

## Meeting report

### New areas of focus at workshop on human diseases involving DNA repair deficiency and premature aging

#### Abstract

Researchers and clinicians interested in human diseases of DNA repair deficiency and premature aging gathered at the National Conference Center in Lansdowne, Virginia on 5–8 September 2006 to attend a workshop co-organized by Vilhelm Bohr (National Institute of Aging) and Kenneth Kraemer (National Cancer Institute). An important feature of this workshop was the participation of representatives from xeroderma pigmentosum (XP), Cockayne Syndrome (CS) and trichothiodystrophy (TTD) family support groups. Studies presented at the workshop described important new insights into the phenotypic complexity of XP, CS and TTD, renewed focus on the neurological manifestations of each of these diseases, as well as keen interest in the role of oxidative stress and mitochondrial dysfunction in neurodegenerative processes and normal and/or premature aging. This workshop report summarizes some of the presentations and outcomes of the workshop.

*Keywords:* Cockayne syndrome; Werner syndrome; Trichothiodystrophy; Xeroderma pigmentosum; Mitochondria; DNA repair; Oxidative stress

#### 1. Overview

Xeroderma pigmentosum (XP)<sup>1</sup>, Cockayne Syndrome (CS) and trichothiodystrophy (TTD) are rare diseases with worldwide incidence estimated at less than 1 per 250,000 births. Yet, there are enough dedicated researchers and clinicians interested in XP, CS and TTD, and enough cooperative patients and family members that remarkable progress has been made recently in understanding the molecular basis of these complex human diseases. This progress was quite evident at a recent workshop<sup>2</sup> held at the National Conference Center in Lansdowne, Virginia (5–8 September 2006) on XP, CS, TTD and other human diseases involving premature aging and/or defective DNA repair. This second in a series of workshops on DNA repair deficiency diseases was attended by approximately 100 researchers and clinicians and by representatives from XP,

CS and TTD patient support groups. The workshop revealed areas in which great progress has been made, areas ripe for future study, and bottlenecks that are inhibiting progress in either basic understanding of the diseases or their clinical management. Five panel discussion sessions and informal poster sessions provided the opportunity for extensive discussion and interaction between workshop participants.

An important and somewhat unusual feature of the workshops in this series was the participation of representatives from XP, CS and TTD family support groups. Workshop participants agreed that this enriched the meeting immensely. Importantly, family support group representatives who attended the workshop clearly indicated their desire to continue to assist clinical and basic research efforts by raising funds, participating in a patient registry and donating samples for disease-specific cell, tissue or brain banks.

XP, CS and TTD are caused by inherited mutations that alter multifunctional macromolecular protein complexes which play essential roles in DNA repair and RNA transcription. The phenotypes of these diseases are very varied, a fact that is consistent with their genetic and molecular complexity. To date, defects in 12 genes have been associated with XP, CS and TTD and related diseases. However, additional as yet unidentified complementation groups may exist. Recent studies described at this workshop demonstrated important new insights into the phenotypic complexity of XP, CS and TTD and renewed focus on the neurological manifestations of each of these diseases. One hypothesis expressed at the workshop proposed that some

<sup>1</sup> Abbreviations: BCC, basal cell carcinoma; CS, Cockayne Syndrome; DRC, DNA repair capacity; HD, Huntington Disease; SCC, squamous cell carcinoma; NMSC, non-melanoma skin cancer; SNP, single nucleotide polymorphism; TTD, trichothiodystrophy; WRN, Werner Syndrome; XP, Xeroderma pigmentosum.

<sup>2</sup> The workshop entitled Xeroderma Pigmentosum and other diseases of human premature aging and DNA Repair: Molecules to Patients was held at the National Conference Center in Lansdowne, Virginia on 5–8 September 2006. The workshop was co-organized by Kenneth Kraemer (National Cancer Institute) and Vilhelm Bohr (National Institute on Aging) and co-sponsored by the NIH Office of Rare Diseases, National Institute of Aging, National Institute of Environmental Health Sciences and the National Cancer Institute.

clinical manifestations of XP and CS may be explained not simply by “defective transcription” or “defective TFIIH” but, more precisely, by a failure to express a specific gene in a specific tissue at a specific time. The role of oxidative stress as a catalyst of mitochondrial dysfunction also came to the forefront at the workshop, being cited as a potentially key factor in the etiology of diseases characterized by neurodegeneration and/or premature aging. The following report summarizes some of the presentations and outcomes of the workshop (Box 1 and Box 2).

## 2. Revisiting genotype/phenotype correlation in XP

Clinicians who study XP have often pointed to a confusing lack of correlation between phenotype and genotype of XP patients. However, with access to larger numbers of patients, suggestive trends have recently emerged that provide rational explanation for the relative severity of disease in some patient sub-groups. In particular, Kenneth Kraemer (National Cancer Institute) and his colleagues conducted molecular and clinical

### Box 1

#### HIGHLIGHTS OF WORKSHOP SESSIONS

##### CLINICAL RESEARCH

- For some XP patient subgroups (XPA, XPB, XPC, XPD, and XPG), the severity of the disease correlates with the severity of the molecular defect in XP-related proteins or mRNA transcripts.
- The ocular abnormalities in XP are caused by sun exposure and/or reflect premature aging while those in TTD and CS reflect developmental abnormalities.
- The classical descriptors of TTD (PIBIDS, IBIDS and BIDS) may not accurately represent the entire clinical spectrum of TTD. Other clinical signs, including ocular and immunological defects, defective DNA repair and pregnancy complications, should also be considered.
- XP patients exhibit cerebral and cerebellar atrophy; in contrast, TTD and CS patients have a defect in myelination of the white matter of the cerebrum.

##### TRANSLATIONAL

- Diagnostic testing, based on functional assays but not on genotyping, is currently available for XP, CS and TTD in the Netherlands, Germany, Italy, Japan, Israel, and the UK, but not in the US. There is strong consensus among researchers and clinicians that CLIA-certified testing for these diseases is urgently needed in the US.
- A topical agent called dimericine, whose active ingredient is a bacterial DNA repair enzyme that stimulates repair of UV-induced skin damage, reduced the incidence of actinic keratoses and basal cell carcinomas on the skin of treated XP patients significantly more than a placebo control. Dimericine is not yet approved by the US FDA for treatment of any disease.

##### DISEASE MECHANISMS

- TFIIH from TTD/XPD<sup>-/-</sup> mice is defective in stabilizing the thyroid receptor on the promoter region of TR-target genes, resulting in dysregulated expression of myelin in the brains of TTD animals. Thus, defects in transcriptional co-activation play a role in the neuropathology of TTD.
- Deficiency in the 8-oxoguanine-specific mouse glycosylase, mOGG1, prevents rapid expansion of CAG repeats in the brain of mice engineered as a model for Huntington Disease. The results suggest that oxidative damage in the brain may be a precipitating factor, and possibly useful marker, for progression in Huntington Disease.
- Knockout mice lacking the NEIL1 glycosylase, an enzyme that specifically excises ring-fragmented purines (*i.e.*, FapyG and FapyA) and some saturated pyrimidines from dsDNA and ssDNA, have a phenotype that strongly resembles metabolic syndrome in humans (*i.e.*, fatty liver disease, dyslipidemia, diabetes, obesity and insulin resistance). It is possible that accumulation of Fapy DNA lesions in mtDNA and transcriptionally-active nuclear DNA lowers the threshold at which cumulative oxidative stress destabilizes metabolic homeostasis.
- Mice that are heterozygous for exonuclease-deficient polymerase  $\gamma$  have a 500- to 1000-fold higher mtDNA point mutation rate than wild type mice of an equivalent age; however, these animals show no signs of premature aging. Thus, although random point mutations in mtDNA are a likely manifestation of aging, they are not a likely direct cause of aging-related phenomenon in otherwise normal mice.

**Box 2****KEY WORKSHOP CONCEPTS**

- The primary defect in XP is in the response to DNA damage. The primary defect in TTD is developmental in nature. CS patients appear to have both types of defects.
- Some clinical manifestations of XP, TTD and CS may be explained by a failure to express a specific gene in a specific tissue at a specific time.
- Mutations that compromise the co-activator function of TFIIH can have highly tissue-specific effects. This result has clear importance for understanding all diseases characterized phenotypically by defective transcription.
- Oxidative stress is a catalyst of mitochondrial dysfunction, which can manifest itself in highly diverse pathological conditions including neurodegeneration, motor dysfunction, retinal degeneration and aging-like phenomena.
- Oxidative DNA damage and deficient BER may contribute significantly to the pathology of CS.
- Susceptibility to metabolic syndrome in humans could be induced by excessive exposure to endogenous and/or exogenous ROS and associated ROS-mediated DNA and cellular damage.

evaluation of 111 XP and TTD patients and their family members. The majority of the patients fell into complementation groups XPA ( $n = 14$ ), XPC ( $n = 43$ ) or XPD ( $n = 33$ ). Patients with XPA mutations fell into three sub-groups characterized by rapid, moderate or slow progression of neurological disease. Molecular analysis revealed the following patterns: patients exhibiting rapid progression of neurological disease carry XPA alleles with a premature stop codon; patients exhibiting a moderate rate of progression of neurological disease carry an XPA allele with a mis-splice mutation that deletes exon 6; patients exhibiting slow progression of neurological disease carry a splicing mutation that allows for normal transcription re-initiation. Thus, for this sub-group of 14 XP patients with XPA mutations, the severity of disease correlates quite well with the severity of the molecular defect in the XPA gene. Sixteen XP patients with XPC defects and their parents were also characterized. The patients produced no detectable XPC protein; but, very low levels of XPC mRNA were detected. Heterozygous disease-free carriers (parents) demonstrated an intermediate level of XPC mRNA relative to normal individuals, due to haploinsufficiency at the XPC locus. The level of XPC mRNA was also quantified in two Turkish XPC kindreds with five affected patients: two patients with severe disease expressed <0.1% of the normal level of XPC mRNA, while three patients with relatively mild disease expressed 3–5% of the normal level of XPC mRNA. Thus, for these patients, the amount of XPC mRNA correlated with the severity of disease. In general, similar patterns were observed for XP complementation groups B, D, and G, although the number of XPB and XPG patients is very small. Together these data suggest that the severity of the molecular defect in XP patients may often correlate with the severity of phenotype. Furthermore, these studies clearly identify molecular markers that appear to retrospectively explain patient phenotype; these and similar studies may in the future provide the means for prognostic assessment of XP patients.

**3. Clinical features of TTD: moving beyond PIBIDS**

Trichothiodystrophy is a clinically diverse disease syndrome. John DiGiovanna and colleagues (National Cancer Institute) conducted extensive analysis of TTD-associated clinical symptoms based on the reported characteristics of 112 TTD patients in 93 published case studies. Classical symptoms of TTD, namely photosensitivity, ichthyosis, brittle hair, intellectual impairment and short stature (PIBIS) were considered, as well as ocular defects, immunological defects, pregnancy complications and DNA repair defects. Decreased fertility was not considered due to the young age of most of the subjects. The data were analyzed to identify clinical patterns in different patient sub-groups. A significant number of patients had a classical pattern of clinical signs, namely, PIBIDS (28%), IBIDS (21%) or BIDS (12%); but a large patient sub-group (39%) had variable non-classical patterns of clinical signs. These data suggest that the classical descriptors of TTD may misrepresent the clinical spectrum of TTD, and that other clinical signs, including ocular and immunological defects as well as DNA repair deficiency and pregnancy complications are frequent symptoms that warrant consideration during clinical evaluation of TTD patients.

**4. Focusing on eye defects in XP, TTD and CS**

Recent studies revealed interesting eye defects in XP, TTD and CS/COFS patients. Because the types of eye defects differ for XP, TTD and CS/COFS, the results may be helpful in understanding and managing the clinical manifestations of these diseases. Brian Brooks and colleagues (National Eye Institute) analyzed the eye defects in 27 XP patients, 9 TTD patients and 4 XP/TTD patients. Eye defects associated with XP included skin malignancy, eyelid abnormalities, conjunctivitis/inflammation, dry eye and limbal stem cell deficiency. In contrast, eye defects associated with TTD

included infantile cataracts, various ocular developmental abnormalities, high myopia and reduced visual acuity, not entirely attributable to the presence of cataracts. Brooks also noted that TTD and XP/TTD patients often exhibit corneal dryness. Helene Dolphus (University of Strasbourg) described eye defects and their clinical management in CS/COFS patients. The primary eye defects in CS patients were congenital cataract, retinal degeneration, optic atrophy and enophthalmus. More severe but similar eye defects were observed in patients with the severe CS-like disease syndrome known as cerebral–ocular–facial–skeletal syndrome (COFS).

Brooks concluded that the eye defects associated with XP reflect premature aging and exposure-induced degeneration, while the eye defects associated with TTD reflect developmental abnormalities. Brooks further proposed that inflammation and an aberrant inflammatory response to eye damage may contribute to the eye phenotype in XP, and that this may reflect a fundamental immune dysfunction in some XP patients. Although eye defects in TTD patients tend to reflect developmental defects, the presence of dry eye in these patients suggests that premature aging-like phenomena or other pathological mechanisms may also affect the eyes of these individuals. Dolphus pointed out that the retina is an extraordinarily sensitive and adaptive tissue with a high metabolic rate and high oxygen consumption, and that retinal pathology is often associated with diseases characterized by mitochondrial and metabolic defects. The eye provides a “window into the brain”. However, it remains possible that the retinal tropism in CS reflects specific requirements for DNA repair and/or transcription in the retina.

### **5. White matter or gray matter, degeneration or development: making sense of the neuropathology in XP, TTD and CS**

The brains of CS patients demonstrate strikingly similar neuropathological features, despite differences in clinical features, patient age and genotype. Four CS brains, from patients who died at ages 6, 14, 17 or 31, were studied extensively by Karen Weidenheim and colleagues (Albert Einstein University, New York). These brains uniformly displayed markedly low brain weight, patchy demyelination, basal ganglia calcification, optic and auditory system defects and cerebellar atrophy with relative preservation of the neocortex. Atherosclerotic and arteriosclerotic changes, typical of aging normal brains, were present. The neuronal changes suggest possible defects in neuronal connectivity. The molecular etiology of these defects are not known. The mechanism by which defective DNA repair and/or RNA transcription may have caused the observed brain pathology is unclear. It remains possible that other factors besides defective DNA repair and/or RNA transcription may lead to molecular defects in the CNS in CS patients.

Nicholas Patronas and colleagues (Clinical Center, National Institutes of Health) performed neuroradiological studies of 14 XP patients, 6 TTD patients and 6 patients with the XP/TTD complex using both MRI and CT scans. The XP patients showed cerebral and cerebellar atrophy and no white matter disease, while TTD patients showed cerebral atrophy and white

matter disease but no cerebellar atrophy. XP/TTD patients showed significant white matter disease, thus resembling TTD patients more than XP patients.

In discussion, the question was raised as to whether and when one can differentiate between a degenerative defect in neural (and other) tissue and a developmental defect. For example, do myelin structures degrade prematurely in the brain of TTD patients, or did the myelin structures form incorrectly or insufficiently during development in these patients? While it is difficult to answer this question in a definitive manner, clinicians lean towards the latter interpretation with regard to the neuropathology in TTD. This view is supported by the studies of eye pathology in XP and TTD patients described above.

### **6. Translational science: bringing research findings to patients and their families**

Diagnostic testing for XP, CS and TTD was discussed during one of the workshop panel discussion sessions. Diagnostic testing based on functional assays, but not on DNA genotyping, is currently available for XP, CS and TTD in the Netherlands, Germany, Italy, Japan, Israel, and the UK. These tests are used to confirm clinical findings and for prenatal diagnosis of at-risk pregnancies. No CLIA-certified laboratory currently performs these tests in the US. However, there is strong consensus among researchers and clinicians that CLIA-certified testing for these diseases is urgently needed in the US. One laboratory in the US currently offers DNA diagnostic testing for mutations in CS patients and for known mutations in XP families.

Dr. Dan Yarosh (AGI Dermatics, New York) described a placebo-controlled, double-blind randomized clinical study of a topical agent called dimericine, whose active ingredient is a bacterial DNA repair enzyme that stimulates repair of UV-induced skin damage. The trial included 29 patients with XP (20 treated, 9 control) who applied dimericine daily to face and arms for 1 year. The clinical endpoint was appearance of new skin lesions, including actinic keratoses, basal or squamous cell carcinoma or melanoma. The results indicate that dimericine reduces the incidence of actinic keratoses and basal cell carcinoma on the skin of treated XP patients more than the placebo control. The results were encouraging, but the trial suffered from small sample size, variable patient characteristics and lack of an appropriate mathematical model for predicting rare skin cancer events. Yarosh indicated that more sensitive surrogate clinical endpoints were needed to assess drug efficacy, and that different study designs (*i.e.*, bilateral, cross-over or before-after) should be considered in future clinical trials. Although dimericine has been approved by the US Food and Drug Administration (FDA) for research testing in severely immunosuppressed kidney transplant patients, it is not yet FDA-approved for treatment of any disease.

### **7. The beginnings and endings of DNA repair in CS and Werner syndrome**

The SWI/SNF and RecQ families of DNA helicases include several human proteins that may play important roles in

promoting genome stability, and whose absence leads to DNA repair defects and symptoms of premature aging. Several of these proteins and their role in genome stability were discussed at this workshop, including CSB, the Werner Syndrome protein (WRN) and RecQ1. Vilhelm Bohr and David M. Wilson III (National Institute of Aging) presented evidence supporting a role for CSB in BER-mediated repair of oxidative DNA lesions, perhaps through specific stimulation of human apurinic endonuclease and short patch BER. CSB, although a member of the SWI/SNF helicase family, lacks detectable helicase activity *in vitro*. Nevertheless, it remains possible that CSB may promote DNA unwinding at or near DNA lesions *in vivo*, possibly in transcribed genes. Consistent with this idea, Leon Mullenders (University of Leiden) used chromatin immunoprecipitation from UV-treated wild type and CSA- or CSB-deficient cultured cells to show that CSB is specifically required to assemble and co-localize a DNA repair protein complex with DNA lesions and stalled RNA polymerase II (RNAPII). CSB assembles first at the transcription blocking lesion, followed by CSA/DDB1/CSN1 and XAB2. Subsequent assembly of NER proteins such as ERCC1, XPA and TFIIH requires CSB but not the CSA/DDB1/CSN1/XAB1 sub-complex. CSB and CSA also play a role in recruiting chromatin remodeling factors p300 and HMG1 to UV-damaged DNA; CSA and CSB are both required to recruit HMG1, while CSB is sufficient to recruit p300. Together, these data support the hypothesis that CSB is specifically required for repair of transcription-blocking lesions in transcribed regions of the genome and that CSB may play a direct role in stimulating global base excision repair. The results are also consistent with the possibility that oxidative DNA damage and deficient BER may contribute significantly to the pathology of CS.

WRN and human RecQ1 are both RecQ family helicases that possess structure-specific DNA helicase activity. Deficiency in WRN leads to many symptoms of premature aging including dementia, brain atrophy, atherosclerosis, cancer, diabetes and loss and or graying of hair. The exact biological role of WRN is not well understood, although it is thought to play a role in double strand break repair via homologous recombination or non-homologous end joining as well as base excision repair. Mark Eller and Vilhelm Bohr (Boston University) described another role of WRN at this workshop: a role in maintaining and repairing telomeres. Eller characterized the effects of T-oligos, short G-rich C-poor DNA oligonucleotides homologous to telomere repeats, on cultured cells. Because T-oligos mimic a destabilized unfolded telomere, they stimulate a telomere-targeted DNA damage response mediated by ATM, p53, and  $\gamma$ H2AX. Eller presented evidence suggesting that the 3'-exonuclease of WRN protein may play a significant role in this DNA damage response. This result is consistent with the cellular phenotype associated with WRN-deficiency.

## 8. Oxidative stress, mitochondrial dysfunction and premature aging

Cynthia McMurray (Mayo Clinic, Rochester, Minnesota), who presented her recent studies on Huntington Disease (HD) at this workshop, proposed that neurodegenerative diseases

including HD can be modeled using two components: toxicity and onset. In the case of HD, toxicity and onset are independent and separable factors that lead to the observed pathophysiology. In particular, the mutant HD protein is cytotoxic and causes a protein trafficking defect that manifests as mitochondrial dysfunction. McMurray further proposed that onset in HD is a function of the rate/level of oxidative DNA damage in the cell, and that the target of oxidative damage in HD is the CAG repeat in the Huntington gene. According to the model proposed by McMurray, Reactive oxygen species (ROS)-induced single strand DNA breaks accumulate in CAG repeats at an increasing rate in the brains of older animals or individuals, and these lesions play a direct role in promoting repeat expansion. Many lines of evidence indicate that oxidative DNA damage accumulates in a tissue-specific, age-dependent and even an organelle-specific manner in mice and humans. This model for progression in HD is consistent with the fact that 8-oxoguanine (8oxoG) differentially accumulates in the brains of 52 week old mice. McMurray showed that the level of 8oxoG correlates with the rate of expansion of CAG repeats, and the rate of expansion is much higher in the brain than in the tail of animals of equivalent age. The proposed model was tested by examining repeat expansion in mice that lack the 8oxoG glycosylase OGG1 (OGG1<sup>-/-</sup> mice). The rate of HD repeat expansion was much lower in the brain and liver of OGG1-deficient mice than in the brain and liver of OGG1 wild type mice.

These data strongly suggest that oxidative DNA damage, and the rate of repair of oxidative DNA damage, may play a significant role in HD and possibly in other neurodegenerative diseases. For different disease syndromes, different mechanisms are likely to be involved in targeting the pathology to neurons or other biological components or structure that impact neurological function. Nevertheless, the hypothesis that oxidative stress is a common precipitating and/or prognostic factor in neurological disease has gained more support through McMurray's recent work on HD.

Lawrence Loeb (University of Washington) described recent experiments that examine the relationship between mitochondrial DNA (mtDNA) damage and aging. Loeb and colleagues adapted their recently developed "Random Mutation Capture Assay (RMCA)" to enable sensitive detection of point mutations in mtDNA. This assay was used to measure point mutation rates in young and old wild type mice, as well as in pol  $\gamma$ -exonuclease-deficient heterozygous or homozygous young and old mice. In agreement with previous studies correlating increased mtDNA damage with aging, Loeb showed that the level of spontaneous point mutations in normal mouse brain increases from  $0.4 \times 10^{-6}$  at 3 months of age to  $14 \times 10^{-6}$  at 24–33 months of age. Similar results were obtained in mouse liver. The mutation spectrum in these mice showed a preponderance of GC:AT transitions, which are characteristic of oxidative DNA damage and/or polymerase  $\gamma$  misincorporation errors. Importantly, Loeb's data demonstrates an approximately 100-fold lower spontaneous mtDNA mutation rate than previously measured. This could reflect the higher sensitivity and accuracy of the assay.

Although it is generally accepted that mtDNA damage accumulates in the tissues of multicellular organisms over time,

it has not yet been shown that such mtDNA damage plays a direct role in any specific aging-related phenomenon. Loeb helped address this question by measuring mtDNA mutations in mice that are heterozygous or homozygous for exonuclease-deficient pol  $\gamma$ . Loeb observed that the heterozygous mice had a 500- to 1000-fold higher mtDNA mutation rate than young wild type mice. These animals, unlike mice that are homozygous for the mutant pol  $\gamma$ , show no signs of premature aging. The most interesting conclusion from this study is that while random point mutations in mtDNA are a likely manifestation of aging, they are not a likely direct cause of aging-related phenomenon in otherwise normal mice. Secondly, these data support the conclusion that oxidative DNA damage accumulates in the genomes of aging animals.

The potential implications of ROS-mediated DNA damage for cellular function and homeostasis, and the need for efficient repair of ROS-mediated damage was underscored dramatically at this workshop by results presented by Stephen Lloyd (Oregon Health and Science University). Lloyd characterized a DNA-repair defective mouse with a deficiency in the NEIL1 glycosylase, an enzyme that specifically excises ring-fragmented purines (*i.e.*, FapyG and FapyA) and some saturated pyrimidines from dsDNA and ssDNA. Male mice homozygous for a null allele of NEIL1 develop severe fatty liver disease, dyslipidemia, diabetes and obesity by 6–8 months of age, with a slightly later onset of insulin resistance. Heterozygous male mice exhibit a milder version of the same phenotype. It is remarkable that the phenotype of the NEIL1-deficient mice strongly resembles metabolic syndrome in humans (although hypertension is a common symptom in patients with metabolic syndrome, it has not been documented in NEIL1-defective mice, primarily because hypertension is not amenable to study in mice). The primary molecular defect in NEIL1-deficient mice is the inability to remove oxidized purines from DNA, as shown by the fact that FapyG and FapyA residues accumulate at significantly higher level in the nuclear and mitochondrial DNA of NEIL1-deficient mice than in normal mice. Although it is not clear why male NEIL1-defective mice develop such striking pathology in energy and glucose metabolism, Lloyd proposed that deficiency in NEIL1 causes accumulation of Fapy DNA lesions in mtDNA and transcriptionally active nuclear DNA, which lowers the threshold at which cumulative oxidative stress destabilizes metabolic homeostasis. This result and its interpretation implies that the constellation of symptoms known as metabolic syndrome in humans could possibly be induced by excessive exposure to endogenous and/or exogenous ROS and the associated ROS-mediated DNA and cellular damage.

In the context of other results discussed at this workshop, perhaps the most interesting aspect of the NEIL1 mouse phenotype is that it confirms an emerging theme; namely, that excessive ROS-mediated cellular damage correlates with increased mitochondrial dysfunction, and that mitochondrial dysfunction can manifest itself in highly diverse pathological conditions including neurodegeneration, motor dysfunction, retinal degeneration and aging-like phenomena. As discussed by McMurray above, different pathologies may result when ROS-mediated events are targeted to different tissues or cell types.

## 9. Defective transcription: the devil is in the details

One of the most difficult questions facing researcher and clinicians studying XP, CS and TTD is why molecular defects in TFIIH cause heterogenous but highly tissue-specific phenotypes; for example, why do TTD patients with XPD mutations exhibit dysmyelination, while brain gray matter remains unaffected in these patients. Jean-Marc Egly (Strasbourg, France) presented a study of TTD/XPD<sup>-/-</sup> mice at this workshop that provides a remarkable explanation for TTD-associated dysmyelination. Egly showed that TFIIH is required to stabilize the thyroid receptor (TR) on its cognate effector sequence in the promoter region of TR-target genes, and that TFIIH from TTD/XPD<sup>-/-</sup> mice is defective in this function. Egly further showed that this TFIIH dysfunction has strong tissue-specific consequences for expression of TR-regulated genes in TTD/XPD<sup>-/-</sup> mice; for example, myelin basic protein is approximately, two-fold over-expressed in the cerebellum and two-fold under-expressed in the striatum of TTD/XPD<sup>-/-</sup> mice, while synaptotagmin-related gene 1 is approximately two-fold overexpressed in the cerebellum but expressed at a normal level in the striatum of TTD/XPD<sup>-/-</sup> mice. After quantifying expression of multiple TR-dependent genes in wild type and TTD/XPD<sup>-/-</sup> mice, Egly deduced that TR-dependent gene expression is highly dysregulated in the TTD animals.

Egly previously showed that TFIIH stimulates gene expression not only by binding to core promoter elements and facilitating initiation of transcription, but also by promoting specific ligand-dependent nuclear receptor-mediated transactivation of target promoters (*i.e.*, PPAR-, RAR- and ER alpha-dependent gene expression). Thus, TFIIH is a tissue-specific transcription factor co-activator, but the tissue-specificity is conferred by the cell type-specific transcription regulatory machinery, not by TFIIH itself. This work underscores the importance of the co-activator function of TFIIH in TTD and XP/TTD. Furthermore, this result has dramatic implications for understanding tissue-specific effects of mutations that may alter the structure/function of TFIIH in many subtly different ways. This result has clear importance in the study of XP, CS, TTD and other diseases resulting from defects in transcription.

## 10. Expanding the target population: significance and prevalence of hypomorphic human DNA repair

XP is a classical example of a disease caused by a gene-environment interaction, where a specific genetic defect causes a functional deficiency in DNA repair which results in a dramatic increase the risk of sunlight-induced skin cancer. Based on this well characterized paradigm, there has been considerable interest in identifying genetic risk factors for non-melanoma skin cancer in the general human population. At this workshop, Stephen Emmert (Georg-August-University Goettingen, Germany) showed that three single nucleotide polymorphisms (SNPs) in the gene encoding XPC (PAT+, intron 11-6A, and Exon 15 2920C), form a haplotype with a relatively high frequency (38.9%) in the population. Individuals homozygous for this haplotype demonstrated an approximately two-fold elevated

melanoma risk. One of the SNPs was shown to lower DNA repair capacity (DRC) significantly. It is not currently known whether heterozygotes that are carriers for rare disease-causing XPC defects demonstrate similar increases in skin cancer risk, although this question clearly warrants further study. Qingyi Wei (M.D. Anderson Cancer Center, Houston, TX) identified five common human SNPs (allele frequency  $\geq 5\%$ ) in NER genes (XPC Ala499Val, Lyx939Gln XPD Asp312 Asn, Lys751Gln, XPG His1104Asp), and showed that the DRC of individuals homozygous for these variants decreased almost two-fold relative to individuals with no variant alleles or who were heterozygous for variant alleles. DRC also roughly correlated inversely with the number of alleles with the variant genotypes. Wei also showed that sub-optimal repair capacity, as measured by a host cell reactivation assay in lymphocytes, correlated with a two- to three-fold higher adjusted odds ratio for basal cell carcinoma or melanoma in a hospital-based case-control study (146 BCC cases, 109 SCC cases, 312 melanoma cases, and 333 controls). Higher risk of squamous cell carcinoma (SCC) was also observed in individuals with low DRC, but the correlation was less strong for SCC in this population sample.

## 11. Perspectives

This workshop on XP, CS and TTD presented an unusual opportunity for clinicians, researchers, patients and family support group members to interact in both formal and informal sessions. All workshop participants appeared to benefit from this interaction. The discussions brought new

insight and focus to the neurotropism of these diseases, which is of equally strong concern and interest to researchers, clinicians, patients and the patients' family members. Finally, it is important to emphasize that study of relatively rare diseases such as XP, CS and TTD may lead to insights that are relevant for more common diseases such as metabolic syndrome, skin cancer and premature aging. Thus, the efforts of the participants at this workshop may contribute to understanding risk factors for common diseases in the general population.

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