Xeroderma Pigmentosum Group C Splice Mutation Associated with Autism and Hypoglycinemia[1]

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A 4 y old boy of Korean ancestry had xeroderma pigmentosum (XP) with sun sensitivity, multiple cutaneous neoplasms, and inability to speak. Neurologic examination revealed hyperactivity and autistic features without typical XP neurologic abnormalities. Cultured skin fibroblasts (XP22BE) showed decreased post-UV survival, reduced post-UV plasmid host cell reactivation and defective DNA repair (16% of normal unscheduled DNA synthesis in intact cells and undetectable excision repair in a cell free extract). *In vitro* and *in vivo* complementation assigned XP22BE to XP group C (XPC) and a markedly reduced level of XPC mRNA was found. Two XPC cDNA bands were identified. One band had a deletion of 161 bases comprising the entire exon 9, which resulted in premature termination of the mutant XPC mRNA. The larger band also had the same deletion of exon 9 but, in addition, had an insertion of 155 bases in its place (exon 9a), resulting in an in-frame XPC mRNA. Genomic DNA analysis revealed a T→G mutation at the splice donor site of XPC exon 9, which markedly reduced its information content. The 155 base pair XPC exon 9a insertion was located in intron 9 and was flanked by strong splice donor and acceptor sequences. Analysis of the patient's blood showed persistently low levels of glycine (68 μM; NL, 125–318 μM). Normal glycine levels were maintained with oral glycine supplements and his hyperactivity diminished. These data provide evidence of an association of an XPC splice site mutation with autistic neurologic features and hypoglycinemia. *Key words: alternative splicing/amino acid metabolism/DNA repair/skin cancer. J Invest Dermatol 111:791–796, 1998*

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease associated with extreme sensitivity to ultraviolet radiation (UV), resulting in a high incidence of skin cancers (≈1000 times that of the general population) and neurologic disorders in about 20% of the patients (Kraemer *et al*, 1987, 1994). All seven different DNA repair genes (XPA-G) involved in XP have recently been identified (reviewed in Bootsma *et al*, 1998). XP complementation group C (XPC) is the most prevalent form among North Americans and Europeans. XPC patients exhibit elevated frequency of skin cancers but rarely have the neurologic abnormalities, which are common in the more severe forms of XP (XPA, XPB, XPD, and XPG). The molecular defects in the XPC gene that account for the repair deficiency and elevated skin cancers have been analyzed in only five XPC patients (Legerski and Peterson, 1992; Li *et al*, 1993), and splice site mutations in the XPC gene have not previously been reported. Here we describe a very young (4 y old) XPC patient (XP22BE) with multiple skin cancers, including melanoma, unusual neurologic abnormalities (namely, normal hearing and normal reflexes with hyperactivity and autistic features), a splice site mutation, and hypoglycinemia.

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Abbreviations: CHO, Chinese hamster ovary; CS, Cockayne syndrome; XP, xeroderma pigmentosum; XPC, xeroderma pigmentosum complementation group C.

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MATERIALS AND METHODS

Cell lines and culture conditions XP22BE (GM013817), XP21BE (GM09942) XPC and normal (AG10107) lymphoblastoid cells, and XP21RO (GM00709) XPC and normal (GM02987C) fibroblasts were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). The patient was studied under a protocol approved by the NIH Institutional Review Board (91-AR-0161). XP22BE fibroblasts were established from a skin biopsy by H.L. Levy. Repair-proficient HeLa S3 cells were obtained from the stock of Lineberger Comprehensive Cancer Center (Chapel Hill, NC). Normal (FS) fibroblasts from an unaffected donor and XP82SF fibroblasts from a patient with the skin and central nervous system symptoms and extremely low repair commonly associated with XPA were established from skin biopsies by J.E. Cleaver. The normal human fibroblasts (CRL1876), repair-proficient Chinese hamster ovary (CHO) parental cell line AA8 (CRL1859), and the XP-G(ERCC5) repair deficient CHO line UV135 (CRL1867) were obtained from the American Type Culture Collection (Rockville, MD). Lymphoblastoid cell lines were cultured in RPMI-1640 medium supplemented with 20 mM glutamine, and 15% fetal calf serum (Gibco-BRL, Gaithersburg, MD). Fibroblast cell lines were grown in Dulbecco's modified Eagle's medium (Gibco-BRL) containing 40 mM glutamine, 15% fetal calf serum, and antibiotics (penicillin, streptomycin; Gibco-BRL).

Post-UV cell survival and DNA repair measurement Cell survival was measured by assessing cell growth in microwell plates following exposure to

UVC doses of 3–12 J per m^2 (Kraemer *et al*, 1989), and was also quantitated by labeling wells with [^{3}H]hypoxanthine (0.5 μCi per ml, 9.1 Ci per mmol) for 2–4 h and extracting incorporated acid-insoluble radioactivity (Cleaver and Thomas, 1988). The number of repair sites per 10^7 Daltons generated intracellularly during 4 h after 13 J per m^2 was determined by blocking sites with inhibitors, followed by size determination in alkaline sucrose gradients (Cleaver, 1981).

Plasmid post-UV host cell reactivation Post-UV host cell reactivation was measured using the UV-treated plasmid pRSVcat (Protic-Sabljic and Kraemer, 1985). The CsCl purified plasmid (0.25 μg) was transfected into 0.15×10^6 fibroblasts using 3 μl Lipofectamine (Gibco-BRL) in a total volume of 1 ml for 5 h, and the chloramphenicol acetyltransferase activity was measured after 48 h (Moriwaki *et al*, 1996). In order to assign the XP22BE fibroblasts to a specific complementation group a simultaneous cotransfection with 0.25 μg pXPC3 (containing XPC cDNA; Legerski and Peterson, 1992) in addition to pRSVcat was performed (Carreau *et al*, 1995).

***In vitro* DNA repair assay** Double-stranded DNA molecules containing a centrally located 2-aminobutyl-1,3-propanediol moiety to be used as a substrate with a cholesterol side chain (Mu *et al*, 1996) were prepared as described (Matsunaga *et al*, 1995) using six partially overlapping oligonucleotides. The cholesterol-containing oligomer was phosphorylated with [γ-^{32} p] ATP such that the 140 bp duplex had ^{32}P label on one strand at the sixth phosphodiester bond 5′ to the 2-aminobutyl-1,3-propanediol-cholesterol lesion. This assay detects the excised damage-containing DNA fragment resulting from dual incisions both 5′ and 3′ to the lesion (Huang *et al*, 1992; Matsunaga *et al*, 1995; Reardon *et al*, 1997). Cell-free extract preparation, reaction conditions, post-excision processing of DNA, and quantitation were performed as described (Reardon *et al*, 1997).

Northern blotting Northern blotting was performed (Legerski and Peterson, 1992) using total cytoplasmic RNA (Khan *et al*, 1996). The intensity of the autoradiographic bands was measured using a laser densitometer (Molecular Dynamics, Sunnyvale, CA).

Reverse transcriptase-polymerase chain reaction (PCR) and DNA sequencing Poly A+ containing RNA was separated from the total RNA using oligo-dT cellulose (Amersham Pharmacia Biotech, Piscataway, NJ). The first strand cDNA was synthesized utilizing 2 μg poly A+ RNA as described (Khan *et al*, 1996), and was utilized to amplify the entire coding region of XPC gene by nested PCR (Li *et al*, 1993). High-molecular-weight genomic DNA was isolated and the region surrounding exon 9 and intron 9 was amplified by PCR. Sequencing was performed using a Sequenase PCR product sequencing kit (USB, Cleveland, OH) or by cycle sequencing employing dideoxy terminator chemistry and an ABI 373A DNA sequencer (P.E. Applied Biosystems, Foster City, CA).

Microsatellite marker analysis Five microsatellite markers, D3S1515 (+), D3S1307 (+), D3S1304 (+), D3S1270 (+), and D3S1297 (+), near the XPC locus on chromosome 3 were selected from a search of GenBank and PCR amplified using the primer pairs indicated in the GenBank listing. Fragment sizes were measured on polyacrylamide gels.

DNA sequence information analysis Sequences were scanned with the donor and acceptor individual information weight matrices and the identified sites were displayed as described previously (Schneider, 1997a, b).

Amino acid measurements Concentrations of amino acids in plasma, urine, and cerebro-spinal fluid were determined by ion-exchange chromatography on a Beckman T300 Amino Acid Analyzer (Beckman Instruments, Spinco Division, Palo Alto, CA).

RESULTS

Case report A 4 y old boy, XP22BE, with XP (born 7 June, 1991) of Korean ancestry was referred to NIH because of sun sensitivity, multiple cutaneous neoplasms, and inability to speak (**Fig 1**). After a trip to Florida at age 9 mo his skin darkened without blistering or other signs of acute sunburn. By 1 y of age he had markedly increased freckling of his face and hands. He began developing skin cancers before 3 y. He had one squamous cell carcinoma, five basal cell carcinomas, one invasive melanoma, two melanoma *in situ*, and several other pigmented lesions with marked atypia removed by his local physicians. He was born with a cleft palate and duplex right kidney. His height was below the fifth percentile and he had hyperactivity with delayed motor development and absent speech. Neurologic examination did not show the neurologic abnormalities typical of XP, such as diminished deep tendon reflexes, reduced hearing, microcephaly, or dilated ventricles of the brain. Instead he had normal reflexes, autistic features with minimal hearing abnormality, and a normal MRI of the brain with minimal ventricular prominence. The patient was adopted and family history is not available.

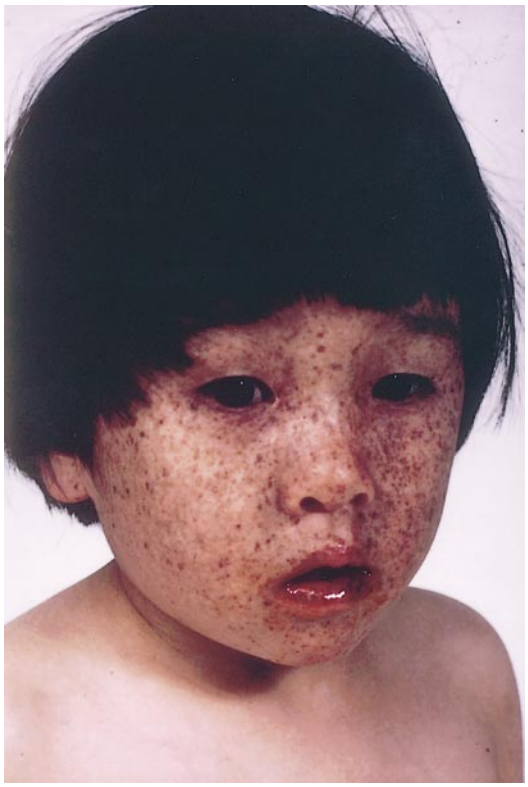

Figure 1. Patient XP22BE shows features of XP. The patient, age 4 y, showing numerous freckle-like pigmented lesions of varying size, shape, and intensity on sun-exposed portion of the face, lips, and ear. Note sparing of the chest and shoulders.

Hypoglycinemia Plasma glycine levels were consistently diminished. The mean value was 68 ± 14 μM (n = 6; normal range 125–318 μM). Conversely, he had mild increases in the plasma levels of serine, valine, isoleucine, leucine, methionine, and tyrosine. Urine glycine levels averaged 771 ± 380 μmoles per g creatinine (n = 4; normal range 1026–4310 μmoles per g creatinine). The glycine level in cerebro-spinal fluid was 3 μM (normal range 1.9–10.1 μM). Glycine supplementation at 120 mg per k per d increased the plasma glycine level into the normal range (166 ± 74 μM). (A detailed account of his clinical and metabolic abnormalities will be presented elsewhere.)

Reduced post-UV survival and DNA repair Post-UV survival of XP22BE fibroblasts was reduced compared with that of normal fibroblasts using two different assays. In the growth inhibition assay following a UV dose of 3 J per m^2, the relative survival of XP22BE cells was 20% of unirradiated cells, whereas that of normal control cells was 73% (data not shown). In the UV sensitivity assay the D_{37} (dose at which 37% of the cells survive) was 5.0 J per m^2 for the XP22BE cells compared with 23–25 J per m^2 for normal control cells and 0.8 J per m^2 for XP82SF cells (data not shown). DNA repair as measured by the number of repair sites accumulated in the presence of polymerase inhibitors during the 6 h after exposure to 13 J per m^2 ultraviolet was 7.5% of normal with XP82SF cells and 16% of normal repair in XP22BE cells. This is in the range for XP complementation group C cells (Bootsma *et al*, 1998).

***In vitro* and *in vivo* DNA repair measurements and assignment to XPC** With cell free extracts prepared from repair proficient human HeLa and CHO AA8 cells, we observed excision of 2.5%–

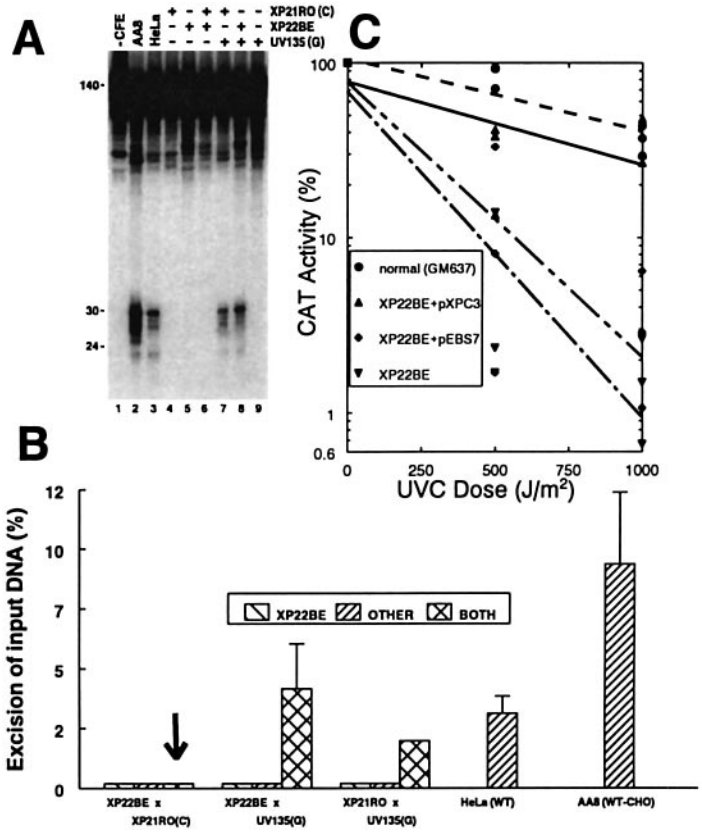

Figure 2. *In vitro* repair and complementation by cell-free extracts and *in vivo* complementation with the XPC gene assigning XP22BE cells to XPC. (*A*) Qualitative analysis of excision of the 2-aminobutyl-1,3-propanediol-cholesterol lesion is shown in an autoradiograph of a sequencing gel showing the excision products resulting from dual incisions during incubation of 8 fmol substrate DNA with wild-type or mutant cell extracts. Substrate DNA alone is shown in *lane 1* (-CFE); dual incisions by repair-proficient extracts resulted in the release of 24–32 nucleotide long oligomers shown in *lanes 2* and *3* (AA8 and HeLa S3 cells); lack of excision by the extract from XP22BE fibroblasts (*lane 5*) is similar to the repair-deficient extracts from XP21RO (XPC) and UV135 (XPG) fibroblasts (*lanes 4* and *9*, respectively). *In vitro* complementation between XP21RO (XPC) or XP22BE and UV135 (XPG) is shown in *lanes 7* and *8*, whereas lack of complementation between XP22BE and XP21RO(XPC) is shown in *lane 6*. (*B*) Quantitative analysis of the data shown in (*A*) and of a second experiment conducted under similar conditions. Detection of radioactivity in the 24–32 nucleotide area is markedly reduced in all three mutant cell lines [XP22BE, XP21RO (XPC), and UV135(XPG)] and is interpreted as absence of excision repair (i.e., below the level of detection with this assay) in comparison with the repair proficient HeLa S3 and AA8 lines. Mixing of extracts from XP22BE or XP21RO (XPC) lines with the UV135(G) showed increased excision of input DNA, indicating complementation of their DNA repair defects. In contrast, mixing the XP22BE extract with the XP21RO (XPC) extract did not increase the excision of input DNA (*arrow*). This lack of complementation indicates that XP22BE and XP21RO(XPC) extracts had similar defects. Thus the XP22BE cells were assigned to XPC. (*C*) UVC-treated pRSVcat was either transfected alone (▼) or cotransfected with pXPC3 (XPC) (▲) or pEBS7 (◆) (control) into triplicate cultures of XP22BE primary fibroblasts. Normal control primary fibroblasts [CRL1876 (●)] were also transfected without XPC. Each symbol represents the relative chloramphenicol acetyltransferase activity in an independent transfection compared with the corresponding control untreated plasmid (Protic-Sabljic and Kraemer, 1985; Moriwaki *et al*, 1996). Specific activity with the unirradiated pRSVcat plasmid in the cell lines used ranged from 0.019 to 0.17 nmol per min per mg protein.

11% of the 2-aminobutyl-1,3-propanediol-cholesterol-A damage (**Fig 2*A***, *lanes 2–3*, and **Fig 2*B***). The XP22BE extract had no detectable excision activity (**Fig 2*A***, *lane 5*) even after a long exposure (data not shown). Similarly, extracts prepared from XP21RO (XPC) and UV135 (XPG CHO) cells showed no excision (**Fig 2*A***, *lanes 4* and *9*).

In vitro complementation was demonstrated between the extract

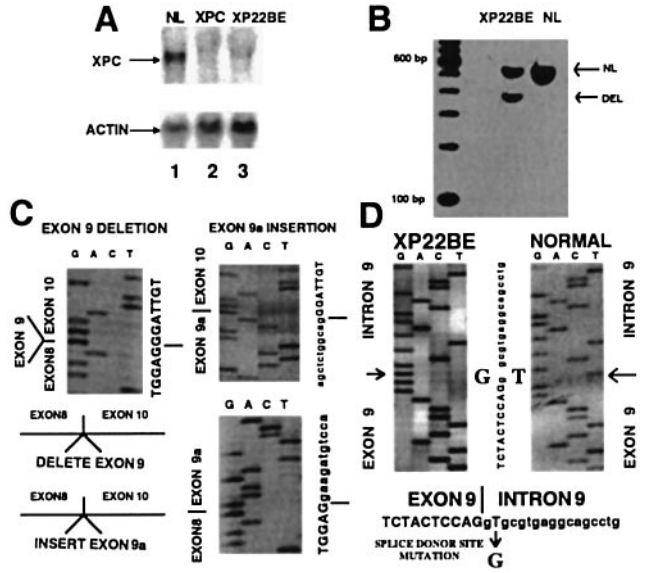

Figure 3. Markedly reduced XPC mRNA by northern blotting of XP22BE cells and analysis and sequencing of cDNA and genomic DNA. (*A*) Total RNA (20 μg) was extracted from normal (AG10107), XP21BE (XPC), and XP22BE cells, separated by electrophoresis and transferred to a nylon membrane for probing with a 3.5 kb XPC cDNA fragment (XPC). The relative amount of RNA transferred was monitored by probing with β-actin cDNA. In comparison with normal cells (*lane 1*) the XPC message level was markedly reduced in the XP21BE (XPC) (*lane 2*) and XP22BE cells (*lane 3*). (*B*) cDNA was prepared by reverse transcriptase-PCR and the XPC region containing exons 8–11 was amplified by use of PCR and separated by agarose gel electrophoresis. *Lane 1*, nucleic acid size markers; *lane 2*, XP22BE cDNA; *lane 3*, normal (AG10107) cDNA. The XP22BE cells show two bands, whereas only one band is present with the normal cells. (*C*) Sequence analysis of cDNA bands from XP22BE cells. The bands from XP22BE cells were extracted from the agarose gel and dideoxy sequencing was performed using Sequenase as described in *Materials and Methods*. Left, the faster migrating band showed deletion of the entire 161 bases of exon 9. Right, the slower migrating band had deletion of exon 9 and replacement with a 155 nucleotide insertion (exon 9a, lower case letters). The 11 nucleotides at the 5′ end and the 11 nucleotides from the 3′ end of the insertion are shown. (*D*) Genomic DNA from XP22BE and normal cells was isolated and amplified by PCR using primers in exon 9 and intron 9. The T in the second position of the 5′ splice donor site of intron 9 in the normal cells was replaced by a G in the XP22BE cells.

from XP22BE and UV135 (XPG) (**Fig 2*A***, *lane 8*) and between the extract from XP21RO (XPC) and UV135 (XPG) (**Fig 2*A***, *lane 7*), indicating that they were not in complementation group G. When the XP22BE extract was mixed with XP21RO (XPC) cell free extracts, we failed to detect excision activity with this *in vitro* system (**Fig 2*A***, *lane 6* and **Fig 2*B***, *arrow*), thus assigning the XP22BE cells to XPC.

We also conducted *in vivo* plasmid DNA repair and complementation studies. The XP22BE fibroblasts showed a reduced post-UV plasmid host cell reactivation in a range typical for XPC (**Fig 2*C***). This assay measures the ability of transfected cells to repair damaged plasmid DNA as reflected in recovery of chloramphenicol acetyltransferase activity after UVC treatment. Similar results were found with XP22BE lymphoblasts (data not shown). Cotransfection with the plasmid containing the XPC cDNA (pXPC3) resulted in an enhanced post-UV chloramphenicol acetyltransferase expression, whereas the cotransfection with pEBS7 (vector without the XPC gene) did not alter the post-UV pRSVcat host cell reactivation (**Fig 2*C***). Although plasmid DNA repair was not fully restored to levels of normal primary fibroblasts, this clearly assigns XP22BE cells to XPC *in vivo* (Carreau *et al*, 1995).

Reduced XPC transcript by northern blotting We compared mRNA from the XP22BE lymphoblastoid cell line to mRNA from a normal donor (AG10107) and from another XPC patient (XP21BE) by northern blot hybridization (**Fig 3*A***). When the XPC cDNA probe was used, a single band ≈3.8 kb in size was detected in the normal

control (*lane 1*), which was consistent with the previously reported (Legerski and Peterson, 1992) description of XPC mRNA expression. In contrast, the 3.8 kb transcript was not detectable or was much reduced in the XP22BE (*lane 3*) and XP21BE (XPC) (*lane 2*) cells. Hybridization of the same membrane with the β-actin cDNA probe revealed normal levels in all three cell lines, indicating that the low levels of XPC transcript in the XP22BE or XPC patients was not a consequence of degradation of RNA samples isolated from these cell lines.

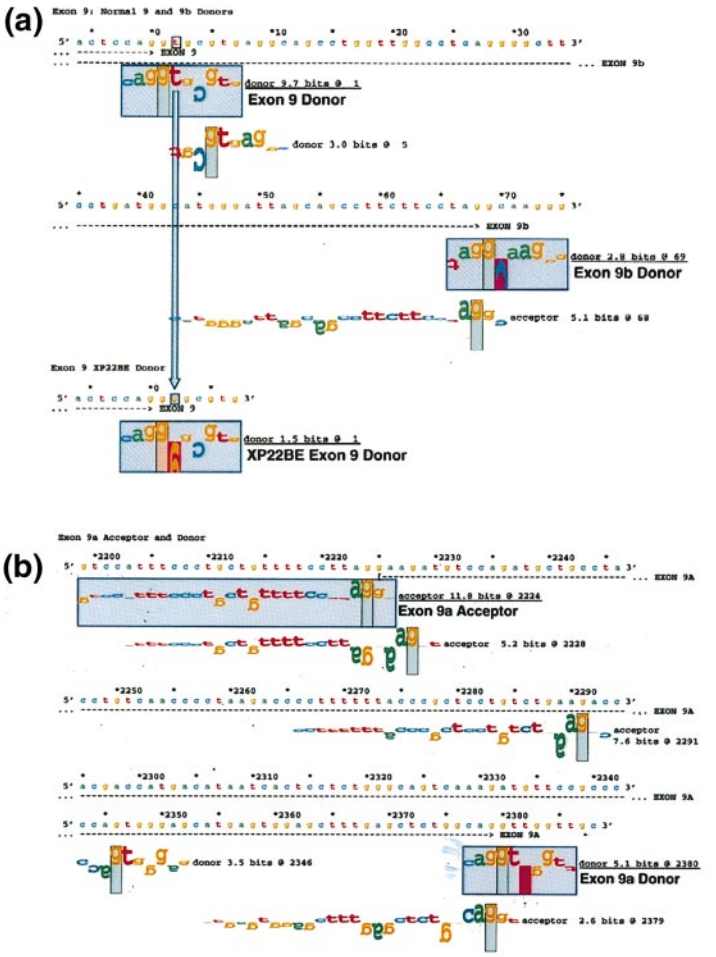

Figure 4. Effects of splice donor site mutation in XP22BE cells. (*A*) Lister map for exon 9 and 9b donors in XP22BE and normal. The locations of donor and acceptor sites are shown by sequence walkers below the corresponding sequence (Schneider, 1997b). In a walker the height of a letter indicates how strongly conserved a base is in natural splice junction binding sites (Stephens and Schneider, 1992). The vertical green or red rectangle is at the zero base of the site and represents a scale extending from +2 bits to –3 bits. The total conservation and location of the zero base are given for each walker for all sites >2.4 bits, the apparent minimal functional value (Rogan *et al*, 1998). The sequences are numbered starting at 1, the first base of intron 9, and marked by an asterisk every five bases. The donor walker at coordinate 1 (underlined) is the normal exon 9 3′ end and has a strong value of 9.7 bits. In XP22BE cells, substituting a G for the T at base 2 (*arrow*) reduces the information content of the exon 9 donor site to 1.5 bits (Schneider, 1997b). This nonfunctional site is indicated by a red rectangle. There is another splice donor site at 69 (exon 9b) with an information content of 2.8 bits. The acceptor at 68 has a high probability (0.2) of being a natural site, but its function, if any, is unknown. (*B*) Lister map for exon 9a. The strong acceptor at 2224 (11.8 bits) and the medium strength donor at 2380 (5.1 bits) are at the 5′ and 3′ ends of 155 bp exon 9a, respectively (underlined). (*C*) Schematic diagram of splicing pattern in XP22BE cells. The T→G mutation in the splice donor site of exon 9 in XP22BE results in three different mRNA isoforms: isoform I has a loss of exon 9; isoform II has an insertion of exons 9b and 9a; isoform III has a deletion of exon 9 and insertion of exon 9a.

Multiple XPC mRNA species The entire 3.5 kb coding sequence of the XPC gene was examined using direct sequencing of PCR-amplified first strand cDNA generated by use of reverse transcriptase-PCR. The PCR products showed the expected size except that primer pairs d1 and d2 resulted in two bands: one of nearly normal size and a second of shorter size (**Fig 3*B*, *C***). The shorter band (isoform I) was missing 161 bases comprising the entire exon 9. The larger band (isoform III) also had the same deletion of exon 9 but, in addition, had an insertion of 155 bases (exon 9a) in its place (**Fig 3*C***).

We amplified cDNA using a new primer derived from this 155 base pair region that was paired with a primer from exon 9. This approach revealed the presence of a third cDNA species in the XP22BE cells (isoform II). This cDNA was comprised of XPC sequences including exon 9 and exon 9a, but in addition also contained a 68 base pair insertion (exon 9b) between exon 9 and exon 9a. Sequencing of the remainder of the coding region of the cDNA from XP22BE patient revealed no other mutations.

The XP22BE lymphoblastoid cell line we used was established by Epstein–Barr virus transformation of peripheral blood lymphocytes from the XP22BE patient. To exclude a possible effect of Epstein–Barr virus transformation, we also analyzed primary fibroblast cultures established from the XP22BE patient. We found the same mutations in the cDNA of the fibroblasts of the XP22BE patient, confirming that these mutations are in his germ line.

An XPC splice donor mutation in genomic DNA The exon/intron junctions were sequenced for exons 8, 9, and 10 in the normal and the XP22BE cells. In addition, the entire intron 9 was sequenced (GenBank accession number AF076952) and found to contain 3862 bp. We found a T→G mutation in the +2 position of the 5′ end of intron 9 (splice donor site of exon 9) in the XP22BE cells (**Fig 3*D***). The sequences at the intron–exon junctions represent strong donor and acceptor sites (**Fig 4*A*, *B***) (Stephens and Schneider, 1992; Schneider, 1997a, b). The genomic DNA PCR products amplified using primer pairs spanning exons 9 and 10 were found to be digested with Hinc II, an enzyme that only cuts within the 155 bp inserted sequence found in cDNA (**Fig 4*B***). The 68 bp insertion (exon 9b in isoform II) and 155 bp insertion (exon 9a in isoforms II and III) in the cDNA were found to be part of intron 9 (**Fig 4*C***).

Homozygous microsatellite markers near the XPC locus on chromosome 3 Because DNA from the parents of XP22BE was not available, we used microsatellite markers to determine whether the region containing the XPC gene on chromosome 3 was homozygous or heterozygous. All the five microsatellite markers we used revealed homozygosity (data not shown).

DISCUSSION

DNA repair and XPC Cells from XP patients with severe defects in nucleotide excision repair are hypersensitive to killing by UV and to induction of mutations in their DNA by UV exposure (Bootsma *et al*, 1998). These studies of XP strongly implicate DNA repair in protection against UV-induced skin cancers (Kraemer *et al*, 1994). The XP22BE patient had multiple skin cancers, including melanoma by age 4 y. The XP22BE cells had characteristic increased sensitivity to killing by UV, reduced post-UV plasmid host cell reactivation, and reduced DNA repair. Cell free extracts from lymphoblasts of this patient did not correct the excision defect in an XPC cell line, and *in vivo* complementation of XP22BE fibroblasts with an XPC gene resulted in an increased plasmid DNA repair capacity in these cells nearly up to normal levels. These results assign the XP22BE cells to the complementation group C.

Neurologic abnormalities and hypoglycinemia About 20% of XP patients show neurologic abnormalities (Kraemer *et al*, 1987). Their neurologic abnormalities are characterized by progressive deterioration and include diminished deep tendon reflexes, reduced hearing, and dilated ventricles of the brain (Kraemer *et al*, 1987; Kraemer, 1998). Most XP patients with neurologic abnormalities are in XP complementation groups A, B, D, or G (Kraemer *et al*, 1987; Bootsma *et al*, 1998; Kraemer, 1998), and have only rarely been reported in group C (Hananian and Cleaver, 1980). We found normal plasma amino acid levels in XP patients with neurologic abnormalities in complementation groups A (one patient), C (one patient), and D (one patient) (K.H.K., unpublished). Nine XP patients in complementation groups B, D, and G (Moriwaki *et al*, 1996) have been identified with a second clinical entity: the XP–Cockayne syndrome complex (Robbins, 1988). These patients have cutaneous abnormalities of XP and neurologic degeneration of Cockayne syndrome (CS) with microcephaly, normal to increased deep tendon reflexes, pigmentary retinal degeneration, progressive sensorineural hearing loss, and calcification of basal ganglia. Pathologically XP/CS patients have the CS type of neurologic changes (dysmyelinization of the brain), which differ from those in patients with XP with neurologic abnormalities (primary neuronal degeneration) (Robbins, 1988).

The XP22BE patient had neurologic abnormalities not usually found with either XP or CS, including autistic features with normal hearing, hyperactivity, normal reflexes, and normal MRI without dilated ventricles or microcephaly. Rather, his neurologic changes may be related to the substantial and persistent hypoglycinemia. In addition, oral glycine supplementation appeared to result in improvement of his marked hyperactivity.

Hyperglycinemia either as a primary entity known as nonketotic hyperglycinemia (Hamosh *et al*, 1995) or secondary to organic acidopathies (Fenton and Rosenberg, 1995), is associated with neurologic abnormalities, the former most likely a consequence of the accumulation of glycine and the latter likely due to the underlying organic acid disorder. Hypoglycinemia, however, has not been reported. We have not defined the metabolic defect producing the hypoglycinemia. A marked decrease in the concentrations of serine and glycine in cerebrospinal fluid and in plasma has been reported to be associated with an early nonspecific mental retardation in 3-phosphoglycerate dehydrogenase deficiency, an inborn error of serine metabolism (Jaeken *et al*, 1996); however, our patient did not have low serine levels. His serine levels were 194 and 184 μM (normal range 71–181 μM) with corresponding glycine levels of 69 and 68 μM, respectively (normal range 125–318 μM), and was 223 μM when glycine supplementation elevated his glycine to 192 μM.

XPC gene and hypoglycinemia The function of the XPC gene is still not fully understood, although the protein encoded by this gene is thought to be involved in repair of damage to bulk (nontranscribed) DNA (van Hoffen *et al*, 1995). XPC forms a stable complex with the human homologs of the yeast Rad23 protein HHR23A and HHR23B in XPC exon 12 (Masutani *et al*, 1994; Li *et al*, 1997), binds the transcription/repair factor TFIIH (Drapkin *et al*, 1994) in exon 14 (R.L., unpublished data), and nonspecifically binds with single-stranded DNA (Reardon *et al*, 1996). Depending on their location, different mutations in a related XP gene (XP-D) are associated with at least three markedly different clinical phenotypes: (i) XP, (ii) XP/CS complex, and (iii) trichothiodystrophy (sulfur deficient brittle hair and mental retardation without skin cancer) (Taylor *et al*, 1997). Although other patients with XP have not had amino acid abnormalities, it is possible that the XPC exon 9 splice mutation has resulted in a new phenotype (XPC with hypoglycinemia), possibly via production of alternatively spliced forms of the XPC message.

XPC splice donor mutation Mutations in splice sites decrease recognition of the adjacent exon and consequently inhibit splicing of the adjacent intron (Talerico and Berget, 1990; Carothers *et al*, 1993). We found a splice donor site mutation (T→G) at the +2 position of the exon 9/intron 9 junction of XPC (**Fig 3*D***). We have analyzed the effects of the mutation on RNA processing using an information theory based approach incorporating information weight matrices that reflect features of nearly 2000 published donor and acceptor sites (Schneider, 1997b) (**Fig 4*A***). This mutation reduced the value of the splice donor site information content from 9.7 to 1.5 bits (Schneider, 1997b). Evidence from analysis of many other splice junction mutations indicates that sites below 2.4 bits are not functional, and often result in skipping of the preceding exon (Rogan *et al*, 1998). Thus the T→G splice donor mutation results in loss of the 161 bp exon 9 (isoform I, **Fig 4*C***), resulting in a predicted XPC protein truncated at 657 aa. A 2.8 bit donor site is located at base pair 69 in intron 9 (exon 9b donor, **Fig 4*A***) and is utilized in isoform II (**Fig 4*C***), resulting in an insertion. The 155 base pair exon 9a is bounded by a strong acceptor of 11.8 bits and a donor of 5.1 bits (**Fig 4*B***). It was found in isoform II along with the insertion of exon 9b (**Fig 4*C***), resulting in a predicted XPC protein truncated at 721 aa. Isoform III contains exon 9a in place of exon 9 (**Fig 4*C***), resulting in an in-frame alteration potentially producing nearly full-length XPC protein (938 aa rather than 940 aa). These altered proteins may have biologic activity that ultimately can also affect the DNA repair pathways as demonstrated for Fanconi anemia cells (Yamashita *et al*, 1996).

Nonsense mutations can enhance mRNA decay rate 10- to 20-fold (Maquat, 1995; Jacobson and Peltz, 1996; Aoufouchi *et al*, 1996). The low level of XPC mRNA in XP22BE cells (**Fig 3*A***) is probably a consequence of the splice site mutation with resulting premature termination of mRNA translation in isoforms I and II.

A new syndrome? In XP22BE cells five microsatellite markers on chromosome 3 near the XPC locus revealed homozygosity. Because we found only one mutation in the XPC cDNA in XP22BE cells, this is evidence that the XPC gene also is homozygous for the T→G splice donor mutation. This splice mutation may have also produced the hypoglycinemia by a presently unknown mechanism. Alternatively, the hypoglycinemia may be the result of homozygous mutation(s) in other, unidentified, gene(s). By analogy with the discovery of the XP/CS complex (Robbins *et al*, 1974), we cannot be certain that the hypoglycinemia is caused by the XPC splice mutation until additional patients are found either with the same XPC mutation or with XP and hypoglycinemia. In conclusion, the association of an XPC splice site mutation, unusual neurologic abnormalities (normal reflexes and normal hearing with autistic features), and hypoglycinemia in XP22BE patient may represent a new XPC syndrome.

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