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# Strict sun protection results in minimal skin changes in a patient with xeroderma pigmentosum and a novel c.2009delG mutation in XPD (ERCC2)

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## Abstract

We examined the clinical, molecular, and genetic features of a 16y old boy (XP2GO) with xeroderma pigmentosum (XP) and progressive neurologic symptoms. The parents are not consanguineous. Increased sun sensitivity led to the diagnosis of XP at 2y of age and a strict UV protection scheme was implemented. Besides recurrent conjunctivitis and bilateral pterygium, only mild freckling was present on his lips. He shows absent deep tendon reflexes, progressive sensorineural deafness, and progressive mental retardation. MRI shows diffuse frontal cerebral atrophy and dilated ventricles. Symptoms of trichothiodystrophy (TTD) (brittle hair with a tiger-tail banding pattern on polarized microscopy) or Cockayne syndrome (CS) (cachectic dwarfism, cataracts, pigmentary retinopathy, and spasticity) were absent. XP2GO fibroblasts showed reduced post-UV cell survival ($D_{37}$=3.8 J/m$^2$), reduced nucleotide excision repair, reduced expression of *XPD* mRNA, and an undetectable level of XPD protein. Mutational analysis of the *XPD* gene in XP2GO revealed two different mutations: a common p.Arg683Trp amino acid change (c. 2047C>T) known to be associated with XP and a novel frameshift mutation c.2009delG (p.Gly670Alafs*39). The latter mutation potentially behaves as a null allele. While not preventing neurologic degeneration, early diagnosis and rigorous sun protection can result in minimal skin disease without cancer in XP patients.

### Keywords

DNA repair; xeroderma pigmentosum; XPD; sun protection; cancer prevention

## Introduction

Three rare autosomal-recessive inherited human disorders are associated with impaired nucleotide excision repair (NER): Xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) (1,2). XP patients are sun-sensitive and have a 1000-fold increased risk of developing sunlight induced skin cancer. About 20% of XP patients

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develop progressive neurologic symptoms (3). Cockayne syndrome and trichothiodystrophy patients are not skin cancer prone (4). Mutations in the *XPD* gene (MIM: 278730) can result in six different clinical phenotypes (Table 1). XP, XP with neurologic symptoms, and TTD are more common than the combined features of XP and TTD (XP/TTD complex), XP and CS (XP/CS complex) (5,6), or the cerebro-oculo-facio-skeletal syndrome (COFS) (2).

In general, each mutational change in the *XPD* gene is specific for a particular clinical phenotype. In this study we performed clinical, molecular, and genetic analyses of a young male XP patient from Switzerland who was found to be compound heterozygous for two different *XPD* gene mutations resulting in a "XP with neurologic symptoms" phenotype.

## Methods

### Cells

XP2GO and XP3GO primary fibroblast cells were established from skin biopsies at the Georg-August-University Goettingen. Normal fibroblasts (GM00637 and AG13145) were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ, USA). Peripheral blood was obtained from both parents and the sister of XP2GO. All clinical studies were conducted according to Declaration of Helsinki principles. All participating family members gave their informed consent. The studies were performed in accordance with protocols approved by the U.S. National Cancer Institute and University of Goettingen Institutional Review Boards.

### Post-UV cell survival, plasmid host cell reactivation (HCR), and XPD mRNA and protein expression analyses

Cell survival was determined by assessing cell growth after UVC irradiation (CL-1000 lamp; LTF Labortechnik, Wasserburg, Germany) as described previously (7). The luciferase reporter gene plasmid pCMVLuc (generous gift from M. Hedayati and L. Grossman, Johns Hopkins University, Baltimore, MD) was used to measure post-UVC HCR as described (7). Real-time quantitative RT-PCR was established using LightCycler technology. A 63 bp XPD cycle product was obtained using ERCC2 (XPD) QuantiTect Primer Assay and QuantiTect SYBR Green kit (Qiagen, Hilden, Germany). For β-actin (219 bp product) forward primer 5'-acactgtgcccatctacgagg-3'and reverse primer 5'-aggggccggactcatact-3' were used. The *XPD* mRNA levels were normalized to the levels of β-actin. XPD protein western blot analysis was performed as described (8).

### Nucleotide sequence analysis

The complete XPD cDNA was sequenced as published (9). Genomic DNA was sequenced using Ex20+21for (5'-ccaactcagacacagcatcc-3'), Ex20+21rev (5'-cagggacagaaggtcattcg-3'), Ex22for (5'-aggctgtttcccgttcattt-3'), Ex22rev (5'-aggggactttctggaggaga-3') primers, and an ABI Prism 310 Genetic Analyzer (ABI Prism, Darmstadt, Germany). The *XPD (ERCC2)* GenBank sequences used were NT_011109.15 for genomic DNA, NM_000400.15 for cDNA and NP_000391.1 for protein. Mutations were described according to the recommendations of the Human Genome Variation Society; http://www.hgvs.org/mutnomen/) and the nomenclature was checked using the mutalyzer website (http://www.lovd.nl/mutalyzer/1.0.1/).

## Results

### Phenotype

XP2GO is a 16 year old Caucasian male (Fig. 1). There is no known consanguinity of his parents (mother Swiss, father Greek). His parents and an older sister are clinically healthy.

Severe sun-sensitivity of XP2GO was noted at 3 months of age. After short sun exposure XP2GO developed a solar dermatitis with persistent erythema and delayed clearing. There was no blistering or edematous swellings. These early childhood exposures affected mainly the face. The sun sensitivity and dry skin led to the clinical diagnosis of XP at 2 years of age. A rigorous sun protection scheme was then implemented including no outdoor activities between 10 a.m. and 4 p.m, sunglasses, hat with neck protection, thick woollen clothing, UV-absorbing films on house and car windows, and use of topical sun block (SPF 50+). The fluorescent lamps in the schoolroom were covered with UV-absorbing material. XP2GO developed mild freckling on his lower lips, conjunctival pterygium, and conjunctivitis but no skin cancer. This represents unusually mild skin and eye involvement for a XP patient including patients with a defect in the *XPD* gene (1). Additionally, XP2GO suffers from progressive sensorineural hearing loss (inner ear, high frequences) since 11y of age, absent deep tendon reflexes, short stature (height $10^{th}$ percentile, weight $25^{th}$ percentile), and progressive mental impairment. MRI with use of contrast material showed diffuse frontal cerebral atrophy and dilated ventricles. Typical symptoms of trichothiodystrophy (TTD) like brittle hair with a tiger-tail pattern on polarized microscopy and of Cockayne syndrome (CS) like cachectic dwarfism, cataracts, pigmentary retinopathy, and spasticity are absent. Taken this together the clinical phenotype of XP2GO can be categorized as xeroderma pigmentosum with neurologic abnormalities (Table 1).

## Reduced post-UV cell survival

XP2GO fibroblasts revealed a markedly reduced post-UV cell survival compared to normal GM00637 and XP3GO fibroblasts (Fig. 2a). XP3GO is a known XP variant patient with a cellular deficiency in translesional synthesis but normal nucleotide excision repair (10,11). The $D_{37}$ (dose that results in 37% cell survival) were 9.8 and 8.1 J/$m^2$ for the normal controls GM00637 and XP3GO, respectively. In contrast, the $D_{37}$ for XP2GO was 3.8 J/$m^2$ indicating an approximately 2.4 fold increased sensitivity.

## Reduced nucleotide excision repair capacity and XPD complementation group assignment

XP2GO fibroblasts showed a reduced nucleotide excision DNA repair capability (Fig. 2b) as reflected by reduced host cell reactivation (HCR) of an UV-treated luciferase reporter plasmid. Normal repair-proficient fibroblasts typically result in a >10 % relative reporter gene expression at 1000 J/$m^2$ UVC. In order to assign XP2GO fibroblasts to a specific XP complementation group cotransfection was performed with plasmids expressing wild-type XP cDNA *(XPA-XPG)* along with the reporter gene plasmid. The cotransfection of *XPD* cDNA exclusively resulted in enhanced reporter gene activities which clearly assigned XP2GO to the XP complementation group D (Fig. 2b).

## Reduced XPD mRNA and protein expression and sequence analysis of the XPD gene

We measured the *XPD* mRNA expression in XP2GO fibroblasts using real-time QRT-PCR. We detected a 50 % reduced *XPD* mRNA expression in XP2GO cells compared to normal GM00637 cells (data not shown). No XPD protein expression was detectable in XP2GO cells (Fig. 2c). Sequencing of the *XPD* gene revealed a novel heterozygous deletion of a guanine in a GGGG run (c.2009delG). This mutation is located on the paternal allele of XP2GO (data not shown). It leads to a frameshift beginning at codon 670 and a predicted early termination of the XPD protein at codon 708 (p.Gly670Alafs*39). Thus, this mutation leads to a truncated and potentially non-functional XPD protein. The second mutation identified in XP2GO is a quite common “hot spot” missense mutation in *XPD*, c.2047C>T. It leads to an amino acid exchange (p.Arg683Trp) and is reported to be associated with a XP phenotype (12). This mutation was derived from the mother (data not shown). The healthy sister of XP2GO was also heterozygous for this mutation (data not shown).

## Discussion

The *XPD* gene is particularly complex, because XPD is a subunit of the basal transcription factor TFIIH, which is critical for basal transcription by RNA polymerase II, and XPD also functions in nucleotide excision repair (NER) (12). Mutations in the *XPD* gene can result in six distinct clinical phenotypes (Table 1) (2). A prevailing theory is that if the defect affects the DNA repair function of TFIIH without affecting its transcriptional role, the clinical features of XP would result. Conversely, if the transcriptional role of TFIIH is affected, patients would develop symptoms of TTD. This implies that the mutations associated with XP or TTD are located at different sites in the *XPD* gene. This was demonstrated in 1997 (12,13). The p.Arg683Trp mutation found in XP2GO has been identified very often in XP-D patients and is associated with XP symptoms. However, it appeared that some identical mutations were found in both XP and TTD patients. These mutations were identified as null mutations and detected in compound heterozygotes where the second mutation determined the clinical phenotype (12,13). The second mutation identified in XP2GO can be considered a null mutation, because it is expected to encode a truncated XPD protein with a stop at codon 708. Another deletion mutation further downstream the XPD protein at codons 716-730 is also considered “null” (13). Another patient, XP17PV, also had a phenotype of XP with neurologic abnormalities and was a compound heterozygote for the common p.Arg683Trp mutation and for another potential null mutation (p.Arg616Pro) (14). Due to rigorous sun protection XP2GO exhibits extremely mild XP skin symptoms (3) without the development of extensive skin pigmentation or skin cancers. Sun protection in general can avoid severe XP skin and eye symptoms (15) but does not prevent progression of the neurological degeneration.

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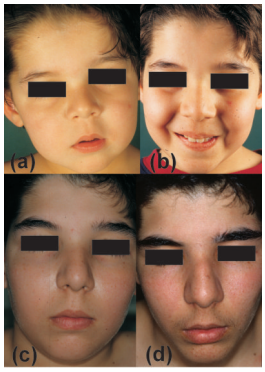


**Figure 1. Clinical pictures of XP2GO at different ages**

Note the absence of typical XP skin symptoms except some very mild freckling on the lower lips at age 3 years (a), 10 years (b), 12 years (c), and 15 years (d).

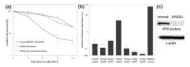

**Figure 2. Post-UV cell survival, assignment of XP2GO to complementation group D, and western blotting**

(a) Reduced post-UV cell survival of XP2GO fibroblasts compared to normal control fibroblasts (GM00637) and known XP variant fibroblasts (XP3GO) after irradiation with an increasing UVC dose. Error bars: mean ± SD (n=2). (b) Reduced luciferase expression in XP2GO fibroblasts is complemented by *XPD* cDNA in a host cell reactivation (HCR) assay. The HCR assay measures the ability of the host cells to repair UV-damaged DNA by assessing the recovery of a reporter gene expression, measured indirectly as enzyme activity of the luciferase reporter gene. The amount of luciferase expression represents the cellular repair capacity for UV-induced DNA photoproducts. Relative luciferase activities (percentage of 1000 J/m$^2$ UVC irradiated vs unirradiated pCMVLuc plasmid) are depicted. (c) No detectable XPD protein expression in XP2GO fibroblasts compared to normal AG13145 fibroblasts. The β-actin expression served as an internal control.

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**Table 1**

Varied spectrum of clinical features of patients with *XPD* gene mutations

| phenotype<br>clinical features | XP without neurologic abnormalities | XP with neurologic abnormalities | TTD | XP/TTD complex* | XP/CS complex** | COFS*** | XP2GO patient |
|---|---|---|---|---|---|---|---|
| **sun-sensitivity** | yes | yes | yes | yes | yes | ? | yes |
| **increased freckling** | yes | yes | | yes | yes | | yes |
| **skin cancer** | yes | yes | | yes | yes | | no |
| **ichthyosis** | | | yes | yes | | | no |
| **brittle hair** | | | yes | yes | | | no |
| **brittle nails** | | | yes | yes | | | no |
| **tiger-tail hair (polarized microscopy)** | | | yes | yes | | | no |
| **sulfur-deficient hair** | | | yes | yes | | | no |
| **progressive cognitive impairment** | | yes | yes**** | yes**** | yes | yes | yes |
| **neuronal degeneration** | | yes | | | yes | ? | yes |
| **sensorineural deafness** | | yes | | | yes | yes | yes |
| **anterior eye abnormalities** | yes | yes | yes | yes | yes | yes | yes |
| **pigmentary retinal degeneration** | | | | | yes | yes | no |
| **loss of subcutaneous tissue** | | | | | yes | yes | no |
| **demyelinating neuropathy** | | | yes, in some | yes, in some | yes | ? | no |
| **cachectic dwarfism** | | yes, in some | yes | | yes | yes | yes |
| **ataxia** | | yes, in some | | | yes | yes | yes |
| **brain calcification** | | | yes, in some | | yes | yes | no |
| **hypertrichosis** | | | | | | yes | no |
| **skeletal abnormalities** | | | | | yes, in some | yes | no |
| **microcephaly/craniofacial abnormalities** | | yes | yes, in some | | yes | yes | no |

* XPD mutation may result in TTD alone or in XP/TTD complex

** XPD mutations may only result in XP/CS complex

*** COFS: Cerebro-Oculo-Facio-Skeletal Syndrome

**** may not be progressive