of SVAS/WMS suggests that elastin plays an important regulatory function in vascular smooth muscle cell development.

Lung Diseases Program

Advance Sleep Phase Syndrome (ASPS)

ASPS is a genetically based sleep disorder characterized by early evening onset of sleep and spontaneous early awakening with normal sleep duration. NHLBI supports basic research to elucidate the biological clock mechanism, the neural pathways through which the clock regulates sleep, immune, and other functions; clinical research on the role of circadian factors and sleep-regulating hormones in sleep disorders and the timing of sleep; and applied research on the role of the biological clock in disturbed sleep and alertness of shift workers, school-age children, and drowsy drivers. ASPS has been linked to a variant of the biological clock gene, *hPer2* (HL59596). This genetic basis of ASPS was determined by linkage analysis of a single family where many related individuals exhibited a large four-hour advance of sleep, temperature, and melatonin rhythms. Genetic studies identified a single base mutation that alters the ability of *hPer2* to interact with other components of the biological clock. The *hPer2* mutation linked to ASPS is hypothesized to advance the biological clock by accelerating the accumulation of other gene products composing the biological clock.

Alpha-1-Antitrypsin (AAT) Deficiency

AAT deficiency is an inherited deficiency of a circulating proteinase inhibitor that is manufactured primarily in the liver. Deficiency states (circulating serum AAT levels below 0.6 mg/ml) are associated with emphysema, presumably from inadequate protection against enzymatic destruction by neutrophil elastase. Fifteen percent of the AAT-deficient population also develop liver disease. NHLBI currently funds a variety of clinical and basic research on AAT deficiency, including study of the molecular mechanisms that impair secretion of AAT, methods of gene therapy delivery, and how to increase the availability of defective but partially active AAT. NHLBI-supported investigators are defining the abnormalities and degradation pathways of the AAT protein and the associated inflammation that leads to disease in various AAT-deficiency states. One new therapy in the early stages of investigation is the enhancement of partially active mutant protein transportation through the liver.

In addition to research that specifically focuses on AAT, NHLBI supports related studies addressing the general causation of emphysema; the function, synthesis, secretion, and interaction of enzymes similar to AAT; animal models of other enzyme deficiencies; gene regulation and therapy; and cellular transport, signaling, injury, and repair. The Institute also continues to support an AAT patient registry.

Asbestosis

Asbestosis, an occupational lung disease, is the interstitial pneumonitis and fibrosis caused by exposure to asbestos fibers. In response to the deposition of asbestos fibers, macrophages and lymphocytes accumulate, type II alveolar epithelial cells and smooth muscle cells proliferate, fibrosis appears in the adjacent walls of respiratory airways, and septa thicken. NHLBI-supported researchers are investigating the molecular and cellular events that trigger cellular accumulation and proliferation and that regulate the remodeling of lung tissue that results in fibrotic lesions.

Bronchopulmonary Dysplasia (BPD)

BPD is a chronic lung disease characterized by disordered lung growth with changes in cell size and shape and a reduction in the number of alveolar structures available for gas exchange. It affects at least 10,000 very-low-birth-weight (VLBW) infants each year and is associated with neonatal intensive care costs of approximately \$30,000 to \$60,000 per individual. The incidence of this disease has increased in recent years due to the increased survival of smaller premature infants.

NHLBI's program in developmental lung biology supports basic and clinical research to close the gaps in our understanding of BPD and identify treatment opportunities. The Collaborative Program for Research in BPD provides a well-characterized primate model of BPD for a multidisciplinary exploration of its etiology. This year NHLBI was able to support two new clinical trials to evaluate the role of nitric oxide (NO) in preventing and treating chronic lung disease in premature infants. This represents an opportunity to confirm findings from the SCOR Program in the Pathobiology of Lung Development that identified NO as an important regulator of lung circulation during development. Together, these clinical studies are expected to yield definitive information about NO's utility and "window of therapeutic opportunity" to prevent chronic lung disease in VLBW premature infants.

Recent progress by the Collaborative Program for Research in BPD includes identification of a new marker (bombesin peptide) that is predictive for the development of BPD and will allow identification of infants at particular risk for BPD. An antibombesin antibody, long-used in cancer treatment, has already been shown to prevent development of BPD in an animal model of the disease and is scheduled for a clinical trial in high-risk human infants. This may ultimately provide a beneficial, cost-effective intervention to decrease the incidence of BPD. Other exciting results indicate that early delivery of a recombinant antioxidant enzyme to human infants at high risk for BPD reduces lung injury when infants are re-examined at age 1. Delivery of a synthetic antioxidant has also been found to be prophylactic against the development of BPD. The recent discovery that retinoic acid, a relative of vitamin A, can stimulate regeneration of alveoli in models of BPD suggests that at least some of the detrimental processes involved in aberrant lung development are reversible. Studies funded by a new RFA program, "Strategies to Augment Alveolization," are also expected to yield information essential to understanding the complex issues surrounding the role of vitamin A and corticosteroids in regulating lung growth.

Churg-Strauss Syndrome

Churg-Strauss syndrome is a rare disorder that was first reported in the 1950s. It is characterized by the formation and accumulation of an abnormally large number of certain white blood cells (eosinophils), inflammation of blood vessels (angiitis or vasculitis), and inflammatory nodular lesions (granulomatosis). Onset typically occurs from ages 15 to 70 years, and the disease affects both males and females. Patients with the syndrome are often affected by asthma. Churg-Strauss syndrome can be severely debilitating and even fatal if untreated, but patients usually respond well to corticosteroid treatment.

More than 90 cases of Churg-Strauss syndrome have been reported in less than 2 years by physicians who had switched asthma patients from corticosteroid therapy to antileukotriene therapy. It is unclear whether the increased reports of Churg-Strauss are the result of an untoward effect of the antileukotriene therapy or a primary eosinophilic disease that had been clinically recognized and treated as asthma but was "uncovered" as Churg-Strauss after the corticosteroid therapy was withdrawn.

NHLBI does not currently support research specifically investigating Churg-Strauss syndrome; however, it does support numerous investigator-initiated grants studying the basic mechanisms of asthma, including

examination of the role of eosinophils. NHLBI also supports clinical studies of severe asthma and of medications used in asthma management, such as antileukotriene therapy.

Congenital Diaphragmatic Hernia (CDH)

CDH is a developmental disorder that occurs once in every 2,400 births. CDH often occurs in isolated fashion, i.e., it is not associated with other life-threatening anomalies or chromosomal aberrations. Neonates with the disorder typically die soon after birth because lung tissue compressed by the herniated viscera is inadequately developed, and hypoplasia of the pulmonary vascular bed leads to pulmonary hypertension or persistent fetal circulation syndrome. NHLBI supports an investigator-initiated clinical study that will test the efficacy of an in utero surgical technique to correct lung hypoplasia in a group of human fetuses with the most severe form of CDH. The goal of the study is to determine whether temporary tracheal occlusion at 24-28 weeks gestation enlarges the hypoplastic fetal lung and improves the odds ratios for survival and various quality-of-life indices.

Cystic Fibrosis (CF)

CF is a multisystem disease characterized by defective transport of chloride and sodium across the cell membrane. More than 25,000 Americans have CF, with an incidence of about 1 in 3,300 among Caucasian births. CF is the leading genetic cause of death for children and young adults, with 90% of the deaths due to pulmonary complications. The responsible gene, the CF transmembrane conductance regulator (*CFTR*), was identified in 1989. More than 800 mutations and DNA sequence variations identified in the *CFTR* gene contribute to a highly variable presentation and disease course. NHLBI supports a vigorous program of basic, clinical, and behavioral research focused on the etiology, pathophysiology, and treatment of CF, specifically in relation to pulmonary manifestations.

Despite the promise of adeno-associated virus (AAV) as a delivery vehicle for human gene therapy, a major shortcoming has been its limited capacity to carry large genes such as the *CFTR*. Recent breakthroughs in the engineering of viral vectors allow larger genes to be delivered by splitting them into two parts and packaging the parts into separate, complementary AAV vectors, one containing the genetic information coding for the protein and the other containing the genetic material for the control elements that cause the therapeutic protein to be expressed. When the two parts of the virus simultaneously enter a cell, their genetic materials “join hands” and allow for high-level production of the therapeutic protein. This approach holds great promise for the future of gene therapy for CF.

Pseudomonas aeruginosa is the main cause of chronic lung infection leading to lung failure in individuals with CF. NHLBI-supported studies are providing insight into how *P. aeruginosa* endures for long periods in the lung and how it can be controlled. The bacteria were recently shown to form, through a process called quorum-sensing, a protective outer layer, or biofilm, on the lungs of CF patients, serving to protect the bacteria from conventional antibiotics and the body’s natural immune responses. The biofilm allows the bacteria to remain in the CF lungs, causing life-long *P. aeruginosa* infections. By studying the biofilm signaling process and how to interrupt it, scientists hope to be able to render the organism susceptible to traditional treatment methods. Importantly, these quorum-sensing signals may serve as a biomarker in screens to identify agents that interfere with cell-to-cell communication and biofilm development.

When used in chewing gum or as a syrup, xylitol, a non-ionic osmolyte that lowers salt concentrations without providing an energy source for bacteria, has been reported to decrease or prevent dental caries and acute otitis media. NHLBI-supported research recently explored the effectiveness of xylitol in

enhancing bacterial killing when applied to the airway surface. Xylitol was found to lower the airway surface liquid salt concentration in both CF and non-CF airway epithelia in vitro, enhancing the activity of endogenous airway antimicrobial substances and decreasing bacteria. Furthermore, in a controlled trial in nasal airway epithelia of normal subjects, xylitol was found to result in a significant decrease in the number of staphylococci present in the nasal passages. Although xylitol may be of little value in treating infections once established, these promising results suggest that it may provide an important new therapeutic to prevent or slow the onset of bacterial infection in CF.

Idiopathic Pulmonary Fibrosis (IPF)

IPF is a rare chronic lung disease of unknown cause affecting between 3 and 30 individuals per 100,000 population. Individuals with IPF develop abnormal, excessive scarring that can cause progressive shortness of breath and coughing. Currently available treatments, most commonly with corticosteroids in combination with other potent drugs, and less commonly with lung transplantation, do little to prevent a relatively rapid death in most patients. NHLBI-supported research on IPF is examining the molecular and cellular events that trigger the inflammation of alveoli seen in the early stages of the disease and that influence progression to the irreversible, fibrotic end-stage. Three NHLBI intramural observational clinical research protocols focusing on the natural history and pathogenesis of IPF are open for enrollment of subjects with familial and non-familial forms of IPF. NHLBI's intramural program has established collaborations with extramural sites and is working with the Pulmonary Fibrosis Association and other patient-support organizations to recruit patients. NHLBI-supported investigators comparing occupational exposures in 248 IPF patients with those in 491 control subjects reported this year on significant correlations with farming, livestock, hairdressing, metal dust, bird-raising, stone-cutting, stone-polishing, vegetable dust, and animal dust. The results confirm previous findings that dusty environments lead to an increased risk of IPF.

Lymphangiomyomatosis (LAM)

LAM is a rare lung disease that affects girls and women from puberty through menopause. Symptoms develop as the result of proliferation of atypical, non-malignant smooth muscle cells in the lungs. Diagnosis is usually made by lung biopsy or by specialized chest radiographs. Common symptoms include shortness of breath, cough, and sometimes coughing up blood. Patients often develop spontaneous pneumothorax (collapse of the lung) or chylous pleural effusion (collection of milky-looking fluid around the lung). Patients may present with abdominal tumors containing abnormal smooth muscle cells similar to those in the lungs. The clinical course of LAM is quite variable, but is usually slowly progressive, eventually resulting in death from respiratory failure. Although no treatment has been proven effective in halting or reversing LAM, lung transplantation is a valuable treatment for patients with end-stage lung disease.

NHLBI supports research on LAM in both its intramural and extramural programs. As part of the intramural program, NHLBI has established a basic and clinical research laboratory at the NIH Clinical Center (CC) to learn more about the cause and progression of LAM at the clinical, cellular, and molecular levels. More than 225 patients have been enrolled in the study. Researchers are determining the characteristics of the unusual smooth muscle cells that damage the lungs of LAM patients.

A prospective study within NHLBI's intramural program demonstrated that a large percentage of women with tuberous sclerosis complex (TSC), a genetically transmitted disease, develop lung lesions identical to those seen in LAM. In some cases, the clinical distinction between TSC and LAM may be difficult. Both men and women with TSC develop lung nodules due to the abnormal proliferation of pneumocytes.

These nodules do not appear to be present in women with LAM but without TSC . Examining genetic mutations found in cells taken from the lungs and kidneys of LAM patients to determine how TSC and LAM are linked, one NHLBI-supported investigator, discovered that mutations in the tuberous sclerosis complex gene *TSC2* can cause pulmonary LAM. This research may lead to new diagnostic and therapeutic strategies.

An important focus of NHLBI's intramural research is learning how growth is regulated in LAM cells. Individual LAM cells isolated from patients have been expanded into colonies. These cells are clonal and have abnormalities in their tuberous sclerosis genes consistent with a role for the genes in the growth of abnormal smooth muscle cells. Although biomarkers have not been identified that predict the course of the disease in any individual patient, the natural history study has demonstrated that time to lung transplant can be estimated from specific pulmonary function tests, lung biopsy, and radiographs.

In other scientific advances of note, an immunohistochemical analysis suggests that the production of proteins that inhibit cell death in LAM cells may be controlled by hormones. Down-regulation of estrogen and progesterone receptors has been reported following therapy. These studies may provide clues to understanding the imbalance that causes cell overgrowth. Also under investigation is how a family of destructive enzymes in the lung, known as matrix metalloproteinases, may lead to progression of LAM.

NHLBI and ORWH support a national LAM Patient Registry that is coordinated by the Cleveland Clinic Foundation. Patients can be enrolled through six major centers (including the NIH CC) or by their personal physicians. By the end of FY 2000, the registry had enrolled more than 200 LAM patients.

Persistent Pulmonary Hypertension of the Newborn (PPHN)

PPHN affects approximately 1 in 1,250 live-born term infants. Due to inappropriate muscularization of fetal pulmonary vessels, the lung arteries of affected newborns fail to dilate after birth to allow for normal blood flow to the lung. These infants are poorly oxygenated and require costly and prolonged medical care, including intubation of the airway, inhalation of 100% oxygen, mechanical ventilation, and, often, heart/lung bypass (extracorporeal membrane oxygenation).

Two NHLBI SCORs on the Pathobiology of Lung Development are focused on identifying the basic molecular mechanisms involved in the development of PPHN. A recently funded clinical study will address maternal risk factors such as cigarette smoking and antenatal exposure to the non-steroidal anti-inflammatory drugs aspirin and ibuprofen. Experimental evidence consistently suggests that maternal exposure to these agents plays a role in the etiology of PPHN and thus influences the incidence of the disorder in term infants. This multicenter, case-control study (560 infants with PPHN with 4 controls each) will also collect and store buccal cell specimens for future genetic analyses, should a relationship be demonstrated.

One experimental therapy that offers promise of less invasive treatment is inhaled NO. Recent studies point to a critical role for endogenous NO as a modulator of levels of vasoactive mediators whose net balance determines pulmonary vascular tone and reactivity. There are three known isoforms of NO synthase (NOS) in mammals, all of which are developmentally regulated in the fetal lung. Understanding the molecular mechanisms by which NO is released under hypoxic conditions may have important implications for the treatment of PPHN.

Other investigators supported by the program have demonstrated that treatment of neonatal rats with the synthetic adrenocortical steroid dexamethasone causes lung hypoplasia, decreases alveolization, and results in an increase in the development of subsequent pulmonary hypertension. Their results demonstrate the importance of temporo-spatial relationships in the coordination of vascularization and cardiopulmonary development and the limits of our understanding of those relationships.

Primary Ciliary Dyskinesia (PCD)

PCD, also known as Kartegener's syndrome or immobile ciliary syndrome, is an inherited disease characterized by defects in the cilia lining the respiratory tract. The result is impaired ciliary function, reduced or absent mucous clearance, and susceptibility to chronic, recurrent respiratory infections, including sinusitis, bronchitis, pneumonia, and otitis media. The disease typically affects children ages 0 to 18, but the defect associated with this condition also has variable clinical impact on disease progression in adults. Many patients experience hearing loss, male infertility is common, and situs inversus (having organs on the opposite side from usual) occurs in approximately 50% of PCD patients. Clinical progression of the disease is variable, with lung transplantation required in severe cases. For most patients, aggressive measures to enhance clearance of mucus, prevent respiratory infections, and treat bacterial superinfections are recommended. Although the true incidence of the disease is unknown, it is estimated to be 1 in 32,000 or higher.

Although PCD has been the focus of many scientific investigations, little progress has been made in identifying its genetic cause. Recent advances in the areas of protein biochemistry and cell culture of airway epithelial cells may now enable new insights into the genetic basis of PCD and increased understanding of its pathogenesis. Several NHLBI-supported studies are seeking to identify defects in the cilia of PCD patients at the level of individual proteins and ultimately to identify mutations responsible for some cases of PCD. Another NHLBI-supported study is identifying and characterizing a regulatory protein, *HFH-4*, expressed specifically in ciliated epithelial cells. Preliminary findings with a mutant mouse lacking this protein suggest that the protein has a role in the regulation of the early development of cilia (ciliogenesis) and that cilia function is critical in left-right body axis symmetry. Mice lacking *HFH-4* did not have cilia and had situs inversus, thus providing the first genetic evidence that ciliogenesis and regulation of left-right symmetry are linked.

Primary Pulmonary Hypertension (PPH)

PPH is a rare progressive lung disorder characterized by a sustained elevation of pulmonary artery pressure. Although PPH can occur in either gender and begin at any age, it usually affects girls and women between puberty and menopause. Estimates of the prevalence of PPH range from 1 to 2 individuals per million population. PPH is associated with structural changes in the small pulmonary arteries and arterioles, resulting in resistance to blood flow. The process eventually leads to an enlarged, overworked right ventricle that is unable to pump enough blood to the lungs, resulting in heart failure and

death, usually within 3 to 5 years of initial diagnosis. Although medical treatment for PPH has improved over the past decade, no therapeutic approach is uniformly accepted or successful.

NHLBI supports basic research on the cellular and molecular events underlying the pathogenesis of PPH. The dominant themes of this research are: 1) isolation and characterization of a familial PPH gene, 2) better understanding of the structural aspects of the disease that cause proliferative and obliterative changes in the vasculature, 3) identification of genetic factors that affect functional and structural changes in the vasculature, and 4) identification and evaluation of more effective treatments.

At least 6% of subjects diagnosed with PPH have a known family history of the disorder. Familial PPH (FPPH) segregates as an autosomal dominant trait with incomplete penetrance, meaning that individuals could inherit the gene but not display symptoms of the disease. Recently, two research groups independently identified germline mutations in the bone morphogenetic protein receptor II (*BMPR2*) gene in patients with the disease. *BMPR2* belongs to a family of proteins responsible for a wide variety of cellular functions in embryogenesis, vasculogenesis, angiogenesis, hematogenesis, immune regulation, and wound healing. The mutations were varied and distributed across the gene, and none of them were detected in control chromosomes.

Familial and sporadic PPH share similar histopathological features and follow a similar clinical course, suggesting a common genetic basis. In some families, parental transmission was observed, showing that family members without the disease can harbor the mutations and suggesting that FPPH is underdiagnosed. Since not all patients with PPH have *BMPR2* mutations, other genetic events may occur in their lungs. A recent study reported that endothelial cells from the lung tissue of sporadic PPH patients acquire somatic mutations from other genes involved in endothelial cell growth and apoptosis.

Pulmonary Alveolar Proteinosis (PAP)

PAP is a rare lung disease characterized by accumulation of lipoproteinaceous material within the alveoli. The congenital form of the disease is fatal and occurs in 1 per 100,000 live births. The pathophysiologic basis of congenital PAP is associated with a mutation in the gene for surfactant protein B, leading to synthesis of inadequate quantities of normally functioning surfactant protein B. The cause of adult onset PAP is not well-characterized and may be associated with the heterozygous condition for a mutation in *SP-B*, a deficiency of granulocyte-macrophage colony-stimulating factor, or a variety of conditions such as silicosis and certain types of malignancies. NHLBI supports investigator-initiated studies to determine the molecular basis for the development of PAP and the incidence of mutations responsible for congenital PAP. Molecular analyses of cord blood specimens collected by health departments in various states and abroad as components of birth and death certificate data are being analyzed to determine the frequency of relevant *SP-B* mutations among populations of varied ethnic composition.

Sarcoidosis

Sarcoidosis is a chronic, multisystem disease of unknown cause in which affected organs, especially the lungs, are invaded by different types of inflammatory cells that become organized into clusters of cells called granulomas. Sarcoidosis affects people of all ages, sexes, and races, although higher incidences are reported for young adults and African Americans. The illness can be self-limited or chronic with episodic outbursts and remissions. In some patients, the disease is characterized by a chronic interstitial pneumonitis accompanied by progressive deterioration of lung function associated with scarring, fibrosis, and permanent destruction of lung tissue. The estimated prevalence of patients with sarcoidosis ranges from 13,000 to 130,000, and the estimated incidence of new cases ranges from 2,600 to 27,000 per year.

Corticosteroids are currently the primary treatment for active pulmonary sarcoidosis, but they often produce undesirable side-effects.

NHLBI's intramural research program has initiated a randomized, double-blind, placebo-controlled clinical protocol to determine if pentoxifylline, a xanthine derivative that has been used for many years in the treatment of peripheral vascular disease, can be beneficial as an adjunct to corticosteroid therapy in patients with pulmonary sarcoidosis. The same group is also attempting to identify genetic polymorphisms and modifier/susceptibility genes that may be important in the development and/or clinical course of sarcoidosis. NHLBI's extramural program supports basic research on sarcoidosis designed to further understanding of granuloma formation, and clinical research intended to improve understanding of the initiating events and natural history of sarcoidosis and the contribution of susceptibility genes to its development.

In recent studies, histologic and clinical similarities between tuberculosis and sarcoidosis have suggested a shared underlying pathophysiology. The *NRAMP1* human protein has been associated with increased susceptibility to tuberculosis in some human populations. Its role in susceptibility to sarcoidosis was studied by comparing variations in the *NRAMP1* gene in 157 African American sarcoidosis patients to variations in 111 African American control subjects. Contrary to the previous findings in tuberculosis patients, the less common gene variations were found more often in control subjects than in sarcoidosis patients, and one variation was actually found to have a protective effect.

Sudden Infant Death Syndrome (SIDS)

SIDS is the diagnosis given for sudden death of an infant younger than 1 year that remains unexplained after a complete investigation, including an autopsy, examination of the death scene, review of the symptoms or illnesses the infant had before death, and consideration of any other pertinent medical history. SIDS is also commonly known as crib death. Abnormalities in neural functions regulating breathing, blood pressure, and waking during sleep are thought in many cases to contribute to SIDS risk. Recent studies indicate that inheritable factors place infants with apneic parents or siblings at increased risk. NHLBI supports basic research on the development of fundamental neurobiological mechanisms regulating chemosensitivity, ventilatory rhythm, and blood pressure, and clinical research to identify genetic and epidemiological factors associated with SIDS risk.

Blood Diseases and Resources Programs

Acute Graft-versus-Host Disease (GvHD)

Acute GvHD is a condition that typically occurs within 3 months after allogeneic hematopoietic stem cell transplantation, when donor T cells react against "foreign" tissue antigens in the recipient. Acute GvHD is characterized by skin rash, liver dysfunction, vomiting, and diarrhea, and often precedes development of chronic GvHD, which may require treatment with immunosuppressive drugs for several years. NHLBI supports basic and clinical research to understand the pathophysiology of GvHD, especially in unrelated transplants, with emphasis on the roles of both major and minor histocompatibility antigens in disease pathogenesis, development of tolerance, function of donor T cells in allogeneic hosts, and mechanisms of GvHD prevention, including depletion of donor T cells from the graft. NHLBI also supports two multicenter clinical studies: the Unrelated-Donor Marrow Transplant Trial of T-Cell Depletion and the Cord Blood Banking and Transplantation Study. Other studies are examining the variables that affect GvHD induction and severity, its effector mechanisms, and whether GvHD can be suppressed while

other necessary immune responses are maintained. Answers to these questions may lead to the design of successful approaches for intervention.

Combining data from their respective registries, the International Bone Marrow Transplant Registry and the Eurocord-Cord Blood Transplant Group compared results of sibling umbilical cord blood (UCB) and bone marrow transplantation for a group of patients younger than 15 years. Their study found that although engraftment (the time for new white blood cells and platelets to grow in the recipient) was longer in the UCB recipients, the incidence of GvHD was lower, and the overall survival was the same. The results suggest that unrelated umbilical cord blood compares favorably with unrelated donor marrow.

Aplastic Anemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH)

AA is a form of bone marrow failure in which hematopoietic cells are replaced by fat, resulting in low blood counts. In PNH, a clone derived from a single hematopoietic stem cell expands, leading to marrow failure, red blood cell destruction, and venous thrombosis. NHLBI's intramural Hematology Branch has a large clinical and laboratory program devoted to bone marrow failure syndromes, including AA and PNH. Bench studies include immunology, cell biology, virology, and molecular biology approaches to the failure to produce blood cells. Clinical studies include therapeutic interventions to reduce autoimmunity in patients with AA. Using sensitive flow cytometry, the Branch has established that an expanded PNH clone is present in a large proportion of patients with AA. In a randomized trial to compare conventional antithymocyte globulin immunosuppression to high-dose cyclophosphamide, the Branch established that the latter treatment is excessively toxic and leads to a high rate of severe fungal infections and increased mortality.

Cooley's Anemia

Cooley's anemia (also called beta-thalassemia, thalassemia major, or Mediterranean anemia) is a genetic blood disease that results in an inadequate production of hemoglobin. Individuals affected by Cooley's anemia require frequent and lifelong blood transfusions. Because the body has no natural means to eliminate iron, the iron contained in the transfused red blood cells builds up over many years and eventually becomes toxic to tissues and organ systems. In addition, many affected children have acquired other diseases such as hepatitis through years of transfusion exposure.

NHLBI's extramural research efforts related to Cooley's anemia include:

- Identification of mutations in the globin gene cluster that lead to the disorder.
- Elucidation of the mechanisms and therapeutic approaches associated with naturally occurring mutations that result in significantly elevated levels of fetal hemoglobin in adult red blood cells.
- Iron chelation.
- Identification of clinically useful therapies and drugs.
- Gene therapy strategies to reduce the morbidity and mortality associated with Cooley's anemia.

Continued support of research protocols for efficient identification and targeting of hematopoietic stem cells, information on how ex vivo manipulation of stem cells alters their biologic properties, and improved vectors will significantly contribute to treatment of Cooley's anemia. NHLBI's strategic approach to the disease also includes programs in therapy development as well as a clinical research network to test new therapies. Another avenue of research is an ambitious study of sibling donor cord blood banking and transplantation for hemoglobinopathy families.

FY 2000 has witnessed a number of important scientific advances in this area:

- NHLBI grantees used a beta-globin gene/beta-locus control region retroviral vector to optimize gene transfer and expression in a mouse transplant model. The results demonstrate high-level, long-term somatic human beta-globin gene transfer into the hematopoietic stem cell of an animal for the first time, and suggest the feasibility of a retroviral gene therapy approach to sickle cell disease and the beta thalassemias.
- New methods of transfusion therapy were developed.
- Less toxic methods of stem cell transplantation that provide potential utility for patients with thalassemia were developed.
- New iron chelators were evaluated.
- A new clinical research network has designed protocols that will provide clinically useful information in the areas of hepatitis and osteoporosis management as well as insights into the potential utility of fetal hemoglobin induction as a function of genotype.
- Several compounds that increase fetal hemoglobin values were described. These include not only hydroxyurea, a compound in routine use in sickle cell disease, but also a number of butyrate-based compounds as well as 5-azacytidine. Whether erythropoietin will have a role in the treatment of the thalassemia remains to be determined.

Creutzfeldt-Jakob Disease (CJD)

CJD is a slow degenerative disease of the central nervous system characterized by motor dysfunction, progressive dementia, and vacuolar degeneration of the brain. The disease is rare but invariably fatal, and has been associated with a transmissible agent. A protease-resistant protein or prion is the hallmark of the transmissible spongiform encephalopathies (TSEs) to which CJD belongs, thus leading to the term “prion diseases” for this group of neurodegenerative illnesses that include bovine spongiform encephalopathy (BSE or “mad cow disease”), scrapie in sheep, and chronic wasting disease in deer and elk. Prion diseases may cross the species barrier, the most notable example being the recent cases of new-variant CJD in humans caused by consumption of beef contaminated with BSE. Classic CJD occurs worldwide at a rate of 1 to 2 cases per million per year. In FY 1999, NHLBI initiated a high-priority program to address the lack of a rapid, sensitive, and specific assay for TSE infectivity. Such assays could form the bases of a blood/tissue donor screening test and could additionally provide a diagnostic test for neurologists, since currently there is no way of detecting disease in the preclinical stage. The assays could also be useful in testing for TSE in animals, especially in domestic animals used for human consumption.

Fanconi Anemia (FA)

FA is an autosomal recessive bone marrow failure syndrome characterized by a decrease in blood cells and platelets (pancytopenia), developmental defects, and cancer susceptibility. Approximately 75% of FA patients can be identified at birth because of congenital anomalies, while the remaining 25% have no birth defects. FA is a clinically heterogeneous disorder and can currently be divided into at least eight different complementation groups, designated A through G. Two FA genes, *FAC* and *FAA*, have been cloned and account for an estimated 75% of all FA patients worldwide. As noted above, eight distinct

complementation groups (designated FAA-FAG) have been reported, suggesting that at least eight genes are involved in the manifestations of FA.

Localization and functional studies to delineate the interrelationship of FA proteins and their functions are a high-priority research area for NHLBI. The Institute supports research on the identification and cloning of the remaining FA genes, protocols for efficient identification and targeting of hematopoietic stem cells, studies of how ex vivo manipulation of stem cells alters their biologic properties, and development of improved vectors to enhance the potential for a cure. NHLBI is currently supporting an investigator-initiated cooperative agreement to conduct sibling donor cord blood banking and transplantation. With the indications for bone marrow transplant of FA patients continually growing and with new evidence of the benefits of cord blood transplants, this promises to be an important endeavor.

NHLBI's intramural Hematology Branch program in FA has three foci: 1) development of hematopoietic stem cell gene therapy for FAC and FAA, 2) analysis of transgenic and knockout mice in order to gain an understanding of the pathophysiology of bone marrow failure and cancer predisposition, and 3) characterization of the molecular function of the cloned FA gene.

A number of studies over the past year have further defined the FA complex of proteins and provided insight into their potential function. Exciting developments include cellular localization of the functional complex and determination of the role of the complex in DNA repair and prevention of mutagenesis. Recent transplantation protocols using Fludarabine have provided new hope that stem cell transplantation may become a therapeutic option for patients with FA.

Hemophilia

Hemophilia is a hereditary bleeding disorder that results from a deficiency in either blood coagulation factor VIII or factor IX. About 20,000 individuals are affected in the United States, all of whom are dependent on lifelong treatment to control periodic bleeding episodes. Approximately 20% of severe hemophilia patients develop antibody inhibitors that specifically neutralize the replacement factor and complicate treatment. The adult hemophilia population has been severely affected by blood-borne infectious agents in plasma-derived replacement products. More than 80% of them have been infected with hepatitis virus, and approximately 20% of them have been infected with HIV.

NHLBI supports a broad spectrum of activities on blood coagulation and its disorders. The research addresses viral and non-viral approaches for gene therapy, mechanisms of antibody inhibitor formation, modification of factors for improved therapeutics, safety of plasma-derived products, and blood product-associated infections. In addition, basic genetic, molecular biology, and protein biochemistry studies of factor VIII and factor IX are supported to improve understanding of their mechanism and regulation.

Gene therapy studies by NHLBI-supported scientists have shown sustained expression of factor IX in mice and hemophilic dogs after muscle injection or intraportal administration of AAV vector-containing factor IX. Preliminary results of the first phase I clinical study for AAV-mediated muscle directed gene transfer of factor IX indicate that the procedure is well-tolerated and show evidence of protein expression. On the basis of pre-clinical safety and efficacy data, a clinical study for intrahepatic delivery of AAV vector-expressing factor IX has been proposed.

Hereditary Hemorrhagic Telangiectasia (HHT)

HHT, also called Osler-Weber-Rendu disease, is a bleeding disorder that is due to weakness of the vascular support structure. HHT's most common manifestations are red spots on the lips and bleeding from mucosal membranes such as in the nose. In an advanced stage, there are often arterio-venous malformations in the lung, brain, gut, and liver. Two gene defects have been identified in patients with HHT. One is in the gene associated with the protein endoglin, and the other is in a gene related to activin receptor-like kinase. A correlation between the gene defect and organ susceptibility to the disease may exist. Diagnosis of patients with HHT, particularly at an early age, is difficult because multiple organs are affected. Establishment of a genetic linkage may allow earlier diagnosis and improved treatment.

NHLBI supports a broad spectrum of research in hemostasis and thrombosis that is focused in part on understanding the biology of platelet activation, the mechanism of clotting, and the interaction of blood with the vascular surface. Progress has been made in determining the underlying molecular basis of HHT, which appears to be a mutation in the genes of two TGF beta-receptor family members on the endothelial cell. The type I receptor (Alk-1) and the type III receptor (endoglin) have been identified, while a rare type II receptor remains unknown. Eight mutations in endoglin leading to HHT have been identified, and a database on genetic mutations related to HHT has been established. This suggests a critical role for the TGF-beta signal transduction pathway in capillary morphogenesis and the pathology of this disease.

Immune Thrombocytopenic Purpura (ITP)

ITP is an autoimmune disease manifested by production of antibodies that react with specific proteins on the surface of platelets. The reaction results in rapid clearance or destruction of the platelets (thrombocytopenia) and clinically significant bleeding. The underlying cause is unknown, but ITP is associated with other autoimmune diseases. Acute (temporary) thrombocytopenic purpura is most commonly seen in young children. About 85% of children recover within 1 year and the problem does not return. Thrombocytopenic purpura is considered chronic when it has lasted more than 6 months. Its onset may occur at any age, adults more often have the chronic disorder, and females are affected two to three times more than males. Most adult patients respond at least transiently to standard therapies, including steroids and splenectomy, but a majority eventually relapse, and some develop very severe chronic refractory ITP.

Part of the NHLBI research program on thrombosis and hemostasis is directed toward understanding the biology of platelet production from megakaryocytes, the function of the growth factor thrombopoietin, and the structure and function of platelet surface glycoprotein antigens. Research in FY 2000 showed that the transcription factor *GATA-1* is necessary for megakaryocyte maturation and platelet production. Subtractive hybridization experiments between megakaryocytes lacking *GATA-1* and controls show that the *4-Ptase 1* enzyme is essential for this process. Application of phage display technology has led to progress in the identifying genetic mechanisms involved in the formation of antibodies to platelets. A transgenic mouse model of thrombocytopenia has been developed and should be useful in research and development of drugs for ITP.

NHLBI's intramural Hematology Branch has enrolled 14 adult patients with severe ITP in a transplantation protocol that consists of intensive immunosuppression using high-dose chemotherapy (cyclophosphamide) followed by autologous peripheral blood stem cell rescue. Although a significant

minority of patients experienced durable complete remissions, further long-term follow-up is needed to assess the general utility and risk/benefit ratio for autologous transplantation in patients with ITP.

Lymphedema

Lymphedema is an accumulation of lymphatic fluid in interstitial tissue that causes swelling, most often in the arm(s) and/or leg(s), and occasionally in other parts of the body. Lymphedema can develop when lymphatic vessels are missing or impaired (primary or congenital), or when lymph vessels are damaged or lymph nodes removed (secondary). When the impairment becomes so great that the lymphatic fluid exceeds the lymphatic transport capacity, an abnormal amount of protein-rich fluid collects in the tissues of affected areas. Left untreated, this stagnant, protein-rich fluid not only causes tissue channels to increase in size and number, but also reduces oxygen availability in the transport system, interferes with wound healing, and provides a culture medium for bacteria that can result in lymphangitis (infection). The incidence of primary lymphedema has been estimated to be between 1 in 6,000 and 1 in 300 live births, so it may be a rare disease, or it may be a more common disease that predisposes to the secondary type and is under-recognized. NHLBI investigator-initiated projects are seeking to identify the developmental, molecular, and cellular defects that contribute to lymphedema and are seeking to design effective therapeutic interventions to treat both primary and secondary lymphedemas.

Myelodysplasia

Myelodysplasia or myelodysplastic syndrome (MDS) is a group of disorders in which the bone marrow overproduces cells that do not mature normally, due in part to an autoimmune suppression of blood cell production and in part to an intrinsic defect in blood cell production. Most patients with MDS are anemic and many have low platelet counts and low numbers of infection-fighting white blood cells (neutrophils). The only curative therapy is allogeneic bone marrow transplantation. The standard treatment for MDS is supportive care, i.e., transfusions and antibiotics as needed. MDS commonly affects older adults. More than 20,000 new cases of MDS are diagnosed per year in the United States and its incidence is increasing. About half of MDS patients die from the consequences of marrow failure, and half die following transformation to a refractory acute leukemia.

NHLBI's intramural Hematology Branch is exploring two treatment approaches for MDS. The first involves immunosuppressive treatment to improve bone marrow function and reverse transfusion dependence. Patients receive two immunosuppressive agents, antithymocyte globulin (ATG) and cyclosporine, and are monitored for transfusion requirements. In the second approach, selected MDS patients up to age 75 are entered into allogeneic stem cell transplant protocols to evaluate curative approaches. The International Aplastic Anemia and Myelodysplasia Foundation has collaborated with NHLBI to recruit patients.

Renal Cell Carcinoma (RCC)

Metastatic RCC is an uncontrolled growth of renal cells, the cells that normally exist in the kidney. These cancerous cells can spread (metastasize) from the original kidney tumor site to other organs such as the bones, lymph nodes, liver, lungs, and brain. Once these organs become involved, the uncontrolled growth of cells can lead to organ failure and death. While there are several treatments available for RCC that can be successful, RCC is rarely curable once it has spread to other organs. Surgery can be used to treat RCC, but in many patients the disease has spread too much to be removed by surgery. Likewise, chemotherapy can be used to treat RCC, but it has been relatively unsuccessful for patients whose cancer has spread to other organs. Bone marrow transplants (BMTs) have been used to treat cancers of the blood and bone

marrow. BMTs are usually combined with powerful doses of chemotherapy and radiation therapy. These additional treatments are associated with toxic side-effects, often making BMTs too dangerous to attempt in many patients. The effectiveness of BMT on solid tumors like RCC has not been well-evaluated.

NHLBI's intramural Hematology Branch is interested in learning more about the potential benefits of modified BMT (allogeneic stem cell transplantation) for patients with advanced RCC. In a current study, the Branch is treating advanced RCC patients with transplanted stem cells from a genetically matched brother or sister. After the stem cells are transplanted, they help to make new blood cells. In addition, immune factors found in the transplant can work to destroy cancerous cells. In order to avoid the toxic side-effects normally associated with BMT, the stem cell transplant is combined with low- intensity chemotherapy. The majority of the cancer-killing effect will be the responsibility of the stem cell transplant rather than the chemotherapy.

Sickle Cell Disease (SCD)

SCD is an inherited disorder that is most common among people whose ancestors come from Africa, the Middle East, the Mediterranean basin, and India. In the United States, SCD affects primarily African Americans, about 72,000 of whom have SCD. As such, SCD is the most common genetic blood disorder in the United States, affecting approximately 1 in 500 African American newborns and 1 in 1,000 Hispanic newborns each year. SCD occurs when an infant inherits the gene for sickle hemoglobin from both parents (sickle cell anemia [SCA]) or the gene for sickle hemoglobin from one parent and the gene for another abnormal hemoglobin from the other parent (SCD types Hb SC, Hb S-Beta thalassemia, etc.) One in 12 blacks carries the sickle cell trait (Hb AS). In SCD patients, the hemoglobin molecules in the red blood cells that carry oxygen throughout the body tend to damage the red cell walls, causing them to stick to blood vessel walls. This leads to sickle cell crises, the painful episodes considered the hallmark of the disease. Chronic end-organ damage occurs to the brain, lungs, kidneys, spleen, and liver and leads to premature death, with death for severely affected individuals occurring between 42 and 48 years.

The current NHLBI SCD research portfolio includes projects to:

- Develop methods for gene transfer and gene replacement in the hematopoietic stem cell.
- Characterize interactions between sickle cells and the vascular endothelium.
- Improve understanding of hemoglobin gene switching to allow increased production of fetal hemoglobin. Develop a transgenic mouse model of sickle cell disease.
- Compare blood transfusions versus conservative therapy to ascertain the time at which blood transfusions are no longer needed to prevent recurrent stroke in children with SCA.
- Conduct a phase III clinical trial of hydroxyurea in children with SCD.
- Conduct an epidemiologic study of the incidence of parvovirus B19 seroconversion in children with SCD.
- Conduct an epidemiologic study of the adult patients who participated in the Multicenter Study of Hydroxyurea (MSH) Trial.

Research published in the past year confirmed that adhesive interactions between individual blood components and between blood components and cells that line blood vessels (endothelium) are likely to be important initiators of sickle cell vaso-occlusive crises. The sticky, stiff, sickle red blood cells provoke inflammation as they obstruct blood flow. Investigators have been able to induce inflammatory responses in sickle mice but not normal mice by removing and then providing oxygen. This was correlated in the sickle mice with oxidant production by vascular endothelial cells and could be completely prevented by prior infusion into mice of an antibody directed toward the P-selectin molecule expressed on vascular

endothelium. These reports have set the stage for development of human therapies, e.g., monoclonal antibodies, designed to interfere with specific adhesion molecules such as P-selectin in order to decrease leukocyte-endothelial cell interactions.

Systemic Lupus Erythematosus (SLE)

SLE (or lupus) is an autoimmune disorder in which the body produces antibodies that harm its own cells and tissues. Typical symptoms of SLE are fatigue, arthritis, fever, skin rashes, and kidney problems. SLE affects more women than men. Patients with SLE have a higher incidence of thrombosis and spontaneous loss of pregnancy. Its cause is unknown and there is no known cure, but the symptoms can be controlled with appropriate treatment and most patients can lead an active life. Recent NHLBI-supported studies have found that some lupus antibodies have catalytic properties and can specifically convert prothrombin to thrombin, thereby creating a hypercoagulable condition that may explain the high incidence of thrombosis in patients with SLE.

Thrombotic Thrombocytopenic Purpura (TTP)

TTP is a potentially fatal disease characterized by low blood platelet levels and widespread platelet thrombi in arterioles and capillaries. Relapse is not uncommon in those who survive the acute phase. Both endothelial cell damage and intravascular platelet aggregation have been suggested in its pathogenesis. Microscopic examination of the thrombi has revealed an abundance of the plasma protein von Willebrand factor (vWf). Despite advances in basic sciences, treatment options and mortality associated with TTP remain unacceptable. NHLBI supports studies on the biology of platelet function, mechanisms of blood coagulation, and the interaction of blood with the vascular surface. An interaction between vWf and the platelet surface glycoprotein complex I (GP I) is believed to be essential for the formation of a thrombus. vWf is synthesized as large polymers and is then cleaved into smaller units by a plasma protease. The presence of inhibitory antibodies to the protease was confirmed in some patients with TTP. Inhibition of the protease results in large amounts of vWf in plasma that can spontaneously aggregate platelets. Understanding the biosynthesis and processing of vWf may offer an opportunity to develop new treatments for TTP.

Rare Diseases Research Initiatives

Ongoing Initiatives

- Clinical Research on Cooley's Anemia.
- Comprehensive Sickle Cell Centers.
- Immunogenetics of Inhibitor Formation in Hemophilia.
- Mitochondrial DNA Mutations in Heart, Lung, and Blood Diseases.
- Specialized Centers of Research (SCORs) in Neurobiology of Sleep and Sleep Apnea, Airway Biology and Pathogenesis of Cystic Fibrosis, and Acute Lung Injury.
- SCORs in Pathobiology of Fibrotic Lung Disease, Pathobiology of Lung Development, and Cellular and Molecular Mechanisms of Asthma.
- Stem Cell Transplantation to Establish Allochimerism.
- Strategies to Augment Alveolization.
- T Cell Depletion of Marrow for Unrelated Bone Marrow Transplantation: Clinical Trial to Ascertain Risk : Benefit Ratio.
- Thrombocytopenia: Pathogenesis and Treatment.

Initiatives Started in FY 2000

Cellular and Molecular Mechanisms of PPH

A renewal of a PA encourages efforts to continue important research in PPH with the emphasis on a mechanistic understanding of the disease. Further studies are encouraged that address, at the cellular and molecular level, mechanisms involved in pulmonary vascular remodeling, pulmonary vascular tone, and the genetic basis of PPH. Priority will be given to research to identify novel genes or vasoactive mediators important in PPH pathology and determine their functional effects on pulmonary vascular cells, on extracellular matrix, and on pulmonary vascular tone. Further, research integrating the relationship between mediators of vasoconstriction and pulmonary vascular remodeling is strongly encouraged. The ultimate goal is to develop new and effective therapies.

Programs of Excellence in Gene Therapy (PEGT)

A new RFA establishes up to five multidisciplinary, collaborative research environments that promote rapid translation of basic, preclinical studies of gene therapy for cardiovascular, pulmonary, and/or hematologic diseases into human pilot experiments. The 5-year awards, using the U01 cooperative agreement grant mechanism, will provide shared access to specialized services such as preclinical toxicology testing, generation of vectors for preclinical and clinical use, large-scale production of biological reagents (e.g., cytokines), and biostatistical support. In addition, the programs will provide training to NHLBI-supported physician-scientists in translational (basic science to clinical application) research for gene therapy. Each program will have a minimum of two clinical projects under way at any one time and four to six training positions. One PEGT will serve as the Coordinating Center and Data Core. A one-time competitive renewal for the existing awardees may be awarded for an additional 5 years.

SCORs in Hematopoietic Stem Cell Biology

A renewal of an RFA extends for a second 5-year period the SCOR program to advance knowledge of basic stem cell biology in areas of stem cell isolation, quantitation by in vivo assay, in vitro and in vivo growth and expansion, gene insertion and long-term expression, and engraftment. This basic knowledge will be applied clinically to develop hematopoietic stem cell therapy that cures both genetic and acquired diseases and to perform successful gene therapy using the hematopoietic stem cell as the target for gene transfection and for lifelong expression of normal genes.

The SCOR mechanism is uniquely designed to support a spectrum of multidisciplinary basic and clinical research in a synergistic fashion such that major therapeutic advances will be realized in the next decade in both gene therapy and stem cell transplantation.

Initiatives Planned for the Future

Blood and Marrow Transplant Clinical Research Network

A new RFA organizes a network in FY 2001 to accelerate research on management of hematopoietic stem cell transplantation, standardize existing treatments, and evaluate new treatments. The network of 16 to 20 clinical centers and a data-coordinating center would provide a coordinated, flexible mechanism to accept ideas and build consensus from the transplant community; develop protocols; expeditiously perform multi-center phase II and phase III clinical trials; provide information to physicians, scientists, and the public; and improve stem cell transplantation therapy for diseases such as leukemia, SCD, thalassemia, and FA.

Genetic Modifiers of Single-Gene Defect Diseases

A new RFA encourages investigators in FY 2001 to identify and characterize the genes responsible for modifying the clinical progression and outcome of heart, lung, and blood diseases due to single-gene defects. Examples of such single-gene defect diseases are CF, SCD, hemophilia, AAT deficiency, glucocorticoid remediable aldosteronism (GRA), Liddle syndrome, and cardiac myopathies, dysplasias, and arrhythmias that result in sudden cardiac death. The modifier genes are likely to encode a wide variety of proteins that either interact directly with the disease gene, influence pathways involving the disease gene, or affect metabolic processes altered as a result of the disease gene defect. Identification of the genes responsible for these differences should lead to a better understanding of disease pathogenesis, early diagnosis, and improved treatment.

Pathogenesis and Treatment of Lymphedema

A new PA encourages efforts in FY 2001 to investigate the pathogenesis of, and new treatments for, primary and secondary lymphedema, the swelling of subcutaneous tissues caused by a breakdown in the regulation of lymphatic drainage. Lymphedema results from fluid accumulation and may arise congenitally or from surgery, radiation, or the presence of a tumor in the area of the lymph nodes. This trans-NIH program announcement seeks to stimulate research on the biology of the lymphatic system; to characterize at the molecular, cellular, tissue, organ, and intact organism levels and the pathophysiologic mechanisms that cause the disease; and to discover new therapeutic interventions. Such knowledge will help to improve early diagnosis of affected individuals, the choice and timing of treatment, and genetic counseling.

Pediatric Heart Disease Clinical Research Network

A new RFA establishes in FY 2001 a network of interactive pediatric clinical research centers to promote efficient evaluation of new treatment methods and management strategies that may benefit children with structural congenital heart disease, inflammatory heart disease, heart muscle disease, and arrhythmias. Therapeutic trials and studies may involve investigational drugs, drugs already approved but not currently used, devices, interventional procedures, and surgical techniques. The network approach, consisting of five to six clinical centers and a data-coordinating center, is an effective, flexible way to study adequate numbers of patients with rare diseases such as congenital cardiovascular malformations. Efficiencies will be achieved through a common infrastructure for recruiting, monitoring, and following patients whose conditions will be characterized in a standard fashion. Approximately 2,000 patients are expected to participate in 6 to 12 different protocols over the 5-year project period. The network will also serve as a

platform to train junior investigators in pediatric clinical research and as a vehicle for rapid and widespread dissemination of findings.

Comprehensive Sickle Cell Centers

A renewal of an RFA in FY 2003 to operate a nationwide network of collaborative comprehensive centers in basic and translational research focused on the development of cures or significantly improved treatments for SCD. The network of 10 centers and a statistics and data management core will carry out basic research, inter-center collaborative clinical research, and local clinical research focused on the most promising biomedical and behavioral therapeutic modalities. The centers will also support career development of young investigators in SCD research and will support services such as patient education and counseling, community outreach, and hemoglobin diagnosis. This is the eighth re-competition of a program established by a presidential initiative and congressional mandate in 1972.

Rare Diseases Program Activities

AAT Deficiency

A June 2000 conference on “Alpha-1 Antitrypsin Deficiency and Other Conformational Diseases,” organized by the Alpha One Foundation and co-sponsored by NHLBI and NIDDK, enabled researchers on these related diseases to exchange scientific information.

BPD

A “Workshop on Bronchopulmonary Dysplasia” was organized by NICHD, NHLBI, and ORD to review the definition of BPD and lung injury in very preterm infants, identify gaps in knowledge of lung development, select the best indicators of outcome for infants with BPD, and prioritize areas for future research. A report of the meeting will appear in the Spring 2001 issue of the *Journal of Respiratory and Critical Care Medicine*.

Churg-Strauss Syndrome

NHLBI, NIAID, and ORD co-sponsored a September 2000 workshop on “The Relationship of Asthma Therapy and Churg-Strauss Syndrome.” More than 20 scientists with expertise in immunology, pharmacotherapy, epidemiology, allergy, asthma pathogenesis and management, vasculitic and eosinophilic diseases, and asthma clinical research met to explore the nature and magnitude of the association between anti-leukotriene therapy and Churg-Strauss syndrome and to identify possible mechanisms. The workshop participants made recommendations for future research directions to shed light on the pathogenesis of asthma and eosinophilic syndromes. Publication of the workshop summary is expected in 2001.

Cooley’s Anemia

A “Working Group on Stem Cell Plasticity” met in March 2000 and developed RFA-HL-01-007, Hematopoietic Stem Cell Plasticity, issued in November 2000.

FA

NHLBI's Hematology Branch participated in the "Symposium on Fanconi Anemia" at the Annual Scientific Meeting of the International Society for Experimental Hematology in Tampa, Florida; and in the Annual International Fanconi Anemia Scientific Symposium in Amsterdam, The Netherlands.

GvHD

A "Forum on Allogeneic Unrelated Cord Blood Banking and Transplantation," co-sponsored by FDA, met in August 2000. Leaders in cord blood banking and transplantation from around the world discussed requirements for collecting, processing, storing, and transplanting unrelated allogeneic umbilical cord blood. The recommended practices included infectious disease screening and testing, determining the number of viable cells post-processing, collecting donor family histories, and maintaining a sample attached to the frozen cord blood unit for follow-up testing. Transplant outcome data suggested that human leukocyte antigen- (HLA-) mismatched transplants result in less GvHD than expected and that larger cell doses tend to result in better engraftment.

LAM

NHLBI and the LAM Foundation co-sponsored the "International LAM Symposium" held at Columbia University in November 1999.

Lymphedema

A May 2000 meeting on "Conquering Lymphatic Disease: Setting the Research Agenda," co-sponsored with the Lymphatic Research Foundation, ORD, and four other NIH Institutes, resulted in PA-01-035, Pathogenesis and Treatment of Lymphedema, released in December 2000.

PPH

NHLBI and the Pulmonary Hypertension Association (PHA) have agreed to joint sponsorship of a program to train clinicians to perform biomedical research related to pulmonary hypertension. The training will be supported by the Mentored Clinical Scientist Development Award (K08) mechanism.

SCD

At the September 2000 "Workshop on Nitric Oxide as a Potential Therapeutic Agent for Sickle Cell Disease and Other Vascular Diseases," a discussion was held on the promise of NO as a possible therapy for SCD-associated acute chest syndrome, respiratory distress in premature infants, and other severe vascular problems. Pilot data in animals and humans were presented that suggest that NO provides a promising therapeutic option for these complex clinical problems.

A September 2000 "Workshop on Central Nervous System Disease in Children with Sickle Cell Disease" was held at NHLBI to discuss current understanding of the effects of SCD on the central nervous system (CNS), contemporary methods of evaluation of the CNS, prophylactic and therapeutic interventions that may alleviate brain damage, and future directions for research. Workshop participants discussed the age that screening for CNS disease should begin; studies needed for screening for CNS disease; the role of continuing blood transfusions versus hydroxyurea in secondary stroke prevention as patients age; the use of anti-platelet drugs and anti-inflammatory drugs as possible therapeutic options for clinical trial testing

in stroke prevention; identification by HLA typing and other risk modifiers of subjects who may be at risk for SCD; establishment of a registry of stroke patients; and establishment of a clinical network to plan various protocols for future studies.

After 20 years and more than 40 publications, the Cooperative Study of Sickle Cell Disease has ended. In September 2000, the investigators met to discuss manuscripts still to be written based on the database and the stored genetic and sera samples.

At the STOP Trial Steering Committee Meeting in September 2000, the STOP investigators met to discuss the STOP II Trial protocol to be submitted for review by the Data and Safety Monitoring Board. The STOP II Trial will attempt to ascertain if it is safe to stop transfusing children for stroke prevention after 30 months.

At the MSH Patients' Follow-up Steering Committee Meeting in September 2000, the investigators met to discuss follow-up of the study cohort for the next 5 years. A paper summarizing survival over the past 8 years is planned. Data from the study suggest that survival is improved if fetal hemoglobin levels are elevated by continuing hydroxyurea therapy.

At the first BABY HUG Steering Committee Meeting in September 2000, investigators discussed plans for protocol development and recruitment. The objective of the clinical trial is to determine if hydroxyurea therapy is effective in preventing chronic end-organ damage in young pediatric patients with SCA. The clinical trial will involve pediatric clinical centers with expertise in treating SCA and a medical coordinating center to supervise drug distribution, central laboratory functions, and data collection.

TTP

At a July 2000 "Workshop on von Willebrand factor and Thrombotic Thrombocytopenic Purpura," TTP investigators gained a clearer understanding of worldwide efforts to address the disorder and laid the groundwork to develop new collaborations. A summary of the workshop is planned for publication.

Problem Areas Related to Rare Diseases

AAT Deficiency

Treatment options for patients with AAT deficiency are limited. Only one product has been approved by FDA, and its approval was based on increased levels of AAT in the blood without evidence of clinical benefit. New products are in various phases of evaluation and there is much debate about whether a clinical trial is needed to assess the clinical efficacy of these new products before they are approved.

AA and PNH

The viral agent in post-hepatitis AA, which is probably the same agent that is responsible for seronegative acute hepatitis and fulminant hepatitis of childhood, needs to be identified using samples of blood, liver, and stool from patients with acute hepatitis. Better immunosuppressive treatment of AA requires large clinical trials, and patients must be recruited to specified research centers rather than

treated haphazardly in private practice. To elucidate the relationship between an autoimmune disease (AA) and clonal expansion of mutated cells (PNH), large numbers of patients must be available for study.

ARVD

A concerted multi-laboratory program combining basic, clinical, and genetic approaches is needed to identify the causes of this highly lethal form of cardiomyopathy so that a rational search for therapies can begin. Additional clinical centers, and perhaps a national registry, would be useful to investigators who are already studying its origins and potential treatments.

CJD

Standardized reference materials to validate assay systems to detect TSEs such as CJD are urgently needed. In April 1999, the WHO recommended the establishment of international reference materials for TSE diagnosis. Standards proposed would include human brain tissue, human blood, animal tissues, and animal blood. These materials would be used to calibrate the in-house reference materials of individual laboratories to the same single, international standard. The need for blind panel validation of all assays—i.e., the validation of the sensitivity, reproducibility, and predictive abilities of any given candidate assay—is emphasized. Without standardized reference materials, it is not possible to evaluate the relative merits of any assay developed or even to know for sure whether they are more sensitive than existing Western blots or ELISAs.

FA

The eight distinct complementation groups represent a high degree of locus heterogeneity, which complicates molecular diagnosis of FA and may make screening cumbersome. However, certain complementation groups prevail in specific populations (FA-C in Ashkenazi Jews, FA-A in Afrikaans-speaking people and Italians), which helps to set priorities for mutation screens. The FA-A and FA-C proteins have no sequence homologs in the current databases, although structural homologs may exist. Thus, resolution of difficulties in FA protein purification and pursuit of the X-ray crystallographic structure of FA proteins is considered a high priority. The function of FA proteins and the nature of their interaction with other proteins in the Fanconi Protein Complex as well as their relation to DNA repair are areas of current study.

GvHD

The nature of the responding cells in GvHD and reliable methods to predict and ameliorate the problem remain elusive. A challenge remains in fostering graft-versus-leukemia or graft-versus-tumor effect while avoiding GvHD. In addition, the basic immunology, biology, and tissue specificities of the response require further definition.

Infectious Myocarditis

A non-invasive test for infectious myocarditis having appropriate sensitivity and specificity is needed. At present, the endomyocardial biopsy, which is invasive and has limited specificity and sensitivity, is the gold standard for diagnosis.

LAM

Scarcity of data and LAM tissue has hindered learning about the etiology and pathogenesis of LAM. The small number of patients makes it difficult to learn about important aspects of the disease such as its prevalence, prognosis, and clinical course, or the effects of various treatments. A lack of animal models makes it necessary to obtain human cells or tissue to do LAM research. The registry will facilitate collection of clinical data and tissue and will identify a cohort of LAM patients who might be contacted in the future if opportunities for clinical studies arise. The LAM Foundation continues to facilitate collection of LAM tissue at the time of lung transplantation. Progress in LAM research has increased demand for this scarce resource. A NHLBI LAM Tissue committee is establishing procedures and guidelines for LAM tissue collection and distribution.

LQTS

Access and identification of sufficient numbers of new patients for studies remain a constant problem. Identification of mutant gene carriers would be greatly facilitated by accurate means of screening individuals in afflicted families for specific founder mutations. Improved means of identifying new mutations in the various genes involved would also be helpful. Investigators are working to increase the visibility of the registry in the African American medical community. It is not known whether this disease is less common in this group or whether African Americans are referred to the registry with less frequency than the Caucasian population.

Lymphedema

As with other rare disease, the main problem is how to interest biotechnology and pharmaceutical companies in undertaking research to find the genetic bases of the disorder and develop treatments, given that the potential profits are small.

National Human Genome Research Institute (NHGRI)

Overview of NHGRI Rare Diseases Research Activities

The mission of NHGRI is to understand the structure and function of the human genome and the role it plays in human health and disease. To that end, NHGRI supports the Human Genome Project (HGP), an international research effort to sequence the human genome and determine the function of the genes contained within the genome. The February 2001 publication of the initial sequence and analysis of the human genome was a historic scientific achievement. The sequence information from HGP has been continuously, immediately, and freely released to the world, with no restrictions on its use or redistribution. This information is a major resource for all the areas of basic and applied biomedical and behavioral research in the 21st century. HGP is already producing research tools and information that are leading to improved detection and diagnosis of genetic disorders, both by our own intramural scientists and scientists in the broader biomedical research community.

Using the information and tools produced by HGP, scientists in NHGRI's intramural research program are developing techniques to study the fundamental mechanisms of genetic disorders and genetic factors involved in common and rare diseases. These cutting-edge approaches are yielding new knowledge about human genetic diseases and their diagnosis, prevention, and treatment.

Recent Scientific Advances in Rare Diseases Research

Tools for Gene Discovery

Human DNA Sequencing

In March 1999, the HGP international consortium launched the full-scale effort to sequence the estimated 3 billion base-pairs that make up the human genetic "instruction book." In the following months, production of the human genome sequence skyrocketed; HGP produced 1,000 bases per second of raw sequence 7 days a week, 24 hours a day. June 26, 2000, marked a historic milestone when leaders of the public HGP and Celera Genomics Corporation announced that both had successfully completed the production of a "working draft" of the human genome.

Public and private research teams published their data in February 2001, including an initial analysis of the main features of the sequence. The intense phase of analyzing the sequence for gene content and a host of other biological features is under way. A list of links to a number of important Web sites that contain information about the human genome sequence, other genome sequences, and other relevant genomic information can be found at: http://www.nhgri.nih.gov/genome_hub.html.

"DNA Chip" Microarrays

The newfound abundance of genomic information is propelling scientists out of the pattern of studying genes individually. Scientists are now able to monitor thousands of genes at a time. For such large-scale analyses, miniaturized "DNA chip" technologies, also called microarrays, can be rapid, efficient, and economical. Microarrays are being used to compare gene activity in people with or without a disorder or with different clinical presentations of the same disorder. This technology will benefit rare diseases research because it can identify altered patterns of gene expression.

"Tissue Chip" Microarrays

In order to determine the importance of any gene in a more physiological setting, a second kind of array, called the tissue microarray, can confirm the importance of each gene that emerges as a candidate. NHGRI researchers have developed a way of arranging some 1,000 tiny cylindrical tissue biopsies in a small paraffin block. Tissue arrays permit researchers to examine the molecular details of many different healthy tissue types or in different stages of disease. NHGRI researchers combined cDNA and tissue microarray technologies to make rapid diagnosis of rare disorders and to better predict how a given patient will respond to available treatments and medications.

Genetics of Human Disease

Scientists in NHGRI's Division of Intramural Research (DIR) apply genomic tools to the study of human genetic diseases, many of which are rare. Research progress took place in the areas discussed below.

Severe Combined Immunodeficiency (SCID)

SCID, also called Bubble Boy disease, is a rare but devastating complete lack of T cell and B cell immunity. NHGRI scientists discovered the gene for the most common form of SCID (the IL2RG gene), which encodes the common gamma chain of receptors for several lymphocyte growth factors or cytokines. When this gene is defective, lymphocytes cannot develop normally, and affected infants therefore have frequent, severe infections that are ultimately fatal unless the immune system can be restored. Scientists are analyzing the expression and function of the common gamma chain protein. Carrier testing and genetic counseling can then be provided, as can prenatal diagnosis, which makes affected infants eligible for improved early treatments.

In addition, scientists have developed and tested methods for correcting the genetic defect in X-linked SCID by gene transfer. Clinical trials of human gene transfer are planned to treat patients with X-linked SCID who were not helped by bone marrow transplant.

Hyper IgE Syndrome (Job Syndrome)

Hyper IgE syndrome, also called Job syndrome, is an enigmatic, rare condition characterized by recurrent skin abscesses, recurrent pneumonia with development of lung cysts, and extreme elevations of serum IgE. Although the specific immune defect has not been discovered, NHGRI scientists have found that the syndrome can be inherited as an autosomal dominant disorder and, therefore, genetic studies may help find the cause. NHGRI and NIAID scientists have arrived at a new clinical understanding of the condition as a multisystem disorder with immune, dental, and skeletal abnormalities. Hyper IgE syndrome has variable expressivity and penetrance. Genome-wide linkage studies show at least three loci in the human genome that may be associated with hyper-IgE syndrome. Scientists are currently investigating the genetic regions and hope to identify disease genes for this condition.

Autoimmune Lymphoproliferative Syndrome (ALPS)

ALPS is a newly discovered syndrome in which patients have large lymph nodes and spleens, autoimmune disease, increased numbers of a rare type of lymphocyte called CD4-/CD8-T cells, and defects in programmed cell death of their lymphocytes. NIH research has shown that people with ALPS have a high risk of lymphoma. NHGRI and NIAID scientists have discovered that most patients with this

condition have inherited defects in the apoptosis mediator *Fas*. The position of mutations within the *Fas* gene influences the severity of ALPS and whether family members with the same mutation are likely to have symptoms. Mouse models for ALPS combined with studies of family members can show how varying genetic backgrounds influence the disease manifestations.

Familial Mediterranean Fever (FMF)

The aim of this study is to use transgenic mice as models to understand the role of the human FMF gene, *MEFV*, in the pathogenesis of the disease. FMF is an inherited disease with periodic fever and abdominal pain. FMF is very frequent in the Middle East and can be fatal if untreated due to amyloidosis and renal failure. NHGRI scientists have cloned this gene through linkage study and positional cloning, and found that patients with FMF have point mutations in this gene. The gene is expressed specifically in maturing granulocytes, one of the target tissues of the disease process for FMF. *MEFV* encodes for a protein of unknown function. The scientists plan to generate transgenic mice with a null-allele of the *MEFV* gene. This will enable understanding the normal function of this gene. NHGRI scientists also plan to generate transgenic mice with point mutations in the *MEFV* gene, mimicking those found in FMF patients. Such mice can be used to confirm the importance of the mutations for the induction of the disease and to study the pathophysiology of the disease, which may lead to a better treatment for FMF patients. Scientists have successfully generated mice carrying targeted interruptions of the *MEFV* gene. Mice with homozygous *MEFV* deletions grew normally and showed no visible defects or suffered any specific diseases. NHGRI scientists are conducting experiments to analyze the defensive function of neutrophils, the target cell type of the FMF disease, and the temperature control of these mice. In addition, scientists are in the process of generating additional mutations in the *MEFV* gene through ES cell gene targeting, which mimics those seen in FMF patients.

Niemann-Pick Type C (NP-C) Disease

The long-term goal of this project is to identify the gene responsible for NP-C and to study its role in the pathogenesis of the disorder. NP-C is an autosomal recessive neurovisceral lipid storage disorder and presents as variable hepatosplenomegaly, vertical supranuclear ophthalmoplegia, progressive ataxia, dystonia, and dementia. Using human positional cloning and crosses with spontaneous mouse models, scientists have identified the gene responsible for this disorder. Scientists have found that one gene, *NPCI*, has a retrotransposon insertion resulting in a loss of function of the normal gene product in mutant mice. Mutations in human individuals with NPC were also found.

Huntington's Disease (HD)

HD is an autosomal dominant progressive neurodegenerative disorder. HD onset is generally in midlife. HD is characterized by chorea, dementia, and neuropsychiatric problems. The mutation lies in the expansion of a polymorphic CAG repeat, resulting in greater than normal length of polyglutamines in the N-terminal end of the HD gene product (*huntingtin*), which is of unknown function. Scientists have created mouse models for HD that recapitulate features of behavioral abnormalities and neuropathological changes inherent in the human disease. The mice carrying the repeat expansions show progressive behavior going from hyperkinesia to hypokinesia and akinesia. Pathologically, neurons show early dendritic changes culminating in apoptotic changes and cell loss. Scientists have identified morphological changes in affected neurons of these mice that mark early events of pathogenesis, and these changes are supported independently by molecular and biochemical approaches using cDNA microarrays and immunocytochemical stains for vesicular proteins and cytoskeleton. The interplay of mutant *huntingtin* with these proteins and how these interactions could lead to neurodegenerative changes

are the subject of intense investigation by this group. These studies will lead to a better understanding of brain function as it relates to cognition, emotions, and neuronal survival, and may lead to better therapeutic regimens for HD and other degenerative disorders of the brain.

Presenile Familial Dementia with Neuroserpin Inclusion Bodies (FDNIB)

This project involves the study of a novel familial neurodegenerative disorder, presenile FDNIB. This disorder, which has a characteristic clinical course of progressive dementia and neurologic involvement, was defined in one extended family. Neuroserpin is a strong candidate gene for this disorder, and a mutation is present in this large kindred. This project will characterize the clinical phenotype, delineate the natural history of the disorder, and explore genotype/phenotype correlation in the index family. Families with immunohistopathologically neuroserpin-positive neuronal inclusion on autopsy/biopsy in an affected member(s) or with familial presenile dementia with neurologic features consistent with the original FDNIB family will be enrolled.

Hirschsprung Disease

Animals heterozygous for Dominant megacolon (Dom/+) exhibit multiple defects in neural crest development, including reduced numbers of melanocytes in the skin and an absence of myenteric ganglion in the colon. A human congenital disorder, Hirschsprung disease, also exhibits rectocolic aganglionosis and can be associated with hypopigmentation. Thus Dom/+ mice, as well as the piebald and lethal spotting mutants, serve as mouse models for Hirschsprung disease. Investigation of the involvement of Dom/+ in Hirschsprung disease will be explored.

Congenital Disorders of Glycosylation (CDGs)

CDGs are a group of metabolic disorders characterized by a wide range of phenotypic presentations, from severe developmental delay and systemic manifestations to only gastrointestinal symptoms and normal development. CDG results from defective N-linked oligosaccharide synthesis, a pathway with approximately 200 steps with different types of CDG resulting from a disruption in any individual step. NHGRI scientists are identifying new patients with CDG and conducting studies to determine the pathogenic basis for novel cases of CDG and to define the relationship between genotype and phenotype in CDG patients. As an outcome of the proposed investigations, NHGRI scientists expect to elucidate the correlation among the phenotype, the glycobiochemistry, and the genes involved.

Smith-Magenis Syndrome (SMS)

Caused by a deletion on the short arm of chromosome 17, SMS is associated with a distinct phenotype of physical features; developmental delay; speech delay with or without associated hearing loss; clinical signs of peripheral neuropathy; and neurobehavioral problems, including sleep disturbance, outbursts, and self-injurious behaviors. More than 200 individuals representing a diversity of ethnic backgrounds have been identified with SMS worldwide. Utilizing existing physical maps and comprehensive clinical analysis of the physical, cognitive, and neurobehavioral aspects of SMS, the SMS Research Team seeks to define the natural history and pathophysiology of SMS across the life span and identify genes in the chromosome 17p11.2 region that contribute to physiologic and functional aspects of human cognition, speech/language development, and behavior. The SMS Research Team is funded by a 1999 Bench to Bedside Award from the NIH CC.

Holoprosencephaly (HPE)

NHGRI researchers are studying HPE, the most common structural disorder of the developing human forebrain. HPE is associated with varying degrees of developmental disability and mental retardation. Scientists have located four genes that cause HPE in humans. These findings suggest that the following genes play an important role in the brain's separating into left and right hemispheres: Sonic Hedgehog (*SHH*), *ZIC2*, *SIX3*, and TG-interacting Factor (TGIF). Maternal diabetes, low maternal cholesterol, and other environmental factors have been associated with abnormal brain development. Analysis of regulation, interaction, and physiological role of these genes and factors will help our understanding of normal and abnormal formation of the central nervous system (CNS).

Batten Disease

Juvenile neuronal ceroid lipofuscinosis (NCL type III), known as Batten disease, is a degenerative neurological disease resulting from a lysosomal storage disorder. The Batten gene, *CLN3*, encodes a protein with a function that is not really known.

NHGRI scientists have created a mouse carrying a deletion of the *Cln3* gene that encodes a transmembrane lysosomal protein of unknown function. The mouse has the same biochemical abnormalities seen in human patients with Batten disease. Work in yeast performed at the University of Rochester has revealed that the yeast ortholog of *Cln3* is a vascular protein that, when deficient, abnormally lowers the vascular pH. NHGRI scientists hypothesized that humans (and mice) deficient in *CLN3* might store lipofuscin in their lysosomes because of abnormal depression of lysosomal pH that interferes with degradative enzyme function. Scientists have started a treatment protocol of mice with chloroquine, an alkaline base that accumulates in the lysosomes, to see if they can correct the biochemical abnormalities seen in Batten disease with this widely used and characterized drug.

Lowe Syndrome

Lowe syndrome is a rare X-linked disorder characterized by congenital cataracts, developmental delay, and Fanconi syndrome of the renal tubules. The defect is a deficiency in an enzyme, a phosphatidylinositol 4,5 bisphosphate 5-phosphatase localized in the Golgi complex, particularly the trans-Golgi network. NHGRI scientists are investigating the relationship between this enzyme deficiency and the clinical phenotype through cellular and animal models.

Idiopathic Scoliosis (IS)

IS is a structural lateral curvature of the spine present in the late juvenile or adolescent period in otherwise normal individuals. Studies from a number of populations have suggested autosomal dominant, X-linked, and/or multifactorial modes of inheritance. As part of a large collaborative study of familial IS, 200 families (1,200 individuals) with at least 2 family members with scoliosis have been ascertained and clinically characterized. A genome-wide screen for 1,200 individuals was performed at the Center for Inherited Disease Research, and preliminary analysis for linkage has been completed for all 1,200 individuals. Several candidate regions have been identified, and flanking markers are being typed to corroborate the findings from the genomic screen.

Camptodactyly-arthropay-coxa Vara-pericarditis (CACP) Syndrome

Scientists have identified mutations in a gene previously known as megakaryocyte growth and stimulating factor, which causes CACP syndrome. CACP syndrome, an autosomal recessive disease with synovial hyperplasia as the basic underlying defect, leads to several clinical phenotypes, mainly loss of proper joint growth and function. Scientists have made a mouse knockout construct and are currently studying these animals to see whether they can replicate the human phenotype in mice. This effort will allow better understanding of joint development and may lead to the identification of the basic molecular components responsible for CACP.

Human Skeletal Dysplasias

Work on this project has focused on an autosomal recessive form of skeletal dysplasia, cartilage hair hypoplasia (CHH). To identify the CHH gene, NHGRI researchers have taken the positional candidate gene approach. Known genes that were mapped to the CHH critical interval were evaluated as possible CHH candidates on the basis of their expression in CHH-affected tissues (cartilage, hair, and T cells) and in various other tissues, and on their involvement in function of relevant tissues, especially involvement in cell proliferation and/or cell cycle control. Candidate genes were screened for mutations by direct sequencing in several unrelated CHH patients of both Amish and non-Amish origin. The following genes have been fully evaluated and excluded: valosin-containing protein (*VCP*), Talin (*TLN*), carbonic anhydrase 9 (*CA9*, data not published), and camp responsive element binding protein 3 (*CREB3*). Several new polymorphic markers from the CHH critical interval became available and have enabled NHGRI scientists to reduce the CHH critical region to an approximately 0.6-Mb interval, between the markers D9S163 and D9S1804.

Hyperparathyroidism-Jaw Tumor (HPT-JT) Syndrome

In collaboration with the University of Utah, NHGRI scientists are attempting to identify the gene responsible for HPT-JT syndrome using genetic and molecular techniques. Scientists have genotyped a large set of families with a large number of genetic markers through the previously identified HPT-JT genetic interval at 1q24-q31. NHGRI scientists have successfully refined the genetic interval from roughly 30 centiMorgans to approximately 7 centiMorgans and are currently mapping and characterizing genes, which have mapped to the HPT-JT critical interval. NHGRI scientists are also using bioinformatics and databases in utilizing large amounts of draft human genome sequence from HGP to identify novel genes using gene prediction algorithms. Scientists will begin mutational analysis of a number of positively mapped genes on a set of HPT-JT patient DNA samples in hopes of identifying mutations in a gene that can be associated with HPT-JT.

Achondroplasia and other Fibroblast Growth Factor Receptor 3 (FGFR3) Disorders

The achondroplasia family of skeletal dysplasias includes three previously recognized diagnoses and one that has been defined under this project. The three well-established conditions are achondroplasia, hypochondroplasia, and thanatophoric dysplasia (TD). Work published has described a new syndrome, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN). All four disorders in this family of conditions are caused by mutations in the gene encoding FGFR3. Work during FY 2000 focused on the creation and characterization of two mouse models for FGFR3 disorders.

Marfan Syndrome and Related Conditions

NHGRI scientists are studying patients with Marfan syndrome and related conditions (Ehlers-Danlos syndrome [EDS] and Stickler syndrome). Studies have documented newly recognized gastrointestinal complications of these disorders, and that chronic musculoskeletal pain is a significant complication of both EDS and Stickler syndrome. Studies have also documented an increased risk of femoral head failure in children with Stickler syndrome. Scientists have developed proposed diagnostic criteria for Stickler syndrome on the basis of NHGRI clinical and molecular studies in this population, and have identified a previously undescribed connective tissue disorder with features resembling Marfan syndrome, Stickler syndrome, and EDS. Chronic musculoskeletal pain is a serious complication of many of the hereditary disorders of connective tissue. During FY 2000, scientists performed a pilot study of the Mindfulness-Based Stress Reduction program to examine its efficacy in the relief of chronic pain in this population.

Alagille Syndrome (AGS)

Scientists at NHGRI have shown that mutations in the Jagged1 (*JAG1*) gene are responsible for AGS, a developmental disorder affecting multiple organ systems, including liver, heart, eyes, face, and vertebrae. In order to understand the role of Jaggeds in vertebrate development and to understand how alterations in their function lead to AGS in humans, scientists have isolated and characterized three homologous genes termed *Jagged 1*, *Jagged 2*, and *Jagged 3* from zebrafish. Expression of dominant negative forms of the Jaggeds and blocking their expression with antisense oligonucleotides are being used to evaluate the function of the Jagged proteins.

Cleft Lip and Palate

NHGRI collaborates on a study of the genetics of oral clefts (cleft lip, cleft palate, or both) with investigators in Syria. Several hundred persons have been studied in Syria, and genotyping and linkage analysis of the first set of families has been completed for a genome-wide set of markers. Regions with suggestive evidence of linkage are currently being studied with fine-mapping techniques in the original set of families and in a new set of recently collected families. These analyses and the collaborative data collection in Syria will continue into FY 2001.

Pallister-Hall Syndrome, Greig Cephalopolysyndactyly Syndrome (GCPS), Polydactyly

This research study encompasses a range of phenotypes that include Pallister-Hall syndrome, the allelic disorder GCPS, and disorders with overlapping phenotypic manifestations. These overlapping disorders include the McKusick-Kaufman syndrome, Bardet-Biedl syndrome, the oral-facial-digital syndromes, and the short-rib-polydactyly syndromes. The clinical manifestations of these disorders include polydactyly, CNS malformations (with or without mental retardation and seizures), craniofacial malformations, and visceral malformations such as renal malformations or congenital heart defects. Scientists are using positional cloning strategies, biochemical approaches, and cell biologic studies to understand the genomic alterations, predict consequences to the proteins, and then integrate these findings at the cellular or embryologic level. These data are then used to develop additional hypotheses that can be investigated at the clinical or molecular level.

McKusick-Kaufman Syndrome (MKS) and Bardet-Biedl Syndrome (BBS)

NHGRI scientists have identified an altered gene responsible for MKS, a rare developmental syndrome found predominantly among the Old Order Amish population. MKS is the first human disorder to be attributed to a mutation in a gene affecting a type of molecule called a chaperonin. Chaperonins are sometimes called "protein cages," and they protect cells by capturing and refolding misshapen proteins that could otherwise interfere with normal cellular functions. Females with MKS are affected by hydrometrocolpos (accumulation of fluids in the uterus and vagina). Both affected males and females have a form of polydactyly (the presence of extra fingers or toes) and congenital heart disease. The disorder is most serious in female infants in whom the hydrometrocolpos can cause death because of lung compression complications. Further research has shown that this gene is also mutated in some persons with BBS, an inherited form of blindness, mental retardation, and obesity. These results suggest that therapies directed at chaperonin function ameliorate these symptoms.

Amish Nemaline Myopathy

This disorder, a progressive muscle disease that has so far only been identified in the Old Order Amish of Pennsylvania, causes muscle wasting that results in death before age 5 years. NHGRI scientists isolated the disease-causing mutation in this disorder in FY 2000. This alteration is in the *Troponin T1* gene, known to play a role in the muscle, but not previously known to cause any human disease. A collaborative group of scientists and physicians is working to translate these results into a potential treatment for this disease.

Lenz Microphthalmia Syndrome

This rare disorder causes small or absent eyes, mental retardation, and other anomalies. Its rarity is matched by its variability and there is a significant confusion and controversy about the range of the phenotype and its overlap with other disorders that cause microphthalmias. In collaboration with doctors at Children's National Medical Center, NHGRI scientists are analyzing a large family with multiple family members affected with this disorder. The results of this research should allow development of accurate diagnostic tests for microphthalmia and Lenz syndrome.

Cataract and Craniofacial Anomalies Syndrome

A new and rare syndrome involving congenital cataracts and craniofacial anomalies has been identified in an inbred Saudi Arabian family. The most prominent feature of the syndrome is a failure of closure of the fontanels and sutures. At birth, the anterior fontanel is large due to open sagittal and metopic sutures. The second major feature is posterior Y-shaped structural cataracts that are congenital or develop over time. Chromosomal and biochemical studies of the family were normal. A genome-wide screen was performed using 387 markers at the Center for Inherited Disease Research on 21 DNA samples. Effects to fine-map the gene are under way.

Rieger Syndrome

A continuing area of interest involves the homeodomain family of proteins, which play a fundamental role in the specification of body plan, pattern formation, and the determination of cell fate. Recent work has focused on two genetic disorders caused by defects in *Pitx2*, which codes for a homeodomain protein. Mutations in the human *Pitx2* gene in 4q25-q26 lead to two eye-related disorders, Rieger syndrome and iridogoniodysgenesis. Sequelae include iris hypoplasia and the eventual development of glaucoma. In

the past, biochemical observations explaining these diseases were documented simply as "loss-of-function," this is the first time that a concrete, structural underpinning for the development of these disorders has been proposed. Continuing work in this area involves another homeodomain protein called *FOXC1* that has also been implicated in Rieger syndrome and iridogoniodysgenesis.

Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is a behavioral disorder of childhood and adolescence, sometimes with serious sequelae in the adult years. Family, twin, and adoption studies all support the hypothesis that ADHD is a genetically influenced brain disorder. A genetic study of ADHD is being undertaken through a genome-wide search for loci linked to ADHD. The long-term goal of this study is to identify ADHD susceptibility genes. These genes will elucidate the pathogenesis of ADHD and ultimately lead to the design of individualized therapeutics. Participating individuals will undergo a battery of psychological tests that meet *Diagnostic and Statistical Manual, Fourth Edition (DSM IV)* criteria as set out by the American Psychiatric Association. To achieve this goal, we have a two-phase recruitment plan. Phase one, currently under way, recruits large, densely affected families from a South American isolate. Phase two will recruit North American affected sib-pairs.

Left-Right (L-R) Axis Malformations

A study of the complex genetics of L-R axis malformations has been undertaken with an emphasis on those genes that are associated with common phenotypes of L-R disorders, including situs inversus, heterotaxia, and organ isomerism. L-R defects can result from either environmental or genetic causes. It is the aim of these investigations to determine the genes responsible for both normal and abnormal L-R axis formation through the study of patients with these disorders. Mutations in genes such as *ZIC3*, *LEFTY A*, and *ACVR2B* have been shown to be responsible for several familial and sporadic cases of heterotaxia. It is anticipated that many additional genes important for L-R development will be identified in the search for genetic causes of laterality disorders. NHGRI scientists recently identified the human *CFC1* gene as causing laterality defects and are studying this and other genes in individuals with cardiac anomalies.

Multiple Endocrine Neoplasia Type 1 (MEN1)

MEN1 is an autosomal dominant familial syndrome characterized by multiple tumors in the parathyroids, endocrine pancreas, and anterior pituitary. Using positional cloning, NHGRI scientists identified the gene responsible for MEN1 in 1997. MEN1 homologous genes from mouse, frog, zebrafish, and *Drosophila* have been identified. A mouse heterozygous knockout model that mimics the human disease phenotype has been developed. Menin, the protein product of the *MEN1* gene, is ubiquitously expressed in all tissues of the mouse, so it is no surprise that the conventional knockout mouse (i.e., the gene product is not functional at all in the mouse) is embryonic-lethal. Studies are under way to evaluate the gene expression changes in mouse embryo fibroblasts grown in culture carrying the three *MEN1* genotypes, +/+, +/-, and -/-.

Rare Diseases Research Initiatives

Rare Diseases Meetings and Workshops

The First NIH Conference on Holoprosencephaly (HPE)

NHGRI, ORD, and The Don and Linda Carter Foundation sponsored an April 3-6, 2000, conference on holoprosencephaly. The conference consisted of two consecutive programs. During the first session, specialists from many different areas of biomedical research were invited to discuss their work. The second session consisted of presentations and activities that deal with the more common problems encountered by families with someone with HPE. The goals of the conferences were to bring parents, researchers, and professionals involved with the care of persons with HPE together to exchange information, ideas, and scientific data, and to share the family experience.

The Second National Conference on Smith-Magenis Syndrome (SMS)

NHGRI, ORD, and the support group Parents and Researchers Interested in Smith-Magenis Syndrome (PRISMS) co-sponsored an educational conference on SMS held September 21-24, 2000. The goal of the conference was to bring parents, researchers, and professionals involved with the care of persons with SMS together to exchange information, ideas, and scientific data, and to share the family experience. Attendees heard from medical experts, geneticists, and parents about the latest information on the medical, social, developmental, and behavioral aspects of SMS, as well as current research efforts into the cause, management, and effective treatment of SMS.

Immediately preceding the educational conference, the PRISMS Professional Advisory Board convened a small SMS research roundtable discussion of basic scientists and clinicians currently working on SMS to share, review, and discuss current findings and plans for needed future research on SMS.

Additional Activities

Genetic and Rare Diseases Information Center

In order to respond to the public's need for information on genetic and rare disorders, NHGRI and ORD plan to establish the NHGRI/ORD Genetic and Rare Diseases Information Center. The Information Center will focus on meeting the information needs of the general public, including patients and their families, health care professionals, and biomedical researchers. The purposes of the Information Center are to: 1) serve as a central, national repository of information materials and resources on genetic and rare diseases, conditions, and disorders; 2) collect, produce, update, and disseminate information on the diagnosis, treatment, and prevention of genetic and rare disorders; and 3) coordinate with organizations and associations interested in genetic and rare disorders to explore networking capabilities, avoid duplication of effort, and identify information gaps.

Office of Clinical Liaison (OCL)

The Office of the Clinical Director, NHGRI, formed OCL in response to the increasing volume of clinical inquiries to NHGRI from the public. OCL centralizes the management of clinical inquiries about rare genetic diseases that are sent to investigators within NHGRI. Most of the 400 inquiries in FY 2000 handled by the staff, which includes two senior genetic counselors and a physician, were from parents and

physicians of children with rare genetic disorders. The goal of OCL is to refer people to resources such as genetic counseling, family support groups, or medical experts in these rare disorders. OCL utilizes established programs, such as the Genetic Alliance and ORD, and customizes each reply.

National Institute of Mental Health (NIMH)

Overview of NIMH Rare Diseases Research Activities

NIMH is dedicated to ensuring and improving the scientific foundation for the diagnosis, treatment, and prevention of mental illnesses. To continue to expand this scientific base, NIMH conducts and supports research in basic and clinical neurosciences, behavioral sciences, epidemiology, and mental health services and service systems. As understanding of the neurobiological and behavioral bases of mental disorders has grown, insight into rare diseases affecting the brain and behavior is being gained.

Recent Scientific Advances in Rare Diseases Research

Anorexia Nervosa (AN) and Bulimia Nervosa (BN)

Eating disorders are often chronic relapsing disorders that have some of the highest death rates of any psychiatric illness. Recent studies have shown that AN and BN run in families, and studies in twins suggest a genetic basis for the high rate of these eating disorders in certain families; that is, 50% to 80% of the factors contributing to the development of eating disorders are genetic. This means that AN and BN may be as heritable as schizophrenia or bipolar disorder, illnesses that have long been regarded as exhibiting a strong degree of genetic vulnerability.

Twin, family, and genetic studies support the possibility that some underlying trait, such as a vulnerability to an imbalance in the serotonin system, place someone at risk for developing an eating disorder. Recent studies have established that the leptin system also is fundamental in the regulation of energy balance and neuroendocrine function and that the leptin and serotonin systems may interact in an important manner to influence ingestion. Current studies are also examining the role of various hormone levels in the modification of taste signals, which could help us better understand the way ingestion-related information is processed in individuals with AN and BN.

Individuals with AN often exhibit symptoms of other psychiatric disorders, such as major depression and obsessive compulsive disorder, which respond favorably to medication. It has been somewhat puzzling that medical treatment for AN has been consistently unsuccessful. One possible reason for the lack of medication effectiveness may be the associated neurochemical changes due to starvation. Currently funded research is under way to examine the utility of medication for patients with AN who have regained body mass into normal range as they leave the hospital. In addition to the cognitive behavior therapy, the effects of fluoxetine, compared to placebo, will be examined with regard to protection against relapse.

Efforts to prevent eating disorders have been limited by difficulties in refining specific risk factors. Two separate randomized prevention trials have identified body image concerns and unhealthy weight control methods as symptoms to target to prevent onset of more severe forms of eating disorders. Each of these trials are school-based (high school and college), target young women, and utilize the Internet as a resource for monitoring attitude and knowledge change, as well as monitoring homework assignments.

Completed Suicide

Suicide is a rare behavior that, nearly 90% of the time, occurs in the context of a mental and/or substance abuse disorder. 1998 statistics indicate that 30,575 Americans took their own lives; however, for every completed suicide, there are an estimated 8 to 25 suicide attempts. Individuals who complete suicide have overlapping, but quite distinct, characteristics from persons who attempt suicide. For example, estimates of attempted suicide indicate that twice as many women attempt suicide as men; however, five times as many men as women actually commit suicide. To best determine risk factors for completed suicide compared to attempted suicide, researchers have focused on postmortem research methods to develop both psychological and biological risk profiles.

The psychological autopsy is a method used to determine risk factors for suicide. NIMH has funded psychological autopsy research for youths and is currently funding a study focused on older adults. Youth suicide victims are more likely to have a mood, conduct, and/or substance abuse disorder, and a history of a past suicide attempts. In contrast, older suicide victims are more likely to have a later-onset mood disorder or a physical illness, but less likely to have a substance abuse disorder or to have made suicide attempts. NIMH-supported research from long-term studies of depression has found that recurrent hopelessness is a key risk factor for poor treatment adherence and consequent death by suicide.

NIMH-supported research on biological risk factors for suicide has resulted in some compelling new findings. Investigators long suspected that mental disorders like depression that have a high risk for suicide may involve measurable changes in brain chemistry. To address this question, several laboratories have looked at the postmortem brains of suicide victims to determine if neurochemical differences occur. While some of the details of these findings are still under investigation, it is clear that there are differences in specific classes of neurotransmitter systems (e.g., serotonin, dopamine, and noradrenaline) in the brains of suicide victims. To further refine our understanding of these differences, researchers are examining the levels of these neurotransmitters in the brain, the expression of their receptor molecules, and the other chemical elements that play critical roles in their synthesis and regulation. Such refined investigations suggest a complex but very interesting conclusion about the neurobiology of suicide. It is clear that serotonin and dopamine levels are significantly different in brains of suicide victims who were also suffering from major depression. This is consistent with earlier findings. There is also, however, a population of suicide victims who were not depressed whose brains also show differences in serotonin and dopamine measures, but these differences are not the same as for depressed suicide victims. This is a very profound finding because it suggests a neurobiological risk factor for suicide that is independent from major depressive illness and other affective disorders. Although it is still early in the discovery process, it is not unreasonable to speculate that these findings will have a major impact in the diagnosis and potential treatments for individuals at risk for suicide.

Rare Diseases Research Initiatives

Completed Suicide

In late 1999, NIMH commissioned two comprehensive reviews of assessment instruments for suicidal behaviors and cognitions, one for children and one for adults. The review for youth is posted on a NIMH Web page (see <http://www.nimh.nih.gov/research/measure.pdf>), and the review for adults will soon be available. Recent NIMH initiatives have also encouraged the inclusion of more diverse samples of patients, including suicidal patients, in treatment protocols. In order to help protect these patients and to encourage more researchers to include suicidal patients in research, NIMH staff have worked with experts

in bioethics and suicide treatment research to develop a paper that describes various approaches to considering the ethical issues in treating suicidal patients, and ways to increase the safety and monitoring of suicidal patients in clinical trials. This paper, "Issues to Consider in Intervention Research with Persons at High Risk for Suicidality," is available on the NIMH Web page (see <http://www.nimh.nih.gov/research/highrisksuicide.cfm>). A workshop scheduled for June 2001, co-sponsored by NIMH, ORD, and the American Foundation for Suicide Prevention, will further consider consent and capacity issues involved in enrolling and treating suicidal individuals in clinical trials.

NIMH, along with NIDA, NIAAA, SAMHSA, and CDC, is funding an Institute of Medicine (IOM) study, "Pathophysiology and Prevention of Adolescent and Adult Suicide."

NIMH awarded a conference grant (R13) to the University of Rochester in FY 2000 to conduct five annual meetings to review and develop scientific consensus on suicide risk factors and prevention strategies for a number of at-risk groups. There are multiple NIH and other agency co-sponsors. The first of these meetings was held in June 2001 and focused on suicide prevention in adolescents and young adults.

Rare Diseases Program Activities

Eating Disorders: Workshop Update

In April 2000, NIMH sponsored a "Prevention of Eating Disorders Roundtable" to discuss the status of efforts in eating disorders, knowledge gaps, and future research activities to be considered, including the state of the science in understanding risk factors such as neuroscience contributions; state of the science in effective preventive interventions for eating disorders and other relevant disorders (depression, substance abuse); and challenges to developing prevention efforts in eating disorders. A summary is in draft form and will be published on the NIMH Web page.

Completed Suicide

NIMH and NIDA continue to receive applications in response to the March 2000 PA, Interventions for Suicidal Youth, to support efforts to develop and test interventions that build on both risk and protective factors for youth suicidal behavior. This PA identifies the need to test the effectiveness of interventions for reducing suicidal behavior for a number of approaches, ranging from broad-based community or school-based prevention efforts, to more targeted approaches that reduce suicidal behavior in youth with identified mental disorders or substance use disorders (SUDs).

Pediatric HIV/AIDS

In the United States, pediatric HIV/AIDS infection has become a rare disease as a result of medication advances and behavioral prevention efforts. Vertical transmission of HIV has been reduced significantly, though not completely, as a result of prenatal and postnatal medication administration. For children born with HIV, the use of antiretroviral therapy (ART) has prolonged life expectancy and quality; however, children with HIV/AIDS face major issues coping with the physical and psychological burden of HIV as they progress through childhood and adolescence.

Research on the mental health consequences of HIV for these children as they enter adolescence is somewhat limited. In order to improve the quality of life for children and youth living with this disease,

the Center for Mental Health Research on AIDS (CMHRA) at NIMH supports the development of research on coping with pediatric AIDS. In FY 2000, CMHRA supported more than 50 grants on research with HIV-seropositive children/adolescents, as well as prevention research for non-infected youths. In order to further develop research in this area, CMHRA and ORD are co-sponsoring a meeting in the fall of 2001 to address the neurological, neuropsychological, psychosocial, and mental health consequences of HIV infection in children and adolescents. Topics will include the long-term physical, cognitive, and behavioral effects of antiretroviral therapy, coping with HIV as a chronic illness, and adherence to treatment. The meeting will help identify research priorities in the area of pediatric AIDS.

National Center on Minority Health and Health Disparities (NCMHD)

Overview of NCMHD Rare Diseases Research Activities

Effective January 16, 2001, the Minority Health and Health Disparities Research and Education Act of 2000 (P.L. 106-525) established NCMHD. The Office of Research on Minority Health (ORMH) within the Office of the Director (OD) was administratively abolished, and its programs were transferred to the new Center. NCMHD conducts and supports research, training, information dissemination, and other programs aimed at reducing the disproportionately high incidence and prevalence of disease, burden of illness, and mortality experienced by certain American populations. These populations include racial and ethnic minorities and groups such as the urban and rural poor who experience disparate health status.

Ethnic and racial minorities may also experience a higher incidence and prevalence of rare diseases than the general population for a variety of reasons, genetic and otherwise. For example, certain conditions such as sickle cell anemia (SCA) and keloid formation are linked to genes that are predominantly found within one racial group. Alternatively, for rare diseases such as systemic lupus erythematosus (SLE) and breast cancer, which tend to occur across populations, there are still significant disparities in risk of disease development, severity of symptoms, and mortality. Indeed, racial and ethnic minorities and other underserved populations are likely to experience even greater barriers to screening, diagnosis, and treatment of rare diseases than for common conditions due to a variety of cultural, socioeconomic, and environmental factors.

This section of this report highlights the rare disease research that is supported by NCMHD in partnership with other NIH Institutes and Centers. All projects are aimed at increasing knowledge of, reducing, and/or eliminating health disparities and include significant racial and minority group participation. Several projects, including the Sudden Infant Death Syndrome (SIDS) Minority Outreach project and the Jackson Heart Study, involve extensive community outreach. The Jackson Heart Study, Collaborative Clinical and Molecular Correlative Studies, and Regional Research Centers for Minority Oral Health emphasize research training for minorities and research capacity-building at minority-serving institutions.

Ongoing Rare Diseases Research and Recent Scientific Advances

NIA/NCMHD

Genetic Epidemiology of Alzheimer's Disease in Hispanics

Alzheimer's disease is a degenerative brain disease that impairs mental and emotional functioning in older adults. Genetic influences of Alzheimer's disease are being investigated among patients and their siblings in the Caribbean Hispanic population in New York City and in the Dominican Republic. The goal is to identify chromosomes containing genes that may increase the risk of Alzheimer's disease in members of this ethnic group. Four hundred and fifty sib-pairs will be included in the study; 225 families have been identified, in which there are up to 213 sib-pairs. Genome screening of each individual is in progress.

NIAMS/NCMHD

Degenerative Spondylolisthesis in Black Women

Degenerative spondylolisthesis is characterized by degenerative arthritis of the spine, resulting in forward slippage of all or part of one vertebra on another in the lumbar spinal region. In the ongoing Study of Osteoporotic Factors, 476 elderly African American women have been radiographed and have completed questionnaires on pain symptoms and related disabilities, with assessment now under way to determine the burden that degenerative spondylolisthesis imposes on this population.

Mapping of Vitiligo Susceptibility Genes

This initiative will examine familial vitiligo to identify the gene or genes associated with this progressive, chronic condition of white skin patches. Vitiligo affects all racial groups but is very psychologically damaging to dark-skinned populations. A probable gene for familial vitiligo has been found. To corroborate this finding, two independent populations in the United States and the United Kingdom are being studied.

Specialized Center of Research (SCOR) in Scleroderma

Scleroderma is a connective tissue disorder that causes tissue scarring within the skin, internal organs, and small blood vessels. NIAMS will begin a fifth year in its study of scleroderma in minority populations. The Choctaw Native American people in Oklahoma have a high prevalence of this disease. A large number of candidate genes have been ruled out, with evidence now indicating a genetic association with fibrillin-1, osteonectin, and *MHC*. In addition, data from a multiethnic longitudinal study of patients with disease duration of less than 5 years indicates potentially significant genetic, sociodemographic, and behavioral background factors among the groups studied.

Arthritis Among Latinos: A Study Based on National Data

Although arthritis means "inflammation of the joints," the term is generally used to refer to more than 100 rheumatic diseases that can cause pain, redness, heat, swelling, stiffness, and decreased joint function. These diseases may affect other parts of the body, including connective tissues, muscles, tendons, ligaments, bones, and internal organs. Involving a secondary analysis of data from the National Health Interview Survey, this 1-year study's goal is to uncover the underlying factors related to observed differences between Latinos and non-Latinos in the prevalence rates of arthritis and of associated levels of disability (both being greater in the Latino population). Ultimately, this information will be used to guide policy and intervention programs aimed at reducing physical disability and improving health promotion in the Latino population.

Molecular Mechanisms for Keloid Formation

This project focuses on investigating the epidemiologic, genetic, and molecular aspects of keloid development, an abnormal wound healing process resulting in tissue scarring (overgrowth of fibroblastic tissue). It is anticipated that the number of subjects participating in the study including probands and family members, will range between 500 and 1,000. The majority of subjects are African Americans, whose incidence of keloids is very much greater than in other populations. To date, no genes have been identified, but results are expected as the research sample grows.

Hypoxia Regulation of VEGF/VEGF Receptors in Keloids

This is a newly funded collaborative study conducted by the Children's Hospital of Los Angeles, the University of Southern California, and NCI to examine the role of vascular endothelial growth factors in keloid vascular homeostasis.

SLE

SLE is an autoimmune disease of unknown etiology in which autoantibodies damage one's own tissues and, potentially, multiple organs. It is primarily a disease of women but is more prevalent in African American, Hispanic, and Native American women than in Caucasian women. Seven studies are in progress. Three focus on identification of genes related to clinical manifestation of SLE, with the hypothesis that the disease is polygenic. Four studies are examining related biochemical phenomena in SLE patients. One study of African American, Hispanic, and Caucasian patients is in the process of assessing relative influences of socioeconomic-demographic, behavioral-cultural, and immunogenetic factors in patients with SLE. The projects are:

- Gene Mapping in Women with SLE.
- Genetics of Childhood-Onset SLE.
- A Genetic Association with Lupus in American Blacks.
- Patient-Oriented Research: SLE.
- Accelerated Atherosclerosis in SLE: Prevalence Factors.
- Ectopic Germinal Center Reaction in SLE.
- Role of Nitric Oxide (NO) in SLE.
- Outcomes of SLE in Minorities: Nature versus Nurture.

NHLBI/NCMHD

Familial Aggregation and Natural History of Sleep Apnea

Sleep apnea is a potentially life-threatening condition characterized by interrupted breathing during sleep (and thus interrupted sleep). Early recognition and treatment of sleep apnea is important because this condition may be associated with irregular heartbeat, high blood pressure, heart attack and stroke. Four hundred African American and Caucasian subjects have participated in a study to determine genetic variation in predisposition toward sleep apnea. It has been demonstrated that African Americans have almost 3.5 times the risk level of Caucasians of developing sleep apnea. Obesity, sinus problems, and asthma, while all predisposing factors, do not account sufficiently for African American children's high risk of the condition. There is evidence of the role of a major recessive gene. In fact, the risk of inheriting sleep apnea was shown to be independent of the risk of obesity. Information gained from this study will be used to improve strategies to reduce related co-morbidities such as hypertension in those populations at genetic risk.

Adrenergic Receptors and Hypertension in Blacks

In this study, the role of alpha-2 adrenergic receptors is being examined: the genetic factors in these receptors' mediation of platelet aggregation and central baroreceptor activity, as well as their role in transport of chloride possibly being a necessary link between the autonomic nervous system and salt-sensitive hypertension. Four hundred African Americans are being studied. Hypertension often has

no symptoms but may lead to stroke, heart attack, kidney malfunction, or blindness if undetected and untreated.

Sarcoidosis Genetic Linkage Consortium

Sarcoidosis is a disease of unknown origin involving formation of granulomatous lesions, especially in the lungs (for 90% of patients), liver, lymph nodes, and skin. The goal of this project is to identify sarcoidosis susceptibility genes and determine how these genes and environmental risk factors affect the manifestation of this systemic disease, of which African Americans have a higher incidence and experience a more severe form. NCMHD funds are being used to increase the planned efforts of an epidemiologist, data coordinator, data entry person, and computer programmer to a level considered sufficient for successful completion of the study.

Cellular Phenotypes of Salt Sensitivity

This study examines the role of cellular calcium turnover rate in salt-sensitive hypertension, which has a high prevalence in the African American population. Approximately 32 African Americans from the study population will be examined in depth as inpatients. A preliminary finding is that a male/female difference in blood pressure regulation is related to the menstrual cycle. Characterization of cellular calcium regulation in men and women on varying sodium intake levels is in progress. This information could ultimately be used as a predictor of the body's response to changing sodium diets.

Jackson Heart Study (JHS)

The JHS is a partnership among Jackson State University, Tougaloo College, the University of Mississippi Medical Center, NHLBI, and NCMHD. The goals of this study are to:

- Establish a single-site cohort study to identify the risk factors for the development of cardiovascular diseases, especially those related to hypertension, in African American men and women.
- Build research capabilities in minority institutions through partnerships.
- Attract minority students to careers in public health and epidemiology.
- Establish a NHLBI field site in Jackson, Mississippi (similar to those established for the Framingham Heart Study and the Honolulu Heart Program), which will include a strong community health education component.

The study will have a sample size of approximately 6,500 men and women aged 35-84 years and will include approximately 400 families. Recruitment began in September 2000. Strategies to increase community and individual awareness have resulted in an increased enrollment rate.

Genetic Analysis of Human Hypertensive End-Stage Renal Disease (ESRD)

The focus of this initiative is a search, through a variety of research approaches, for genes that predispose the African American population to hypertension-associated ESRD. NCMHD support has been targeted to add a sample of the Mexican American study population through the support of a full-time recruiter. In addition, funds will purchase an additional piece of equipment to increase efficiency and to handle the increased number of samples that generate genetic data.

NIDDK/NCMHD

African American Study of Kidney Disease and Hypertension

This multicenter clinical trial is investigating treatment interventions to slow the progression of kidney disease in African Americans with hypertension. A total of 1,094 patients were randomized to one of three antihypertensive medications. The patients taking antitension-converting enzyme inhibitor (Ramipril) were less likely to have progressive worsening of kidney function than those on the calcium channel blocker, so the latter regimen was eliminated. NCHMD has provided substantive support to the trial since FY 1992 and is now providing ongoing support for three minority/majority collaborations in the full-scale trial, and also for the Howard University Center.

Gestational Diabetes Component of Diabetes Prevention Program

A total of 3,234 subjects have been enrolled in a 3-arm study with 2 active treatment groups (medication and lifestyle). Sixty-eight percent are women, and 165 of these have or have had gestational diabetes. More than 45% of the study participants are from minority groups. NCMHD funds have provided significant recruitment and retention resources to randomize women, those with gestational diabetes, and minorities into the program. The high level of compliance by volunteers will enhance research staff's ability to assess prevention and intervention strategies.

Birth Weight and Gestational Age as Predictors of Chronic Disease Risk

This investigation is examining the effects of a woman's growth before her own birth on her risk for gestational diabetes and for pregnancy-induced hypertension as an adult. Information on approximately 70,000 women has been collected and is being analyzed. These women are of a variety of ethnicities, were born in New York State after 1959, and delivered a singleton infant between 1991 and 1996. Results are expected in FY 2001.

Hepatitis C Antiviral Long-term Treatment against Cirrhosis Trial: Enhanced Minority Recruitment

Hepatitis C is a blood-borne virus that inflames and damages the liver, causing a contagious chronic liver disease that may result in cirrhosis or liver cancer. With some minority subpopulations at a higher risk for hepatitis C, enhanced recruitment for a cirrhosis treatment study was warranted. Of a total recruitment goal of 1,350 patients, with 20% from racial minorities, 222 have been enrolled, 22% being minority patients. Contacting minority physicians at the study sites for potential enrollees has contributed to the improved minority enrollment.

Magnesium and Sickle Cell Disease (SCD)

In this 5-year study (1 year supported by ORMH/NCMHD), clinical and cell studies are indicating that magnesium, in preventing the loss of potassium, is a possible therapy for SCD. Potassium loss, causing red cell dehydration, leads to the characteristic sickling of the cells, preventing their uptake of oxygen. Use of magnesium not only reduces cell dehydration, but also reduces the occurrence of painful crises in sickle cell patients.

NCI/NCMHD

Cellular Responses to Mutagens in Lung Cancer Case and Controls: A Focus on Gender and Race

This intramural initiative seeks to determine if mutagen sensitivity, p53 induction, and apoptosis in cultured lymphocytes will be predictive of lung cancer risk and if this predictability varies by gender or race. Lung cancer is the most common cause of cancer-related deaths in African Americans and Caucasians, and it has been proposed that African Americans have a higher risk than Caucasians for developing the disease at a given level of smoking. Two hundred of each population group with confirmed lung cancer are being compared to 2 control groups, 1 hospital-based and 1 population-based, with a total of 400 of each of the 2 ethnicities.

Collaborative Clinical and Molecular Correlative Studies in Minority Populations Involving Genitourinary and Gynecologic Malignancies

The purpose of this project is to assess the relationships between a series of molecular markers found in tumor tissue samples and defined clinical outcomes in African Americans and Hispanic Americans with genitourinary (e.g., prostate) or gynecologic malignancies. Three institutions are providing both research materials and investigators: Howard University, Louisiana State, and the Medicine Branch. Both minority and majority investigators are involved in this study.

Gene-Environment Interactions for Breast Cancer Risk and Survival in Different Racial and Ethnic Groups

This intramural project is an exploration of gene-environment interactions for breast cancer risk and survival in Caucasians, African Americans, and Hispanics. The study will allow for direct comparison of risk factors for both initiation and aggressive disease.

Study of Breast Cancer, Breast Disease, and Pesticides Among a Population from Triana, Alabama

This study is a continued evaluation of pesticide (DDT) exposure, a potential risk factor for breast cancer, in a rural, low-income, and predominantly African American cohort of women.

NIDCR/NCMHD

Oral Infections, Carotid Atherosclerosis, and Stroke

This is a prospective study to determine the contribution and impact of periodontal infections on the risk of atherosclerosis and cardiovascular disease. Participants from three ethnic groups have been drawn from the Northern Manhattan Stroke Study; they are being screened at baseline and will receive telephone follow-up for 3 years. Pathological outcomes to be focused on are thickening of the carotid artery wall and the amount of time before a cerebrovascular event occurs.

NICHD/NCMHD

Back to Sleep Campaign (Sudden Infant Death Syndrome [SIDS] Minority Outreach)

SIDS is the leading cause of death in infants between 1 and 12 months of age, and it appears that lying supine (face-up) rather than prone (face-down) reduces the likelihood of this occurrence. This project is focused on the development and distribution of culturally appropriate educational materials for African American and Hispanic communities, and on partnering with community-based organizations for the conduct of community outreach activities targeting SIDS. A variety of materials are now in use, including a resource kit and bus advertisements. NCMHD funds are also being used to support the National Black Child Development Institute's activities regarding SIDS.

NIEHS/NCMHD

The Hormonal and Environmental Risk Factors for Systemic Lupus Erythematosus (SLE): The Carolina Lupus Study

This is the first population-based epidemiologic study in the United States of SLE, a chronic, disabling disease that disproportionately affects African American women. The study provides the opportunity to examine occupational and environmental risk factors in a previously understudied population. Efforts may help to illuminate etiologic pathways and to develop targeted preventive measures. Sixty percent of subjects are African Americans.

NEI/NCMHD

Myopia Development in Children

Myopia Development in Children is an expansion of the Orinda Longitudinal Study of Myopia initiated in 1989, which focuses on an investigation of ethnicity and the development of myopia. Three new study populations have been added: African American, Asian American, and Hispanic American children. While fewer than 2% of children beginning elementary school in the United States are nearsighted, or myopic, the prevalence of myopia increases to more than 15% among middle school graduates, and to 25% of the adult population.

National Institute of Neurological Disorders and Stroke (NINDS)

Overview of NINDS Rare Diseases Research Activities

NINDS conducts and supports research on the causes, diagnosis, treatment, and prevention of hundreds of disorders that affect the nervous system. Most of these disorders can be considered rare. The sheer number of rare diseases that affect the nervous system, combined with their profound impact on the individuals and families affected, presents a significant challenge to NINDS. We must individually address each disorder to determine the cause, underlying pathophysiology, and potential treatment, while also supporting research that may more broadly impact multiple fields.

Because many of the diseases studied by NINDS are rare disorders, the following highlights represent only a snapshot of the involvement of NINDS in this area of research.

Ongoing Rare Diseases Research and Recent Scientific Advances

Lysosomal Storage Disorders (LSDs)

Lysosomes are structures inside cells that contain enzymes capable of breaking down molecules such as carbohydrates, fats, and proteins. The LSDs are a large family of diseases that share a common feature: a specific enzyme is defective in each, resulting in the toxic buildup of cellular products that would normally be destined for destruction. Many of these disorders result in neurological dysfunction, as well as a range of effects throughout other organs in the body. The LSDs include Tay-Sachs disease; the lipofuscinoses, one of which is known as Batten disease; Gaucher disease; and Fabry's disease. The two most common LSDs, Gaucher disease and Fabry's disease, are both caused by a build-up of fatty molecules in the body.

NINDS intramural scientists have played a significant role in making progress in treating these disorders, beginning a decade ago with the development of enzyme replacement therapy that could successfully treat type I Gaucher disease. Gaucher disease is characterized by a build-up of the fat glucocerebroside, and it affects the spleen, liver, lungs, bone marrow and, in some cases, the brain. Similarly significant contributions in developing effective therapies for Fabry's disease have been made by NINDS intramural researchers in FY 2000. In Fabry's disease, accumulation of the lipid globotriaosylceramide can cause damage to the kidneys, heart, and blood vessels of the brain, leading to death by the fourth or fifth decade of life. Previous work had demonstrated that the critical lipid breakdown enzyme needed for these patients could be isolated from placental tissue, and had shown that administration of this enzyme to individuals with Fabry's disease reduced the levels of globotriaosylceramide in the blood. Lack of sufficient quantities of the enzyme hampered further tests, however. To overcome this limitation, the researchers developed a procedure to prepare the enzyme using DNA technology and human cells in culture. With the resulting adequate supply of enzyme, NINDS researchers conducted a phase I safety and dose-escalation clinical trial showing that this enzyme therapy was safe and that it reduced globotriaosylceramide in the liver, blood, and urine. Moreover, several of the patients were able to permanently discontinue the medications they were taking for the pains in their hands and feet. This study, published in January 2000, provided the basis for a double-blind placebo-controlled phase II clinical efficacy trial of enzyme replacement therapy in Fabry's disease that recently confirmed the reduction of pain in the hands and feet in subjects, as well as improvements in kidney and heart function.

Publication of these results was expected in June 2001. Additional clinical trials are presently in the planning phase.

In collaboration with NICHD, NINDS has also provided support for a recent study that shows promise in developing a treatment for the infantile form of neuronal ceroid lipofuscinoses (or INCL). INCL is caused by a failure of enzymes called lysosomal proteases to break down specific lipid-protein linkages, leading to a build-up of these molecules. The result is degeneration of vision and brain death within the first several years of life. A recently published study (based in part on work conducted in FY 2000) has shown that the drug phosphocysteamine, a compound that has been used for two decades to treat cystinosis, another LSD, may also have potential in treating INCL. In test preparations, phosphocysteamine enhanced the depletion of the abnormal lysosomal deposits that are characteristic of INCL and inhibited cell death. In addition to its potential efficacy in treating INCL, phosphocysteamine is also known to be relatively non-toxic, has a long record of clinical safety, and is believed to cross both the blood-brain and the fetoplacental barriers. A clinical trial of this therapy was initiated in March 2001 by NICHD intramural researchers with collaboration from NINDS and NEI.

Trinucleotide Repeat Diseases

The trinucleotide repeat diseases are a family of disorders caused by abnormal expansion of specific pieces of genetic information called nucleotides. The sequence of nucleotides in each gene directs a cell to produce a specific protein. When an inappropriate expansion of a coding region occurs, the normal protein is not produced. Fifteen neurological disorders are believed to be caused by expansions of nucleotide "triplets," including Huntington's disease (HD), the spinocerebellar ataxias, and Friedreich's ataxia (FRDA). The degree to which these sequences are expanded, and the gene in which the expansions are located, affect the onset, symptoms, and severity of the disease. Many of these expansions affect the genes, and subsequently the neurons, that regulate movement. Thus ataxia, a problem with motor coordination, is one of the most significant features of this family of diseases.

NINDS intramural and extramural researchers are actively involved in research on the pathophysiology and treatment of these disorders. Intramural scientists are currently developing therapies for FRDA, the most common hereditary ataxia, which affects several thousand individuals in the United States. FRDA causes progressive damage to the nervous system resulting in symptoms ranging from muscle weakness and speech problems to heart disease. FRDA typically leads to increasing debilitation and death in early adulthood. At a recent international scientific workshop on FRDA co-hosted by NINDS, French investigators reported promising preliminary research results from a study of the drug compound idebenone based on the hypothesis that the drug may act on iron metabolism pathways in FRDA. Idebenone was originally developed as a neuroprotective antioxidant for treatment of stroke and dementia, but did not show efficacy in these uses. The research results were sufficiently encouraging in reducing the severity of heart disease in individuals with FRDA that NINDS plans to initiate a study to determine the effectiveness of idebenone in the treatment of the disorder. Sufficient supplies of the drug have recently been secured, and an IND application for a phase I dose-escalation trial is currently being prepared for submission to FDA.

NINDS-funded extramural researchers are also exploring many other trinucleotide repeat disorders. A recent report from one group, published in February 2000, has provided new insight into the mechanisms by which abnormal nucleotide repeats may cause neuronal damage in a mouse model of spinocerebellar ataxia-1 (SCA1). In this disease, the presence of the mutant form of the ataxin-1 protein in brainstem and cerebellar neurons leads to cell death and subsequent behavioral symptoms. The mechanism by which

the expanded gene sequences initiate these degenerative events has not yet been characterized, however. To address this issue, investigators explored the possibility that changes in the expression of other specific genes may be an initial effect of mutant ataxin-1, and that these events may play a role in cell survival/death. Using animal models of SCA1, researchers identified several genes that were down-regulated. These genes were localized to a type of cell in the cerebellum that is essential for proper movement control. Moreover, the genes were affected early in the disease process and, in some cases, were down-regulated in postmortem SCA1 human tissue as well. Although the relationship of abnormal ataxin-1 to these changes in gene expression is still poorly understood, these events may be of use in characterizing the very earliest stages of the disease process.

Animal Models of Rare Diseases

Genetic mutations cause a great number of the rare neurological diseases studied by NINDS. Examples of these types of disorders include Rett syndrome, several forms of muscular dystrophy and the LSDs, the hereditary ataxias, Canavan disease, and neurofibromatosis. Although correction of these genetic defects is a difficult task, a critical step following gene discovery is the mutation of these genes in appropriate experimental animals. The resulting models are useful for evaluating both disease pathology and preclinical therapies. NINDS continues to play an active role in supporting the development of disease models. For example, Canavan disease is a rare inherited neurological disorder caused by inherited defects in the enzyme *aspartoacylase* encoded by the gene *ASPA*. This defect impairs the normal growth of myelin, leading to a spongy degeneration of the brain (in which the white matter is replaced by microscopic fluid-filled spaces). Symptoms include mental retardation, loss of previously acquired motor skills, feeding difficulties, abnormal muscle tone (i.e., floppiness or stiffness), poor head control, and megaloccephaly (abnormally enlarged head). Paralysis, blindness, or hearing loss may also occur. The disease is not currently treatable, and death usually occurs by 4 years. Gene therapy has been attempted in this disorder, but the development of an animal model of the disease was greatly needed to evaluate possible treatment delivery strategies. In the spring of 2000, NINDS-funded researchers published a study of the development and characterization of a mouse model for Canavan disease, generated by "knocking out" the *ASPA* gene. This model will offer clinical researchers an opportunity to further understand the disease, and to refine gene therapy vectors and other strategies that may be useful in clinical trials.

The neurofibromatoses are genetic disorders that cause tumors to grow on nerves and produce other abnormalities, including skin changes, optic gliomas, malignancies, vision loss, learning disabilities, and bone deformities. There are two distinctly different classifications of the disorders: neurofibromatosis type 1 (NF1), the more common type; and neurofibromatosis type 2 (NF2). Both are transmitted in an autosomal dominant fashion, although the *NF1* gene is located on chromosome 17, and the *NF2* gene is on chromosome 22. Both genes encode tumor suppressors whose functions are disrupted in the disorders.

NF1 patients can develop a variety of symptoms, the most common of which are benign peripheral nerve sheath tumors (neurofibromas). Animal models of this disorder are critical to improving our understanding of this disease. Although an animal model of the learning disabilities in NF1 had been developed, this model did not exhibit the same predisposition for tumor formation that is characteristic of humans with the disease. During FY 2000, two teams (one NINDS-supported) reported the development of a mouse model for NF1 that develops neurofibromas as well as malignant transformations. It had been previously hypothesized that combined mutations in the *p53* and *Nf1* genes would be required for tumor formation, and these recently published data provide support for this hypothesis. It is hoped that these

improvements in the modeling of NF will advance our understanding of how these tumors develop and how interventions might be designed.

Rare Disorders with Public Health Impact

The scientific community is becoming increasingly aware that rare diseases may yield clues to other more prevalent conditions. In addition, disorders may be rare when first recognized, but have the potential to cause much more widespread effects on public health. For example, the small number of case presentations of the disorder ultimately identified as AIDS would have placed it into the "rare disease" category in the early 1980s, whereas its prevalence soon exceeded this classification.

Creutzfeldt-Jacob disease (CJD) is a disorder that fits both of these categories. Caused by a proteinaceous infectious agent, or prion, it is notable for its rarity (one in a million individuals is affected) and its unusual pathology. The mechanisms that underlie the degenerative effects of prions, however, are being increasingly compared to inappropriate protein-processing mechanisms that cause more widespread neurodegenerative diseases such as Alzheimer's disease. Furthermore, the increasing prevalence of new-variant CJD, a form of the disorder linked to "mad cow" disease in Great Britain, is also cause for public health concern for Europeans and Americans. NINDS has a long-standing commitment to the study of the prion diseases, starting with the work of Dr. Carleton Gajusek and the late Dr. Clarence Gibbs on Kuru in the 1960s, and continuing with the significant contributions of Drs. Gibbs and Paul Brown of the intramural Laboratory of Central Nervous System Studies. A number of NINDS extramural projects are also supported in this area of research, including two contracts (funded in FY 2000) that will help develop rapid diagnostic tests for human prion diseases.

Rare Diseases Research Initiatives

Program Actions

FY 2000 Solicitations

"Rett Syndrome: Genetics, Pathophysiology, and Biomarkers" (PAS-99-037, revised as HD-00-001, jointly with NICHD, released 1/18/2000)

"Spinal Muscular Atrophy, Amyotrophic Lateral Sclerosis, and Other Motor Neuron Disorders," (RFA-NS-01-004, released 3/9/2000)

"Exploratory Grant in Pediatric Brain Disorders: Integrating the Science" (PAS-99-080, revised as NS-00-009, jointly with NICHD, NIMH, released 7/14/2000) [includes FRDA, ataxia telangiectasia, Batten disease, Duchenne muscular dystrophy, fragile X syndrome, Sturge-Weber syndrome, holoprosencephaly, and many others]

"Development of Innovative Treatment Approaches to Autism" (RFA-MH-01-010, jointly with NIMH, NICHD, and NIDCD, released 11/29/2000) [includes Rett syndrome]

As a result of these solicitations, NINDS hopes to enhance its support of research on rare diseases. The Institute has awarded, and will continue to award, the majority of its grants to investigators who submit unsolicited proposals. These investigators often have the greatest insight into the critical questions facing a particular field of research, and their proposals may be highly targeted to these areas. This is true for

rare diseases research, as well as for research on more common disorders. In FY 2000, new grants were awarded to researchers exploring the following rare diseases: trinucleotide repeat diseases, Lyme disease, LSDs (including Batten disease and mucopolysaccharide disorders), Canavan disease, cyclic vomiting syndrome, Rett syndrome, spinal muscular atrophy, Tourette syndrome, and Duchenne muscular dystrophy.

Workshops and Meetings

In FY 2000, NINDS sponsored workshops in a number of rare diseases areas. These activities are often joint ventures with other NIH Institutes, and frequently involve the participation of outside disease advocacy organizations.

Events of this type that were sponsored or co-sponsored by NINDS in FY2000 include:

"Brain Fatty Acid Uptake, Utilization and Relevance to PB," held March 2-4, 2000, jointly sponsored by NINDS, NIDDK, NICHD, and ORD.

"Symposium on Hereditary Spastic Paraplegia," held March 16-18, 2000, jointly sponsored by NINDS, NICHD, and ORD.

"Conference on Cause and Treatment of FSH Dystrophy," held May 8-9, 2000, jointly sponsored by NINDS, NIAMS, and ORD.

"Workshop on Therapeutic Approaches for Duchenne Muscular Dystrophy," held May 15-16, 2000, jointly sponsored by NINDS, NIAMS, and ORD.

"First Scientific Workshop of Hallervorden-Spatz Syndrome," held May 19-20, 2001, jointly sponsored by NINDS, NICHD, and ORD.

"Cerebral Blood Flow and Development Metabolism," held June 8-11, 2000, sponsored by NINDS.

"The Olfactory Model System and Rett and Kallmann Syndromes: Sniffing Out Insights into Brain Development," held September 12, 2000, jointly sponsored by NINDS, ORD, NIDCD, NIMH, and NICHD.

"International Conference on the Neuronal-Lipofuscinosis," held September 20-24, 2000, jointly sponsored by NINDS, NIDDK, and NICHD.

Upcoming (Post-FY 2000) Workshops

"Gene Therapy for Neurological Disorders," October 23-24, 2000, jointly sponsored by NINDS and ORD. [Gene therapy for metabolic storage disorders, such as Batten and Fabry's disease, was a focus of the meeting.]

"From Gene to Function in Dystonia," January 19-21, 2001, sponsored by NINDS.

"Hypertonic Movement Disorders Workshop," April 22-24, 2001, jointly sponsored by NINDS and ORD. [Relevant to disorders in children that cause increased muscle tone, leading to rigidity, spasticity, and dystonia.]

"Strategies for Therapy of MPS and Related Diseases," June 21-24, 2001, jointly sponsored by NINDS, NIDDK, and NICHD.

"2001 CAG Triplet Repeat Disorders," a Gordon Conference, July 15-19, 2001, jointly sponsored by NINDS and NIA.

"Mucopolysaccharidosis, TRPs and Human Disease," September 8-10, 2001, jointly sponsored by NINDS, NIDDK, NICHD, NIMH, and ORD.

"Fourth International Dystonia Symposium," September 20-23, 2001, jointly sponsored by NINDS, NIA, NIAMS, and ORD.

"Neurobiology of Disease in Children Conference," October 17, 2001, jointly sponsored by NINDS, NIAMS, NICHD, and NIMH.

In addition to these meetings, a workshop on pediatric neurotransmitter diseases is also planned, as is a joint workshop on Hutchinson-Gilford progeria syndrome, an exceedingly rare disease of premature aging in children. The latter meeting is being organized by NINDS, NHLBI, NIAMS, ORD, and NIA; it is expected to aid participating Institutes in identifying promising lines of investigation in this field, and in stimulating research on this disorder.

National Institute of Nursing Research (NINR)

Overview of NINR Rare Diseases Research Activities

NINR supports clinical and basic research to establish a scientific basis for the care of individuals across the life span: from management of patients during illness and recovery to the reduction of risks for disease and disability, the promotion of healthy lifestyles, promoting quality of life in those with chronic illness, and care for individuals at the end of life. NINR's rare diseases research portfolio investigates strategies to control, manage, and prevent biobehavioral complications of such pathologies.

Recent Scientific Advances in Rare Diseases Research

Gilles de la Tourette Syndrome (TS)

NINR-funded research is examining the social-emotional functioning in children with Gilles de la Tourette syndrome (TS) alone and children with TS and attention deficit hyperactivity disorder (ADHD). In addition, the contribution of family functioning to social competence is being examined. Findings reported in the *Journal of Child Psychology and Psychiatry* (2000) state that children with TS and ADHD demonstrated more externalizing and internalizing behavior problems and poorer social adaptation than children with TS only or unaffected controls. Children with TS only were not significantly different from unaffected controls on most measures of externalizing behaviors and social adaptation, but did exhibit more internalizing symptoms. Tic symptom severity was not associated with social, behavioral, or emotional functioning among children with TS, even after stratifying by medication status. ADHD diagnosis, obsessional symptom severity, and family functioning, however, were significantly associated with social and emotional adjustment among TS children. Moreover, family functioning was associated with social and emotional adjustment even after controlling for TS and ADHD diagnostic status. These findings demonstrate that much of the social and behavioral dysfunction in children with TS is ADHD-specific, and children with TS alone have a very different social-emotional profile than do those with TS plus ADHD. Finally, social-emotional adjustment in children with TS is best understood within the family context.

Acute Respiratory Distress Syndrome

NINR researchers are studying optimum ways to wean patients diagnosed with acute respiratory distress syndrome from mechanical ventilation. A study published in *Biological Research in Nursing* (2000) investigated the immediate transition from positive pressure mechanical ventilation to spontaneous ventilation in order to detect cardiopulmonary hemodynamic alterations based on weaning mode. The study compared hemodynamic responses associated with the initial transition to three modes of ventilator weaning (spontaneous ventilation/T-piece, pressure support [PS], and continuous positive airway pressure [CPAP]). Right ventricular hemodynamic responses were evaluated with a thermodilution pulmonary artery catheter, while left ventricular hemodynamic responses were measured by a transducer-tipped Millar catheter and conductance catheter.

Two groups of canines were studied. Both groups significantly increased cardiac output in response to T-piece. Right ventricular stroke work was also significantly increased with T-piece and CPAP in both groups of subjects. Left ventricular response depended on baseline ventricular function. Baseline ventricular function influenced hemodynamic response to the immediate transition from mechanical to spontaneous ventilation. There were also differential hemodynamic responses based on the ventilatory

mode. Consideration of baseline cardiac function may be an important factor in the selection of an appropriate mode of spontaneous ventilation following controlled mechanical ventilation. NINR-funded investigators anticipate that the conclusions of this study will provide insight into methods of optimizing cardiovascular and respiratory function in critically ill patients and the selection of an appropriate model to facilitate spontaneous ventilation.

Fibromyalgia

Fibromyalgia is a complex syndrome, primarily of women, characterized by chronic pain, fatigue, and sleep disturbance. NINR-funded researchers are studying the possible links to hormonal disturbances in order to form a basis for possible treatment strategies such as sleep therapies, hormone augmentation, and stress reduction to manage the symptoms of fibromyalgia. Altered function of the somatotrophic axis has been documented in fibromyalgia patients, but little is known about nocturnal levels of prolactin (PRL). As part of a laboratory study of sleep patterns in fibromyalgia, serum concentrations of growth hormone (GH) and PRL were reported in the *Journal of Clinical Endocrinology and Metabolism* (2001). Sleep efficiency and amounts of sleep or wake stages on the blood draw night were not different between groups. There was a modest inverse relationship between sleep latency and PRL and a direct relationship between sleep efficiency and PRL in fibromyalgia. There was an inverse relationship between age and GH most evident in control women. Insulin-like growth factor I levels were not different between the groups. These data demonstrate altered functioning of both the somatotrophic and lactotropic axes during sleep in fibromyalgia and support the hypothesis that dysregulated neuroendocrine systems during sleep may play a role in the pathophysiology of fibromyalgia.

Irritable Bowel Syndrome (IBS)

In *Digestive Diseases* (2000), NINR researchers reported an association between sleep disturbance and gastrointestinal symptoms in women with IBS. Daily diary data were used to estimate the association between sleep disturbance and gastrointestinal symptoms, both across women (i.e., whether women with high average sleep disturbance have higher average gastrointestinal symptoms) and within the woman (i.e., whether poorer than average sleep on one night is associated with higher than average gastrointestinal symptoms the following day). Regression coefficients showed little change when daily psychological distress or stress was controlled for, the one exception being the coefficient for the across-women effect in the IBS group, which decreased substantially but still remained highly significant. Because it is possible that gastrointestinal symptoms could, in fact, cause poor sleep, the investigators also fitted the temporally reversed model to evaluate the association between gastrointestinal symptoms on one day and sleep disturbance that night. The within-subject regression coefficients were not significant in both the IBS and control groups. These results are consistent with the hypothesis that poor sleep leads to higher gastrointestinal symptoms on the following day among women with IBS.

Cystic Fibrosis (CF)

Advances in biomedical sciences and technology have made longer life spans possible for children with CF. The current generation of adolescents and young adults with CF presents new management challenges for health care providers. NINR researchers are testing the effectiveness of an intervention to improve the quality of life of children with CF (ages 8 to 12 years) by teaching them life skills for managing the psychosocial demands of chronic illness that impact their ability to understand and manage the physiologic and functional demands of CF. A recent publication in the *Journal of Pediatric Nursing* (2000) reported that knowledge of the progression of CF and increasing social interactions with peers

with CF during hospitalization helped children with CF learn that the disease is lifelong with relentless demands. The research is continuing with interventions focusing on strategies to promote peer support, a positive attitude, and the hope to create a sense of belonging, social competence, and well-being.

Down Syndrome

NINR-funded rare disease research contributes to a better understanding of the role that health care providers play in individual and family adaptation to chronic conditions. In *Research in Nursing and Health* (1999), NINR-funded research described parental perceptions of family-provider relationships and explored links between parental perceptions of family-provider relationships and well-being in families with children who have Down syndrome. Results indicated that when mothers of children with Down syndrome believe that their family's relationship with health care providers is positive and family-centered, they feel more satisfied with the care their child is receiving, and they are more likely to seek help from health care providers. In addition, when a discrepancy exists between what mothers want the family-provider relationship to be and what they believe the relationship is, mothers feel less satisfied with the care their child is receiving. Finally, higher levels of individual and family well-being are reported by mothers who 1) want, and believe they have, positive family-centered relationships with providers, and 2) feel more satisfied with the care received.

Rare Diseases Research Initiatives

Program Activities

NINR participated in a rare diseases research initiative in FY 2000. This workshop, "Increasing Nursing Postdoctoral Opportunities in Rare Diseases," was held May 1-2, 2000. The purpose of the workshop was to discuss how to increase the pool of scientists into areas of rare disease research. Scientists and clinicians in fields such as bioengineering, pharmacology, nursing, medicine, and genetics participated. The workshop concluded with a plan of action to develop a cadre of scientists from Ph.D. programs who will be able to develop programs of research in areas such as HIV, CF, end-stage renal failure, juvenile diabetes, hereditary hypercholesterolemia, and syndrome X. Colleagues from the Office of the Director (OD), NIH, and NHGRI participated in the meeting.

National Center for Research Resources (NCRR)

Overview of NCRR Rare Diseases Research Activities

NCRR develops and supports critical research technologies that underpin health-related research to maintain and improve the health of our Nation's citizens. NCRR supports shared resources, sophisticated instrumentation and technology, animal models for study of human disease, clinical research, and research capacity building for under-represented groups.

Through its support of multidisciplinary research, NCRR is uniquely positioned to provide either primary research support or resource support in partnership with other NIH Institutes and Centers (ICs) to address emerging clinical and basic research needs such as the study of rare diseases. Expansion of NCRR's current efforts in new biotechnologies and instrumentation, development of animal models, and clinical research will foster interdisciplinary collaborations and advance NIH's efforts in the study of rare diseases in children and adults.

Recent Scientific Advances in Rare Diseases Research

FY 2000 Research Activities

Animal Models

Kallmann Syndrome

Kallmann syndrome is a neurogenetic disease that affects an estimated 1 in 10,000 males. Patients with this disease suffer from anosmia (inability to smell) and retarded sexual development. These conditions arise because both olfactory nerve axons and the luteinizing hormone-releasing hormone (LHRH) neurons that migrate with them fail to make contact with the brain. Loss-of-function mutations in a gene located on the X (female) chromosome *KALI* cause Kallmann syndrome. No useful mouse model for this disease is available.

A monkey model is currently being developed by NCRR-supported investigators at the University of Nebraska Medical Center in collaboration with the Oregon Regional Primate Research Center. The researchers have obtained the sequence of the complete coding region of the monkey *KALI* gene. Monkey *KALI* protein is 97.4% similar to human *KALI*. The researchers have begun analysis of *KALI* expression in the developing rhesus macaque brain.

The researchers propose to obtain the basic information and to establish the necessary methodology to develop a functional model of Kallmann syndrome in the rhesus macaque. The production of such animals would provide: 1) proof that this methodology could be used for the development of primate models of human diseases, 2) the first in vivo model of Kallmann syndrome, and 3) a primate model to study the role of LHRH neurons in reproductive function.

Niemann-Pick Type C (NP-C) Disease

A storage disorder, NP-C is characterized by abnormal accumulation of fatty substances in small bodies within the cells known as lysosomes. NP-C presents with a variety of clinical features, including enlarged organs, jaundice, seizures, delayed mental and motor development, and premature death. The

primary defect is associated with processing intracellular cholesterol. Onset of NP-C can occur over a wide age spectrum, resulting in its classification as infantile, juvenile, or adult (the adult form is quite rare). Progression is usually slower in patients with later onset. Approximately 1,000 children are affected in the United States.

NCRR-supported investigators at Colorado State University have identified and characterized the gene responsible for the most common type of human NP-C (*NPCI*) and a corresponding gene in cats. They have further demonstrated that these genes are similar in 91% of their components. These findings will enable the investigators to characterize the cat NP-C model on molecular, biological, and physiological levels and to evaluate various treatment modalities for the human disease. The most promising avenue of further research will be an evaluation of the efficacy of a ganglioside synthesis inhibitor as potential NP-C therapy.

Biomedical Engineering and Instrumentation

Sickle Cell Anemia (SCA)

SCA is the most common inherited blood disorder in the United States, affecting about 72,000 predominantly African American (1 in 500) individuals in this country. SCA is characterized by episodes of pain, chronic anemia, and severe infections, usually beginning in early childhood. SCA is caused by an error in the gene that tells the body how to make hemoglobin (the oxygen-carrying component of the blood). This mutation results in the production of structurally abnormal hemoglobin chains, which instead of remaining separate in the cell, clump together into large inflexible complexes. This abnormal aggregation deforms the red blood cells into curved ("sickle") shapes, causing them to block and rupture tiny blood vessels, depriving organs and tissues of oxygen and causing severe damage.

NCRR-supported investigators at the Rockefeller University have developed a method for producing structurally authentic recombinant hemoglobin molecules in yeast to assist in the study of the interactions between hemoglobin chains. The use of recombinant hemoglobin has allowed researchers to ask very specific questions about how changes in protein sequence affect protein interactions. The yeast system produces a recombinant sickle hemoglobin that is identical by about a dozen biochemical and physiological criteria with the natural sickle hemoglobin purified from the red cells of SCA patients. More important, the gelling concentration of this recombinant sickle hemoglobin is the same as that of the sickle hemoglobin purified from human sickle red cells. In addition, fundamental studies are being conducted into the interactions of normal and fetal hemoglobin in order to better understand the correlation between amino acid sequence changes and hemoglobin function. Results obtained thus far show that this system will be very helpful in defining the interactions in normal and sickle hemoglobin chains. This new model system will allow significantly more complex studies of protein interactions in normal and sickle hemoglobin. The more sophisticated understanding of basic biophysical processes at work in SCA may lead to more effective treatments.

Osteogenesis Imperfecta (OI)

OI is a disease that is caused by mutation in the alpha-1 or alpha-2 genes of type I collagen, a structural protein important in bone formation. Mutations can occur in many different positions, and there is currently no known correlation between position of mutation and disease severity. An understanding of

the nature of the factors that promote stability in the collagen triple helix will provide insight into the pathology of the disease.

Mutations in the collagen protein chain change its structure and, therefore, its interaction with other chains to form these important larger structures. NCCR-supported investigators at the University of California at San Francisco (UCSF) have developed computer modeling that has contributed to an understanding of how normal collagen molecules interact to form larger, more stable structures in order to determine the factors that contribute to a loss of stability in that interaction when mutations occur. Molecular dynamics simulations of collagen-like peptides show average structures and internal coordinates similar to X-ray crystallographic structures. These results demonstrate that molecular dynamics can be used to reproduce the experimental structures of fibril proteins by adaptation of software originally designed to model more conventional globular proteins. New information on protein interactions and structure have been reported.

Although in its infancy and fundamental in nature, this work extends the use of computational techniques to the realm of fibril proteins and has provided insight into the way collagen chains interact. Ultimately, an understanding of what factors are responsible for variations in the severity of OI may lead to effective treatments.

Barrett's Esophagus

Barrett's esophagus is caused by an inborn defect in the valve between the esophagus and stomach, which protects the esophagus from reflux of highly acidic stomach contents. Chronic reflux of these acidic stomach contents into the esophagus can lead to cancer of the esophagus, a disease responsible for more than 12,000 deaths in the United States each year. Almost all of these cases occur in patients with Barrett's esophagus, but most patients with Barrett's esophagus do not go on to develop cancer. Ways to discriminate between irritant and cancerous changes in the esophagus are therefore crucial to monitoring these patients. The only way to detect cancer in these patients is through endoscopic surveillance coupled with random biopsies, an expensive method prone to error.

NCCR-supported investigators at the Massachusetts Institute of Technology developed a device to measure light scattering in vivo that was used in an attempt to diagnose patients with cancer of the esophagus. Development of the device builds on the observation that cancerous cells scatter light differently than normal cells. Using a small fiberoptic probe, light was delivered to and collected from small areas of the esophagus. The results of the light-scattering experiments were then compared with the results of biopsies taken from exactly the same location. These experiments were performed on 49 different patients, and multiple experiments were done with each patient. The sensitivity and specificity of light scattering for detecting cancer of the esophagus were very high when compared to the results of the biopsies.

Prion Diseases

Prions are a novel class of "infectious" pathogens distinct from viruses with respect to both their structure and the neurodegenerative diseases that they cause. Prion diseases are manifested as sporadic, inherited, and infectious disorders, including scrapie and bovine spongiform encephalopathy (BSE, also called mad cow disease) of animals as well as kuru, Creutzfeldt-Jakob disease (CJD), and fatal familial insomnia of humans. An important early step in understanding how prions cause these disease states is to determine their structure.

Using magnetic resonance spectroscopy and advanced graphics techniques, researchers at the NCCR-supported Computer Graphics Laboratory at UCSF have solved the structure of the infectious component of the scrapie prion protein. Multiple conformations of prion protein have been constructed. These graphical images can be accessed through the Web at <http://www.cgl.ucsf.edu/home/>.

Clinical Research

SCA

As discussed above, SCA is the most common inherited blood disorder in the United States. NCCR-supported investigators at Emory University noted that some patients with SCA develop multiorgan failure, while others have relatively few end-organ complications. The investigators have also noted that renal albuminuria (protein in the urine) is a very sensitive marker for sickle kidney disease and a predictor of future renal complications. Using sophisticated genetic testing, these investigators were able to track the occurrence of these clinical complications to the presence of a specific gene mutation. Presence of a different gene mutation (known as a microdeletion) was actually associated with protection from the kidney complications.

The mechanism by which microdeletions protect SCA patients from renal disease is unknown. This mechanism is not related to hemoglobin level, degree of anemia, or new red blood cell production. Patients with microdeletions have a tendency toward lower systemic blood pressure, decreased red blood cell volume, and renal vasodilation. Furthermore, erythrocytes from these patients have higher deformability and decreased adhesiveness to vascular endothelium, thereby possibly decreasing vascular damage and blockade that could lead to kidney inflammation and damage.

Peroxisomal Disorders

NCCR-supported investigators at the Johns Hopkins University have described an assay useful in testing for the presence of very long chain fatty acids, a blood abnormality observed in the so-called peroxisomal disorders such as Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum syndrome. These investigators extended the test to amniocytes and the outermost cells of the membrane surrounding the fetus, thus providing a prenatal test to predict the likelihood of the fetus being affected by these diseases. The investigators report results of 255 prenatal assays identifying 63 affected males. Five families elected to continue the pregnancies and the abnormality has been confirmed in all these offspring. Among the fetuses that were aborted, the diagnosis was confirmed on autopsy in all. Among those determined by the assay to be unaffected, Zellweger syndrome has been ruled out in all. After 10 years of follow-up, no case of adrenoleukodystrophy has been manifested. Due to the heterogenous presentation of the latter disease, however, these findings are promising but not definitive. Thus, a sensitive and discriminating assay appears to be available for prenatal diagnosis of these serious rare diseases of childhood.

CF

CF is an inherited disease affecting transport of secretions. Thick lung secretions make affected children particularly vulnerable to lung infections. *Pseudomonas aeruginosa* infections are particularly problematic in this population. NCCR-supported investigators at the University of Washington reported the results of two multicenter, double-blind, placebo-controlled trials of intermittent administration of tobramycin, an inhaled antibiotic. A total of 520 patients with CF and pulmonary infection with *P. aeruginosa* infection were randomly assigned to receive either 300 mg of inhaled tobramycin or

placebo twice daily for 4 weeks, followed by 4 weeks with no study drug. Patients received treatment or placebo in three off-on cycles for a total of 24 weeks.

The patients treated with inhaled tobramycin experienced improved lung function (a 10% increase in FEV1, a measure of airway compliance), while patients receiving placebo had a statistically significant 2% decline in this measure. In the tobramycin group, the density of *P. aeruginosa* in the sputum decreased by an average of 0.8 log colony-forming units, compared with an increase of 0.3 log units in the placebo group. These differences were statistically significant. The patients in the tobramycin group were 26% less likely to be hospitalized for antibiotic treatment of *P. aeruginosa* infection than those in the placebo group. Inhaled tobramycin was not associated with detectable toxicity of the ear or kidney or with accumulation of the drug in serum. In summary, inhaled tobramycin was well-tolerated, caused no serious side effects, improved pulmonary function, decreased the density of *P. aeruginosa* in sputum, and decreased the risk of hospitalization.

Severe Combined Immunodeficiency

NCRR-supported investigators at Duke University summarized their experience with 89 consecutive patients treated for inborn errors of the immune system classified as severe combined immunodeficiency. Patients were treated with bone marrow transplantations and were surveyed between 3 months and 16.5 years posttransplantation. Of particular interest was the relative outcomes of patients receiving genetically matched (HLA-identical) versus genetically half-matched (HLA-haploidentical) donations of marrow. That is to say, while some patients have a sibling with the identical histocompatibility genes, others do not and must rely on a donation from a parent who is only half-identical or haploidentical. The exactness of the match is important. Although the patient, having no immune system, cannot reject the donated marrow, after the immune system is reconstituted, the marrow can recognize the recipient's body as foreign and cause a serious condition known as graft-versus-host disease (GvHD).

The hope is to maximize the chance for rebuilding the immune system while minimizing the chance of rejection. It was theorized that if the donor bone marrow was first depleted of T cells (those cells associated with GvHD) before transplantation both conditions would be satisfied. In reviewing the data, the investigators reported an overall 81% survival rate. Those 12 patients receiving transplantations with immunologically identical donors were all alive. Of those who received half- identical donations, 60 of 77 (78%) were alive. The latter group included two of three who received placental blood as a source of stem cells in addition to the bone marrow. Other factors favoring survival were gender, ethnicity, and age at transplant. All the transplanted girls survived; whites had a better survival than blacks or Hispanics; and 95% versus 76% of children transplanted before the age of 3.5 months survived. No deaths were attributed to GvHD, although one recipient (of a half-matched donation plus placental blood) is receiving continuous cyclosporine treatment for chronic GvHD.

Workshops Co-sponsored with ORD

NCRR did not co-sponsor any workshops with ORD in FY 2000.

National Library of Medicine (NLM)

Overview of NLM Rare Diseases Research Activities

NLM provides information resources for rare disease research and to those seeking information about conditions affecting themselves or their families.

Examples of pertinent NLM resources are provided below:

- Citations to articles on rare diseases have long been available in the MEDLINE database, now accessible to researchers, health professionals, and the public through NLM's free Web-based PubMed system.
- ORD and NLM staff work closely to incorporate information on clinical trials for rare diseases into the ClinicalTrials.gov database (see <http://clinicaltrials.gov>).
- MEDLINEplus, NLM's consumer health information service, has a general rare diseases information page, which has been effective in referring members of the public to ORD (see <http://www.nlm.nih.gov/medlineplus/rarediseases.html>). Over the past year, NLM has also added individual health topics pages for more than 60 specific rare diseases.
- The Online Multiple Congenital Anomaly/Mental Retardation (MCA/MR) Syndromes© is NLM's database of structured descriptions of congenital abnormalities (many of them rare) associated with mental retardation (see http://www.nlm.nih.gov/mesh/jablonski/syndrome_title.html).
- In 2000, NLM published a special bibliography, "Phenylketonuria (PKU): Screening and Management," in the Library's popular Current Bibliographies in Medicine series (see <http://www.nlm.nih.gov/pubs/cbm/pku.html>).
- NLM chairs the Communications Working Group of the Multilateral Initiative on Malaria (MIM), which began in 1997. MIM's objective is to support African scientists and the ability of malaria researchers to connect with each other and sources of information through full access to the Internet and the resources of the World Wide Web, while creating new collaborations and partnerships (see <http://www.mimcom.net>).

Rare Diseases Research Initiatives

National Center for Biotechnology Information (NCBI)

A division of NLM, NCBI serves as a national public resource for molecular biology information. In this capacity, NCBI establishes and maintains various genomic databases and develops software tools for mining and analyzing these data, all of which are freely disseminated to the biomedical community to facilitate a better understanding of the processes affecting human health and disease.

The Human Genetic Map

NCBI is responsible for collecting, managing, and analyzing the growing body of data being generated from the sequencing and mapping initiative of the Human Genome Project (HGP). These data are made available without restriction to the scientific community and have expedited the decoding of various human chromosomes. By analyzing the DNA sequence of a chromosome, scientist may begin to understand the causes of certain rare diseases. Scientists can determine the organization of the genes on a chromosome, how these genes are expressed, how changes in a gene's DNA sequence give rise to a disease-causing mutation, and how a chromosome is duplicated and inherited. Scientists have used these strategies to find clues about gene defects on chromosomes 21 and 22 that lead to a variety of rare diseases, including Down syndrome, Usher syndrome, DiGeorge syndrome, and Ewing's sarcoma. NCBI investigators have also played an instrumental role in the identification and analysis of other disease genes and genetic loci, including analysis of genetic data leading to scientific advances in several rare diseases and disorders, such as the identification and analysis of the genes for Kallman syndrome and neurofibromatosis (*NFI*). Examples of other diseases currently being studied by NCBI investigators include ataxia telangiectasia, breast cancer, hyper-IgE syndrome, nemaline myopathy, and obesity.

Genetic Analysis Software

NCBI investigators are working to develop, implement, and disseminate high-performance computational tools and application software packages for the analysis and linkage of genetic data. FASTLINK is a software program designed by NCBI investigators for conducting genetic linkage analyses, a statistical technique used to study the association of genes and genetic markers that lie near each other on a chromosome. Genes and other genetic markers that are linked tend to be inherited together and therefore can be used to identify and map the location of a particular disease gene. NCBI investigators have used FASTLINK to study hyper-IgE syndrome, a rare immunodeficiency characterized by recurrent skin abscesses, pneumonia, and highly elevated levels of serum IgE. Using FASTLINK, researchers were able to find evidence linking this syndrome to chromosome 4. FASTLINK has been cited in more than 400 other published genetic studies, including studies of macular dystrophy, type 1 hereditary sensory neuropathy, and Alstrom's syndrome.

CASPAR (Computerized Affected Sibling Pair Analyzer and Reporter) is a software program designed by NCBI investigators to study the genetics of complex diseases or diseases involving the interaction of multiple genes. CASPAR allows the user to explore various hypotheses about how different factors may be involved in disease susceptibility. NCBI investigators have used CASPAR to study linkage analysis in patients with a form of diabetes.

The PedHunter software was developed to query genealogical databases to determine a connection between a set of relatives that are afflicted with the same disease and to construct a pedigree suitable for input to genetic linkage analysis. NCBI investigators are using PedHunter to query the Amish Genealogy database to collect information on various genetic diseases, including nemaline myopathy. Nemaline myopathy is a rare genetic neuromuscular disorder that is usually apparent at birth and characterized by extreme muscle weakness. Using PedHunter in combination with other genetic analysis software, NCBI investigators have demonstrated that in the Amish, this disorder is caused by a mutation in the gene for the sarcomeric thin-filament protein slow skeletal muscle troponin T (*TNNT1*). *TNNT1* maps to chromosome 19 and has been previously sequenced. Further analysis resulted in the identification of a stop codon that segregated with the disease. Researchers concluded that Amish nemaline myopathy is a distinct, heritable, myopathic disorder caused by a mutation in *TNNT1*.

The comparative genomic hybridization (CGH) analysis software package is being used by NCBI investigators to model the process of tumor formation in various forms of cancer. The focus of the software is to develop models that relate genetic aberrations with tumor progression. Investigators have used CGH as part of a larger project to search for and identify possible susceptibility loci involved in breast and bladder cancer. Investigators have also published the results of a case study in which CGH was used to analyze chromosomal abnormalities in a large collection of ovarian cancer samples.

Three-Dimensional Structure Database

NCBI's Structure Research Group maintains a database of experimentally determined three-dimensional biomolecular structures, as well as tools for visualizing and analyzing these structures. Three-dimensional structures provide a wealth of information on biological function of a molecule, mechanisms linked to function, and evolutionary history of and relationships between macromolecules, all valuable clues leading towards better understanding rare diseases. For example, in 1995, the structure of *leptin*, the protein coded for by a gene linked to obesity and forms of diabetes, was predicted by NCBI investigators using the structure database. After the discovery of *leptin*, researchers analyzed the protein's sequence and determined that it exhibited no similarities to other known proteins. NCBI investigators hypothesized that *leptin* was ancestrally related to at least one other protein whose sequence had diverged such that only a comparison of three-dimensional structures might detect a relationship. Investigators conducted a search of the database to determine whether this protein might adopt a similar fold pattern, or structure, to that of a protein structure already stored in the database. They discovered that *leptin's* sequence was compatible with the structure of a family of known proteins and predicted a structural model based on these results. Subsequently, this early prediction was confirmed by cloning of the protein's receptor, and more recently, by X-ray structure determination. Now that the structure of *leptin* has been confirmed, future studies of *leptin*, as well as other *leptin*-regulated genes, may reveal the mechanisms by which *leptin* exerts its effect on the body.

Malaria Genetics and Genomics

Malaria is by far the world's most important tropical parasitic disease. The causative agents in humans are four species of a single-celled parasite from the genus *Plasmodium*, *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Of these, *P. falciparum* accounts for the majority of infections and is the most lethal. Malaria is a curable disease if promptly diagnosed and adequately treated. Therefore, much NIH research focuses on the treatment and prevention of malaria.

In collaboration with NIAID, NCBI supports the efforts to sequence and analyze the complete genome of *P. falciparum*, thereby providing researchers with access to information relative to all of the genes found in this parasite. Genetic investigations of malaria require a genome-wide, high-resolution linkage map of *P. falciparum*. A collaborative team of NIH investigators, including researchers from NCBI, constructed such a map. The markers and map, as well as other parameters, are facilitating genome sequence assembly, localization of determinants for traits such as virulence and drug resistance, and genetic studies of parasite field populations.

NCBI's Malaria Genetics and Genomics Web page (see <http://www.ncbi.nlm.nih.gov/Malarial>) serves as a resource for data and information relevant to *Plasmodium* in general and more specifically to *P. falciparum*, *P. vivax* (the second most prevalent form of human malaria), and various forms of rodent malaria. From NCBI's site, researchers may access genome maps, linkage markers, and information about genetic studies. Links are also provided for other malaria sites and for genetic data on related parasites, including NIAID's Malaria Research and Reference Reagent Resource Center (MR4), a central source for

quality controlled malaria-related reagents for the international research community. Users may also link to information concerning malaria epidemiology, taxonomy, molecular tools for data analysis, and various malaria research projects being conducted at NIH.

Additional Human Genome Resources

NCBI makes available a number of other resources to facilitate the widespread use of human sequence data. The Human Genome Resources Web page serves as an integrated, one-stop genomic information infrastructure for biomedical researchers from around the world. From the Human Genome Resources Web page, researchers can access the NCBI Map Viewer, which presents a graphical view of the available human sequence data as well as cytogenetic, genetic, physical, and radiation hybrid maps. Researchers may search for a gene, or a gene marker, of interest by querying against the entire human genome, or by querying one chromosome at a time. Query results link to a graphical display of where the gene or gene marker may be viewed in the context of additional data.

NCBI's Genes and Disease Web page (see <http://www.ncbi.nlm.nih.gov/disease>) is designed to introduce a visitor to the relationship between genetic factors and human disease. Genes and Disease provides information for more than 60 genetic diseases, including numerous rare diseases and disorders. The Online Mendelian Inheritance in Man (OMIM) database is a continuously updated catalog of inherited human disorders and their causal mutations, authored and edited by Dr. Victor A. McKusick and colleagues and developed for the Web by NCBI (see <http://www.ncbi.nlm.nih.gov/Omim/>).

One of the many reasons for sequencing the human genome was to gain an understanding of the role of a gene, or genes, in human disease. By studying the sequence of a disease gene, whether it be from humans or other model organisms, researchers can gain important insights into the genetic and environmental basis of disease.

The advances outlined here demonstrate the importance and utility of NCBI's computer databases, data analysis tools, and software algorithms in identifying and understanding human disease genes, and pave the way for the development of novel strategies to diagnose, treat, and ultimately prevent all forms of disease.

Office of Research on Women's Health (ORWH), Office of the Director, NIH

Overview of ORWH Rare Diseases Research Activities

Research Funded During FY 2000

During FY 2000, ORWH supported 17 grants that focused on disorders classified as "rare diseases" by NIH. These disorders include gestational diabetes mellitus, fibromyalgia, rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE), and osteoarthritis.

Because ORWH does not have direct funding authority, these grants were funded through established collaborations with several NIH ICs. Specifically, ORWH supported these 17 grants by working collaboratively with NIAID, NIDDK, NIAMS, and NIEHS.

Recent Scientific Advances in Rare Diseases Research

Gestational Diabetes Mellitus (GDM) Grants

Working with NIDDK, ORWH supported three grants that focused on GDM, two of which focused on Hispanic and Native American populations. Two hundred thirty-seven Hispanic women with a history of GDM were enrolled in an intervention trial designed to evaluate if an insulin-sensitizing agent could prevent or delay the onset of type 2 diabetes. Preliminary results, with a median of 27 months' follow-up, show the intervention has reduced the onset of diabetes by 50%. The study involving Native American women concentrates on reducing modifiable risk factors for diabetes through culturally specific interventions. The primary interventions include modifying diet by reducing fat intake and increasing vegetable consumption, increasing physical activity, and increasing the percentage of mothers who will breast feed their infants for at least 2 months.

With NIDDK, ORWH provided partial support to fund a large multicenter clinical trial designed to determine whether type 2 diabetes can be prevented or delayed in a population of high-risk individuals, 50% of whom represent minority populations. Of the total number of participants, 13% are women with a history of GDM.

Fibromyalgia Grant

This project, co-funded by ORWH and NIAMS, focuses on improving clinical treatment of fibromyalgia by increasing the understanding of the effectiveness of new drug regimens and the factors affecting patients' responses to these treatments. The investigators have developed a method for effectiveness research that uses patient-focused N-of-1 trials and then combines these trials' results to 1) obtain population estimates of treatment effectiveness and 2) assist in treatment decision-making for an individual patient.

Rheumatoid Arthritis (RA) Grants

ORWH and NIAMS supported two grants in this area: a clinical research grant and a longitudinal registry of African American women diagnosed with early RA.

The clinical research study builds upon recent data that reveal that the excess mortality experienced by people with RA may result from increased rates of coronary heart disease (CHD) among RA patients compared to the general population. Recent findings also point to data that show chronic systemic inflammation, such as that which occurs in RA, plays an important role in the pathogenesis of CHD. The ORWH-funded study will examine the incidence of acute myocardial infarction in RA subjects compared to controls. The possible interactions between RA and major CHD risk factors such as smoking, hyperlipidemia, and exogenous estrogens will be examined. In addition, studies on archived autopsy heart tissue will be conducted to test the hypothesis that coronary atherosclerosis is more extensive in RA subjects compared to matched controls.

ORWH funded the lead research group that is establishing a multi-institutional longitudinal registry of African American women diagnosed with early RA in order to identify genetic and non-genetic prognostic factors of disease outcome to permit prospective analyses of factors predictive of the clinical phenotype and outcomes.

MS Grant

This grant, funded by ORWH and NIAID, supports a clinical trial that focuses on identifying subsets of patients to permit evaluation of treatment outcomes within these various subsets. The investigators categorized MS patients on the basis of whether they have clearly defined relapses, relapsing-remitting MS, or whether they are progressing. Patients who are progressing are further categorized on the basis of whether they initially experienced relapses, or whether they deteriorate slowly without evidence of relapses or remissions.

SLE Grants

A total of nine grants that focused totally or partially on SLE were funded by ORWH and NIAID, NIAMS, and NIEHS.

Two grants explored epidemiological aspects of SLE, including evaluating the potential role of environmental chemicals in the development of lupus nephritis and elucidating how these exposures might influence the racial and gender disparity observed in this disease. Compared to white Americans, the incidence of lupus in African Americans is much higher, the disease develops at an earlier age, and has increased morbidity and mortality, especially relating to renal involvement.

The second grant, a case-control study, focused on determining androgen receptor, estrogen receptor, and cytochrome P450 genotypes in SLE cases as compared to control subjects.

Three Autoimmune Centers of Excellence grants, co-funded by ORWH and NIAID, support large translational studies that bridge basic science with clinical research. Within these large center grants, SLE-specific studies are undertaken, along with a variety of studies focused on other autoimmune disorders such as RA and MS.

Four grants focus on basic science research that uses animal models to elucidate the pathogenesis of SLE. Several studies are exploring the association of selected antigens with other tissue-specific proteins found in affected organs such as the skin and the heart. Findings may increase scientific understanding about ways to prevent, modulate the course of, or improve the treatment of SLE.

Osteoarthritis Grant

Supported by ORWH and NIAMS, this translational research is aimed at developing new therapies for elderly women who, at present, have only non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate their symptoms. Unfortunately, this age group is at greatest risk of developing serious side-effects from NSAIDs. Building upon their basic research findings, researchers have shown that prophylactic oral administration of doxycycline markedly reduces the severity of cartilage damage in a canine model of osteoarthritis. Beneficial protective effects were apparent, even when therapy was initiated after cartilage lesions were established. Similar results have been noted in other animal models of osteoarthritis, such as in guinea pigs and rabbits. Based on the encouraging basic research findings, a randomized placebo-controlled clinical trial will examine the effect of doxycycline and its ability to prevent the progression of early knee osteoarthritis in elderly women.

Rare Diseases Research Initiatives

New and Planned Extramural Research Initiatives

Continuation of Support for Several Rare Disease-specific RFPs or RFAs for FY 2000

Hyperaccelerated Award/Mechanisms in Immune Disease Trials
(NIAID/NIA/NIAMS/NIDDK/NINDS/ORWH)

Environment/Infection/Gene Interactions in Autoimmune Diseases
(NIEHS/NIAID/NIDDK/NIDCR/ORWH)

New Imaging Technologies for Autoimmune Diseases
(NIAID/NIDCR/ORWH)

Autoimmunity Centers of Excellence
(NIAID/NIDDK/NIAMS/ORWH)

Basic and Clinical Research on Fibromyalgia
(ORWH/NIAMS/NIDR/NINDS/NCCAM (formerly Office of Alternative Medicine [OAM]/Office of Behavioral and Social Sciences Research [OBSSR], Office of the Director, NIH)

Rare Disease-specific Meetings

Dr. Pinn attended the following meeting:
March 2, 2000, Autoimmune Conference.

Activities with Voluntary Rare Diseases Organizations to Stimulate Research

Dr. Pinn attended the following foundation-related meetings:
November 15, 1999, Lupus Foundation Award.
June 27, 2000, met with representative from the Temporomandibular Joint Disorders Association.
August 3, 2000, Fibromyalgia Foundation meeting.
September 13, 2000, met with representative from the Arthritis Foundation.

Office of Rare Diseases (ORD), Office of the Director, NIH

Overview of ORD Rare Diseases Research Activities

The main objective of ORD is to stimulate and coordinate research on rare diseases to bring hope to patients who may be affected by any of the approximately 6,000 rare diseases known today. A rare disease is defined as a disease, condition, or syndrome for which there are fewer than 200,000 affected persons alive in the United States.

Rare Diseases Research Initiatives

Highlights of Current ORD Activities

As part of its ongoing support of rare diseases initiatives, ORD:

- Developed and maintained the Rare Diseases Clinical Research Database (RDCRD) and incorporated it into the ORD Web site. In FY 2000, ORD worked with NLM to merge the existing database with the new ClinicalTrials.gov database. The NLM database describes research protocols and provides contact information for principal investigators (PIs), thereby facilitating public access to clinical trials and studies. ORD will continue to work with NLM to ensure inclusion of rare diseases clinical studies in the database.
- Developed and supports the Medical Genetics and Rare Disorders subfile of the Combined Health Information Database (CHID), which provides information available from voluntary patient support groups. Information on approximately 1,500 voluntary patient support groups is available through the ORD and CHID Web sites.
- Responded to requests for information on highly technical matters and matters of public policy and public interest on rare diseases research.
- Prepared the NIH Director's annual report to Congress on rare diseases research activities sponsored by NIH. All reports are published on ORD's Web site (see <http://rarediseases.info.nih.gov/ord>).

Scientific Workshops

Since 1995, ORD has co-sponsored 264 scientific workshops with NIH Institutes. In FY 2000, ORD co-sponsored 52 workshops. A list of these workshops appeared in last year's annual report. Workshops on rare diseases research are funded if a particular scientific opportunity exists or if very little (if any) research is currently under way. Preliminary findings from an evaluation of the workshops show that the workshops are an effective means of generating research ideas and grant applications in rare disease areas that might not otherwise attract much attention.

In FY 2001, ORD sponsored or is committed to co-sponsoring the following 55 workshops with the primary sponsoring NIH Institute(s):

NIA

- Ehlers-Danlos Syndrome
- Hutchinson-Gilford Progeria Syndrome

NIAAA

- Risk from Alcohol: SIDS (Sudden Infant Death Syndrome)
- Cellular and Molecular Mechanisms of Alcoholic Hepatitis
- Genetic and Molecular Markers in Fetal Alcohol Syndrome: High-Risk Pregnancies

NIAID

- Development of Effective Therapies and Prevention of West Nile, Dengue, and Other Flavivirus Infections (U.S./Japan Meeting)
- Animal Models of Autoimmune Disease: Current Models versus Advanced Technology
- Gene Therapy—A Promising Treatment for Primary Immunodeficiency Disease
- Bare Lymphocyte Syndrome (BLS) and Gene Expression of the Class II Major Histocompatibility Complex (MHC)
- The Innate Immune System and its Involvement in Autoimmune Diseases

NIAMS

- Pemphigus as a Model Organ-specific Autoimmune Disease

NCI

- Gene-Environment Interactions in the Etiology of Childhood Cancers
- Advances in Optics for Biotechnology, Medicine, and Surgery
- Childhood Atypical Teratoid/Rhabdoid Tumors of Central Nervous System
- Imaging in 2020, 2nd Series (Molecular Advances)
- (Mapping for) Admixture Linkage Disequilibrium Technique
- Pancreatic Ductal Cancer: Mouse Models
- Explore Potential of Protein Transduction Strategies in the Treatment of Cutaneous T Cell Lymphoma
- Molecular and Cellular Biology of Leukocyte Regulatory Receptors (Keystone Symposia)
- Basic Science of Pediatric Osteosarcoma

NICHD

- Research on Chromosome 18
- Lab and Research in Osteogenesis Imperfecta (OI)
- Endocrine Hypertension

NIDCR

- Treatment of Salivary Gland Disorders: Alternative Approaches

NIDDK

- Cooley's Anemia: Non-Invasive Measurement of Iron
- MPS and Related Diseases: Strategies for Therapy

NIEHS

- Low-Dose Effects of Endocrine Disruptors
- Childhood Respiratory Disease: Early Life Factors
- Immunologic Issues Concerning Genetically Modified Food

- Developmental Toxicology: Multidisciplinary Approaches Using Model Organisms and Genomics
- Lactoferrin
- Gordon Research Conference on “Genetic Toxicology”
- DNA Repair: U.S. and Japan
- Development of Outcome Measures and Consensus on Design Issues for Myositis Clinical Trials

NEI

- Ocular Toxoplasmosis: Epidemiology, Diagnosis, and Reconsideration of Etiologic Mechanism
- Craniofacial Muscle Specialization and Disease

NHLBI

- Alpha 1-Antitrypsin Deficiency: The Challenge of a Genetic Condition
- Sickle Cell Disease: Host Response
- Pulmonary Health and Disease: Protein Processing and Degradation

NHGRI

- Congenital Disorders of Glycosylation Type 1A

NIMH

- Ethical Issues in Suicide Research
- Effects of ART on HIV Disease of Nervous System in Children

NINDS

- Human Neuroborreliosis (in Lyme Disease)
- Chemistry Libraries for Drug Discovery in Neurological Disorders
- Hypertonic Symptoms in Children
- Neurobiology of Craniofacial Pain
- Mucopolidosis IV (MLIV): From Gene to Function
- Dystonia International Symposium
- Reflex Sympathetic Dystrophy
- Wilson’s Disease
- CAG-Repeat Conference

NINR

- Informal Caregiving: State of the Science
- Setting Research Agenda for End-of-Life Issues
- Cystic Fibrosis: Increasing Number of Nurse Scientists

ORD/OD

- Trimethylaminuria

Rare Diseases Information Center

ORD currently provides information on rare diseases through the ORD Web site and in response to telephone or written inquiries. Because the number of direct requests is ever-increasing, ORD and NHGRI are in the process of establishing an Information Center to respond to inquiries about rare and genetic disorders. The Information Center will provide access to existing information and develop new

materials to be included in an interactive Web site. Information for health care providers and the public will include:

- Information about the disease or condition.
- Locations of genetic counseling centers available for consultation.
- Summaries and locations of current and planned research related to rare diseases and genetic disorders.
- Names, locations, and types of printed or audiovisual materials provided by voluntary patient support groups.
- Disease-specific fact sheets.

In addition to its Web site, the Information Center will operate a toll-free telephone information service to respond to inquiries about rare diseases and genetics disorders for those without access to the Internet or e-mail.

Acronyms

AA	Aplastic anemia
AARP	American Association of Retired Persons
AAT	Alpha-1-antitrypsin
AAV	Adeno-associated virus
ACRIN	American College of Radiology Imaging Network
ADHD	Attention deficit hyperactivity disorder
ADPKD	Autosomal dominant polycystic kidney disease
ADRP	Autosomal dominant retinitis pigmentosa
AGS	Alagille syndrome
AIDS	Acquired immune deficiency syndrome
ALD	Alcoholic liver disease
ALPS	Autoimmune lymphoproliferative syndrome
ALS	Amyotrophic lateral sclerosis
ALTS	Atypical squamous cells of undetermined significance (ASCUS)/low-grade squamous intraepithelial lesion (LSIL) Triage Study
AML	Acute myelogenous leukemia
AN	Anorexia nervosa
<i>apoB</i>	Apolipoprotein B
APS	Antiphospholipid syndrome
ARF	Acute rheumatic fever
ARPKD	Autosomal recessive polycystic kidney disease
ARVD	Arrhythmogenic right ventricular dysplasia
ART	Antiretroviral therapy
ASCUS	Atypical squamous cells of undetermined significance
ASPS	Advance sleep phase syndrome
AT	Ataxia telangiectasia
ATG	Antithymocyte globulin
ATM	Ataxia telangiectasia mutated
ATRX	Alpha thalassemia mental retardation X-linked (syndrome)
BA	Biliary atresia
BAMSG	Bacteriology and Mycology Study Group
BBS	Bardet-Biedl syndrome
BLNK	B cell linker protein
BLS	Bare lymphocyte syndrome
BMT	Bone marrow transplant
BN	Bulimia nervosa
BPD	Bronchopulmonary dysplasia
BPES	Blepharophimosis – epicanthus inversus – ptosis syndrome
BPH	Benign prostatic hyperplasia
<i>BPMR2</i>	Bone morphogenetic protein receptor II (gene)
BS	Bloom syndrome
BSE	Bovine spongiform encephalopathy
BWS	Beckwith-Wiedemann syndrome
<i>CA9</i>	Carbonic anhydrase 9 (gene)
CACP	Camptodactyly-arthropay-coxa vara-pericarditis
CAM	Complementary and alternative medicine

CASG	Collaborative Antiviral Study Group
CASPAR	Computerized Affected Sibling Pair Analyzer and Reporter
CC	Clomiphene citrate (NICHD)
CC	Warren Grant Magnuson Clinical Center, NIH
CDGs	Congenital disorders of glycosylation
CDH	Congenital diaphragmatic hernia
CF	Cystic fibrosis
<i>CFTR</i>	Cystic fibrosis transmembrane conductance regulator (gene)
CGD	Chronic granulomatous disease
CGH	Comparative genomic hybridization
CHD	Coronary heart disease
CHH	Cartilage hair hypoplasia
CHID	Combined Health Information Database
CJD	Creutzfeldt-Jakob disease
CLL	Chronic lymphocytic leukemia
CMHRA	Center for Mental Health Research on AIDS
CMV	Congenital cytomegalovirus
CNC	Carney complex
CNS	Central nervous system
COMS	Collaborative Ocular Melanoma Study
CPAP	Continuous positive airway pressure
CRADA	Cooperative Research and Development Agreement
<i>CREB3</i>	Camp responsive element binding protein 3 (gene)
CRF	Corticotropin-releasing factor
CRP	C-reactive protein
CS	Cockayne syndrome
CT	Computed tomography
DA	Dopamine
DDG	Drug Development Group
DES	Diethylstilbestrol
DFO	Desferrioxamine
DGCR	DiGeorge chromosomal region
DGS	Di George syndrome
DIR	Division of Intramural Research
DM	Myotonic dystrophy
DMD	Duchenne muscular dystrophy
DNA	Deoxyribonucleic acid
<i>DSM IV</i>	<i>Diagnostic and Statistical Manual, Fourth Edition</i>
DTR&D	Division of Treatment Research and Development
DXN	Doxorubicin
E/CC	Endometrioid and clear cell (cancer)
EDA	Ectodermal dysplasia (anhidrotic)
EDRN	Early Detection Research Network
EDS	Ehlers-Danlos syndrome
ELST	Endolymphatic sac tumor
EnaC	Epithelial Na ⁺ channel
ERD	End-stage renal disease
EST	Expressed sequence tag
FA	Fanconi anemia

FAS	Fetal alcohol syndrome
FDA	Food and Drug Administration
FDNIB	Familial dementia with neuroserpin inclusion bodies
FGFR3	Fibroblast growth factor receptor 3
FHBL	Familial hypobetalipoproteinemia
FHC	Familial hypertrophic cardiomyopathy
FHH	Familial homozygous hypercholesterolemia
FHL	Familial hemophagocytic lymphohistiocytosis
FMF	Familial Mediterranean fever
FPLD	Familial partial lipodystrophy
FPPH	Familial primary pulmonary hypertension
FRDA	Friedreich's ataxia
FSGS	Focal segmental glomerulosclerosis
FSHD	Facioscapulohumeral muscular dystrophy
GALT	Galactose transferase
GAS	Group A streptococci
GAVI	Global Alliance for Vaccines and Immunization
GBS	Group B streptococci
GCBS	Greig cephalopolysyndactyly syndrome
GDM	Gestational diabetes mellitus
GEB	Genetic Epidemiology Branch
GH	Growth hormone
GH-IGF	Growth hormone-insulin-like growth factor
GIST	Gastrointestinal stromal tumors
GM-CSF	Granulocyte-macrophage colony stimulating factor
GP I	Glycoprotein complex I
GRA	Glucocorticoid remediable aldosteronism
GvHD	Graft-versus-host disease
HbF	Fetal hemoglobin
HCM	Hypertrophic cardiomyopathy
HCV	Hepatitis C virus
HD	Huntington's disease
HDL	High-density lipoprotein
HGP	Human Genome Project
HHCS	Hereditary hyperferritinemia cataract syndrome
HHT	Hereditary hemorrhagic telangiectasia
<i>Hib</i>	<i>Haemophilus influenzae</i> type b
HIE	Hyperimmunoglobulin E recurrent infection syndrome
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPE	Holoprosencephaly
HPS	Hantavirus pulmonary syndrome
HPT-JT	Hyperparathyroidism-jaw tumor (syndrome)
HPV	Human papilloma virus
HSV	Herpes simplex virus
HUS	Hemolytic uremic syndrome
IBS	Irritable bowel syndrome
ICs	Institutes and Centers
ICF	Immunodeficiency, centromeric decondensation, and facial anomalies (syndrome)

IDF	Immune Deficiency Foundation
INCL	Infantile neuronal ceroid lipofuscinoses
IND	Investigational New Drug
IOM	Institute of Medicine
IP	Incontinentia pigmenti
IPF	Idiopathic pulmonary fibrosis
IR	Ionizing radiation
IS	Idiopathic scoliosis
ITP	Immune thrombocytopenic purpura
JHS	Jackson Heart Study
JRA	Juvenile rheumatoid arthritis
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma herpes virus
LAM	Lymphangioliomyomatosis
LDL	Low-density lipoprotein
LH	Luteinizing hormone
LHON	Leber's hereditary optic neuropathy
LHRH	Luteinizing hormone-releasing hormone
LSDs	Lysosomal storage diseases
LQTS	Long QT syndrome
L-R	Left-right (axis malformations)
LSDs	Lysosomal storage disorders
LSIL	Low-grade squamous intraepithelial lesion
LVAS	Large vestibular aqueduct syndrome
MCA/MR	Multiple congenital anomaly/mental retardation
MDD	Medications Development Division
MDMA	Methylenedioxymethamphetamine
MDS	Myelodysplastic syndrome
MEN1	Multiple endocrine neoplasia type 1
MCH	Major histocompatibility complex
MIM	Multilateral Initiative on Malaria
MITF	Microphthalmia-associated transcription factor
MKS	McKusick-Kaufman syndrome
MLIV	Mucopolidosis IV
MM	Multiple myeloma
MODY	Maturity-onset diabetes of the young
MR4	Malaria Research and Reference Reagent Resource Center
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSG	Mycoses Study Group
MSH	Multicenter Study of Hydroxyurea (Trial)
MTP	Microsomal triglyceride transfer protein
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	Nijmegen breakage syndrome
NCCAM	National Center for Complementary and Alternative Medicine
NCBI	National Center for Biotechnology Information
NCI	National Cancer Institute
NCMHD	National Center on Minority Health and Health Disparities (Office of the Director, NIH)
NCRR	National Center for Research Resources

NDA	New Drug Application
NEC	Necrotizing enterocolitis
NEI	National Eye Institute
NER	Nucleotide excision repair
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type 2
NHGRI	National Human Genome Research Institute
NHLBI	National Heart, Lung, and Blood Institute
NIA	National Institute on Aging
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NIAID	National Institute of Allergy and Infectious Disease
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases
NICHHD	National Institute of Child Health and Human Development
NIDA	National Institute on Drug Abuse
NIDCD	National Institute of Deafness and Other Communication Disorders
NIDCR	National Institute of Dental and Craniofacial Research
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIEHS	National Institute of Environmental Health Sciences
NIMH	National Institute of Mental Health
NINDS	National Institute of Neurological Disorders and Stroke
NINR	National Institute of Nursing Research
NLM	National Library of Medicine
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric acid synthase
NP-C	Niemann-Pick type C disease
NSAIDs	Non-steroidal anti-inflammatory drugs
OAM	(Former) Office of Alternative Medicine (now NCCAM)
OBSSR	Office of Behavioral and Social Sciences Research, Office of the Director, NIH
OCL	Office of Clinical Liaison
OD	Office of the Director, NIH
OI	Osteogenesis imperfecta
OMIM	Online Mendelian Inheritance in Man (database)
ORD	Office of Rare Diseases (Office of the Director, NIH)
ORMH	Office of Research on Minority Health
ORWH	Office of Research on Women's Health (Office of the Director, NIH)
OTC	L-2-oxothiazolidine-4-carboxylic acid
PA	Program Announcement
PAP	Pulmonary alveolar proteinosis
PCD	Primary ciliary dyskinesia
PCNA	Proliferating cell nuclear antigen
PCOS	Polycystic ovarian syndrome
PCP	Phencyclidine
<i>PDS</i>	Pendred syndrome (gene)
PEGT	Programs of Excellence in Gene Therapy
PET	Positron electronic tomography
PHA	Pulmonary Hypertension Association
PHHI	Persistent hyperinsulinemic hypoglycemia of infancy
PI	Principal Investigator

PKD	Polycystic kidney disease
PLS	Papillon-Lefevre syndrome
PNH	Paroxysmal nocturnal hemoglobinuria
POF	Premature ovarian failure
PPH	Primary pulmonary hypertension
PPHN	Persistent pulmonary hypertension of the newborn
PPNAD	Primary pigmented nodular adrenocortical disease
<i>PREB</i>	Prolactin regulatory element binding (gene)
PRISMS	Parents and Researchers Interested in Smith-Magenis Syndrome
PRL	Prolactin
PS	Pressure support
PXE	Pseudoxanthoma elasticum
RA	Rheumatoid arthritis
RAID	Rapid Access to Intervention Development
RCC	Renal cell carcinoma
RDCRD	Rare Diseases Clinical Research Database
rhGH	Recombinant human growth hormone
RNA	Ribonucleic acid
RP	Retinitis pigmentosa
RPA	Replication protein A
SADDAN	Severe achondroplasia with developmental delay and acanthosis nigricans
SAMHSA	Substance Abuse and Mental Health Services Administration
SBIR	Small Business Innovative Research
SCA	Sickle cell anemia
SCA1	Spinocerebellar ataxia-1
SCD	Sickle cell disease
SCID	Severe combined immunodeficiency disorder
SCOR	Specialized Center of Research
SCT	Stem cell transplantation
<i>SHH</i>	Sonic Hedgehog (gene)
SIDS	Sudden infant death syndrome
SIV	Simian immunodeficiency virus
SLE	Systemic lupus erythematosus
SLO	Smith-Lemli-Opitz (syndrome)
SMS	Smith-Magenis syndrome
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SOD1	Superoxide dismutase 1
SpeB	Streptococcal pyrogenic exotoxin
SPECT	Single-photon emission computed tomography
SPIRCAP	Strategic Program for Innovative Research on Cocaine (and other Psychomotor Stimulants) Addiction Pharmacotherapy
SUD	Substance use disorder
SVAS	Supravalvular aortic stenosis
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TCR	Transcription-coupled repair
TD	Thanatophoric dysplasia
TFOs	Triplex-forming oligonucleotides
TGIF	TG-interacting factor

TMD	Temporomandibular disorders
TNF	Tumor necrosis factor
TS	Gilles de la Tourette syndrome
TSC	Tuberous sclerosis complex
TSEs	Transmissible spongiform encephalopathies
TTD	Trichothiodystrophy
TTP	Thrombotic thrombocytopenic purpura
UCB	Umbilical cord blood
UCSF	University of California at San Francisco
UV	Ultraviolet
VA	Department of Veterans' Affairs
VCFS	Velo-cardio-facial syndrome
VCP	Valosin-containing protein
VHL	von Hippel-Lindau (disease)
VLBW	Very-low-birth-weight
VLDL	Very low-density lipoprotein
vWf	von Willebrand factor
WAS	Wiskott-Aldrich syndrome
WASP	WAS protein
WHO	World Health Organization
WM	Waldenstrom's macroglobulinemia
WMS	Williams syndrome
WS	Waardenburg Syndrome (NIDCD)
WS	Werner syndrome
XHIM	X-linked hyper-IgM syndrome
XP	Xeroderma pigmentosum

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