

## Cerebro-Oculo-Facio-Skeletal Syndrome with a Nucleotide Excision–Repair Defect and a Mutated *XPD* Gene, with Prenatal Diagnosis in a Triplet Pregnancy

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Cerebro-oculo-facio-skeletal (COFS) syndrome is a recessively inherited rapidly progressive neurologic disorder leading to brain atrophy, with calcifications, cataracts, microcornea, optic atrophy, progressive joint contractures, and growth failure. Cockayne syndrome (CS) is a recessively inherited neurodegenerative disorder characterized by low to normal birth weight, growth failure, brain dysmyelination with calcium deposits, cutaneous photosensitivity, pigmentary retinopathy and/or cataracts, and sensorineural hearing loss. Cultured CS cells are hypersensitive to UV radiation, because of impaired nucleotide-excision repair (NER) of UV-induced damage in actively transcribed DNA, whereas global genome NER is unaffected. The abnormalities in CS are caused by mutated *CSA* or *CSB* genes. Another class of patients with CS symptoms have mutations in the *XPB*, *XPD*, or *XPG* genes, which result in UV hypersensitivity as well as defective global NER; such patients may concurrently have clinical features of another NER syndrome, xeroderma pigmentosum (XP). Clinically observed similarities between COFS syndrome and CS have been followed by discoveries of cases of COFS syndrome that are associated with mutations in the *XPG* and *CSB* genes. Here we report the first involvement of the *XPD* gene in a new case of UV-sensitive COFS syndrome, with heterozygous substitutions—a R616W null mutation (previously seen in patients in XP complementation group D) and a unique D681N mutation—demonstrating that a third gene can be involved in COFS syndrome. We propose that COFS syndrome be included within the already known spectrum of NER disorders: XP, CS, and trichothiodystrophy. We predict that future patients with COFS syndrome will be found to have mutations in the *CSA* or *XPB* genes, and we document successful use of DNA repair for prenatal diagnosis in triplet and singleton pregnancies at risk for COFS syndrome. This result strongly underlines the need for screening of patients with COFS syndrome, for either UV sensitivity or DNA-repair abnormalities.

### Introduction

Although initially reported as early as 1971, by Lowry et al., the cerebro-oculo-facio-skeletal syndrome (COFS syndrome [MIM 214150]) was delineated by Pena and Shokeir in 1974, as an autosomal recessive, progressive brain and eye disorder leading to microcephaly, with cerebral atrophy, hypoplasia of the corpus callosum, hypotonia, severe mental retardation, cataracts, microcornea, optic atrophy, progressive joint contractures, and postnatal growth deficiency. Even in the earliest reports, there ap-

peared to be severe perinatal lethal forms, with similarities to other early-onset disorders, such as Cockayne syndrome (CS [MIM 216400–216411]), CAMFAK syndrome (CAMFAK [MIM 212540]), and, possibly, Neu-Laxova syndrome (NLS) (NLS [MIM 256520]). Other researchers reported entities at the milder end of this phenotypic spectrum, such as Martsolf syndrome (Martsolf [MIM 212720]), CAHMR syndrome (CAHMR [MIM 211770]), and MICRO syndrome (MICRO [MIM 600118]). All these entities appear to be inherited in an autosomal recessive fashion. COFS syndrome and CS are both associated with neurodegeneration and cataracts, and they are usually both considered within the same differential diagnosis, but COFS-syndrome eye defects (i.e., microcornea with optic atrophy) appear to be more severe than those usually associated with CS (i.e., pigmentary retinopathy). Also, cutaneous photosensitivity was not noted in the patients originally diagnosed as hav-

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ing COFS syndrome, so these entities have been considered to be distinct from each other, until the past few years.

A biochemical defect and an associated genetic mutation were first found, in a patient with COFS syndrome, by Hamel et al. (1996). This severe case showed prenatal-onset growth deficiency, severe microcephaly, microphthalmia with no cataracts, cleft palate, cutaneous photosensitivity, and brain atrophy with no calcifications. Skin fibroblasts revealed extreme cellular sensitivity to UV, comparable to that in classic xeroderma pigmentosum (XP [MIM 278700–MIM 278810]). Such XP-like defective nucleotide-excision repair (NER), in combination with the clinical symptoms, suggested a diagnosis of the very rare XP-CS complex (Moriwaki et al. 1996). Complementation analysis indicated that the *XPG* gene was affected in this patient, and this was confirmed by mutation analysis (Noussipik et al. 1997). Sigmundsson et al. (1998) reported another, similar patient with COFS syndrome, with both microphthalmia and cataracts and with an *XPG*-gene defect.

Families related to the Manitoba Aboriginal population within which COFS syndrome originally was reported have also demonstrated cellular UV sensitivity consistent with CS, with irreversible transcription inhibition and normal global genome NER (GG-NER) after UV exposure. Such patients present with both prenatal-onset growth deficiency and microphthalmia with cataracts but with no obvious cutaneous photosensitivity. They manifest a homozygous 2-nt deletion in the *CSB* gene, resulting in a truncated polypeptide, and identical mutations have been identified in their parents and in archival pathology tissue from other patients with COFS syndrome who are from this region (Meira et al. 2000). Recently, a second child was reported with COFS syndrome and a homozygous 177-bp deletion in the *CSB* gene (Powell et al. 2000). We now report a child with COFS syndrome who has a unique mutation in a third DNA-repair gene, *XPD*, and we demonstrate, in a triplet pregnancy and in a subsequent singleton pregnancy, how the finding of abnormal DNA repair can be used for prenatal diagnosis.

## Subjects, Material, and Methods

### Clinical Report

This male infant was the prenatally growth-deficient 1.4-kg product of a 37-wk pregnancy and was born to nonconsanguineous Ashkenazi Jewish parents who previously had experienced two spontaneous abortions. The mother gained only 5 kg during the pregnancy, and amniocentesis performed for maternal age of 35 years revealed a normal 46,XY karyotype. Results of prenatal ultrasonography at 16 and 21 wk gestation were thought to be normal. There was spontaneous early labor, with

meconium-stained amniotic fluid after rupture of membranes, and this male infant was delivered vaginally, with Apgar scores of 9 and 9.

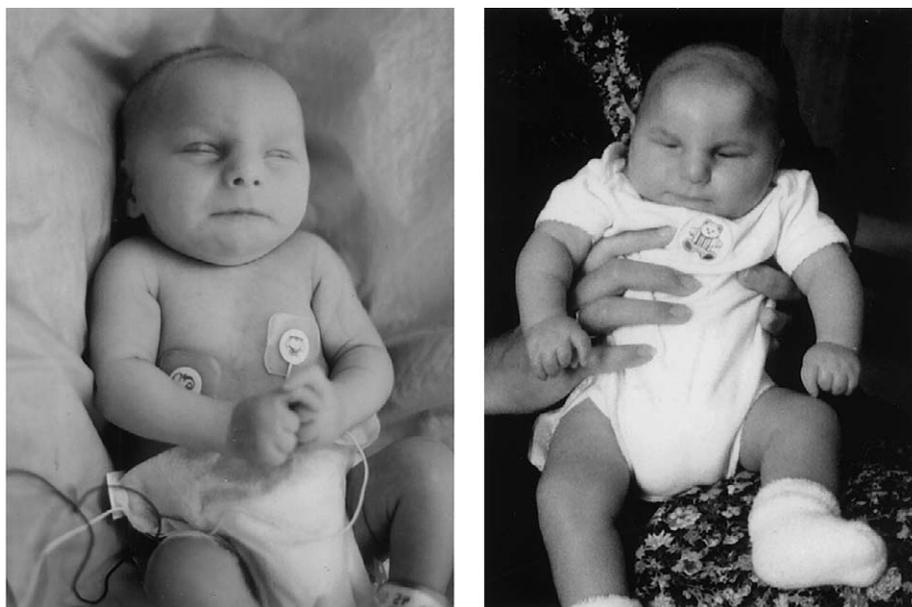
Examination revealed severe intrauterine growth deficiency, microcephaly, deep-set small eyes with bilateral cataracts, a prominent beaked nose, micrognathia, micropenis with scrotal hypoplasia, finger contractures with a simian crease on the right, kyphoscoliosis, and rocker-bottom feet (fig. 1, *left*). Results of repeat chromosome analysis and metabolic studies, including analyses of organic acids, were all normal. The diagnosis of COFS syndrome (versus early-onset CS) was made, and skin-fibroblast analysis for sensitivity to UV radiation was initiated. At age 17 d the infant had a weight of 1.5 kg, length 39.5 cm, and occipital-facial circumference (OFC) 27.5 (all values are 5–6 SD below the expected means for a term birth), and brain-stem auditory-evoked responses suggested profound bilateral hearing impairment. By age 4 mo the cataracts had been removed, and the infant had a weight of 3 kg, length 46 cm, and OFC 31 cm; at age 9 mo there had been little improvement in his growth (fig. 1, *right*). On follow-up at age 13 mo he was noted to be profoundly growth/developmentally delayed, with sparse hair and a deeply sunburned face suggesting cutaneous photosensitivity. No abnormal skin pigmentation or signs of skin cancer were evident; his sparse hair was not examined microscopically, nor was the sulfur content determined. He had severe microcephaly with small, deep-set eyes, micrognathia, and extensive joint contractures. He had repeated hospital admissions, for respiratory difficulties secondary to his neurodegenerative disease, and he died at age 3½ years.

### Cell Cultures and Strains

Response to UV radiation was evaluated in human diploid fibroblast cell cultures derived from skin-biopsy cultures after the parents had signed informed-consent forms approved by local institutional review boards. Cell strains used were 96RD362 (proband), C5RO (normal fibroblasts), XP25RO (XP complementation group A [XPA]), XP1BA (complementation group B [XP-B]), XP21RO (complementation group C [XP-C]), XP6BE (complementation group D [XP-D]), XP2BI (complementation group G [XP-G]), and CS1AN (CS-B).

### UV-Survival Curves

Survival assays were performed at Rotterdam, as described by Hamel et al. (1996). Sparse petri-dish cultures were exposed to graded UV doses and were further incubated for 4–6 d. Subsequently, proliferative activity was determined by incorporation of tritiated thymidine measured by scintillation counting (Rotterdam). Survival was also studied at the Armed Forces Institute of Pathology, by means of six-well-cluster dishes inoculated



**Figure 1** Clinical appearance of patient with COFS syndrome. Patient is shown at age 3 wk (left) and at age 9 mo (right).

with 20,000 cells/well, with irradiation after 1 d of culture, and the cells were counted by a particle counter after an additional 3–5 d. Results of both methods were similar and were identical to those of classic cloning assays.

#### *Unscheduled DNA Synthesis (UDS) and Complementation Analysis*

GG-NER activity was assessed as UV-induced incorporation of tritiated thymidine (i.e., UDS), as described elsewhere (Vermeulen et al. 1993). Fixed cells were processed for autoradiography, and silver grains were counted above 25–50 non-S-phase cells. Complementation analysis was performed as described by Vermeulen et al. (1993) and Hamel et al. (1996), by means of pairwise fusion of cell strains preloaded with different types of polystyrene beads. UDS was determined 2 d after fusion in heterokaryons, and mononuclear cells were differentiated on the basis of their bead content.

#### *Inhibition of DNA Synthesis, by UV*

Cell cultures were pre-labeled overnight with [<sup>14</sup>C]-thymidine and were exposed to various doses of UV. To assess recovery from inhibition of nucleic-acid synthesis, cells were cultured for an additional 16 h, and the rates of S-phase-dependent DNA synthesis were measured by liquid-scintillation counting of pulse-label-incorporated [<sup>3</sup>H]-thymidine. Relative rates of DNA synthesis were assessed on the basis of [<sup>3</sup>H]:[<sup>14</sup>C] ratios and were expressed as percentages of unirradiated cells, as described elsewhere (Sijbers et al. 1998).

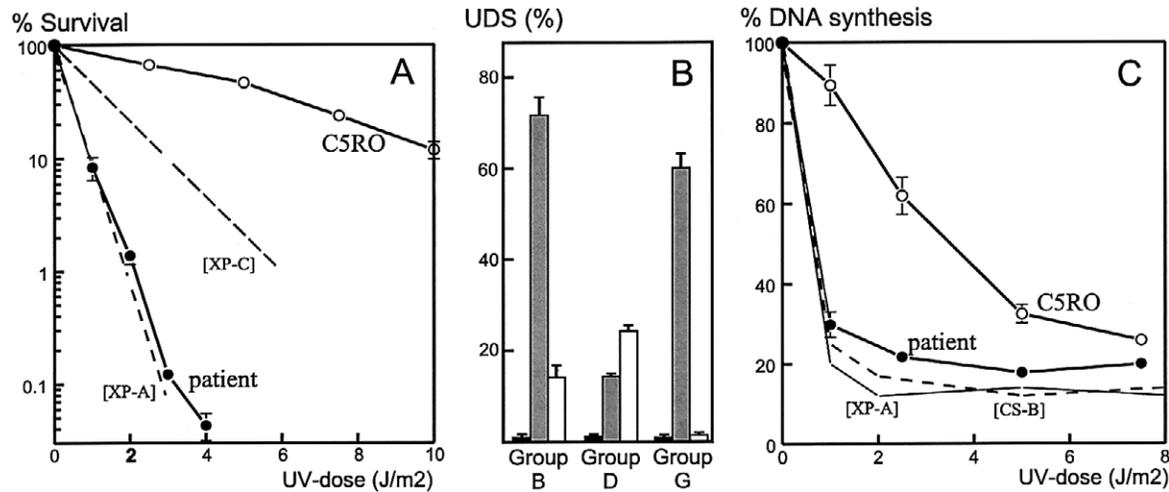
#### *Mutation Analysis*

Initial mutation screening was performed by means of restriction-enzyme fingerprinting (Liu and Sommer 1995). Four overlapping XPD-gene cDNA fragments were obtained by reverse transcriptase-PCR and were digested with various mixtures of restriction enzymes. The combinations used were as follows: fragment 1 (covering codons –17 through 188), *NcoI-AvaII-SacI* and *NcoI-NspI-MboII*; fragment 2 (codons 175 through 346), *AluI-DdeI* and *BglII*; fragment 3 (codons 335 through 557), *NcoI-BglII-BpmI* and *NcoI-AvaII-PvuII*; and fragment 4 (codons 553 through 760+4), *BamHI-AccI* and *RsaI-HhaI*. Digested fragments were end-labeled by means of  $\gamma$ -[<sup>32</sup>P]-ATP, were heat-denatured, and were separated on native polyacrylamide gels. Standard robotic bidirectional sequencing of cDNA fragments 3 and 4 was performed by BaseClear.

#### **Results**

##### *DNA-Repair Studies*

*UV sensitivity of cells.*—An initial study (by the Armed Forces Institute of Pathology) showed the cells to have  $D_{37}$  of 0.6 J/m<sup>2</sup> and  $D_{10}$  of 1.4 J/m<sup>2</sup>, versus 4–8 and 10–17 J/m<sup>2</sup>, respectively, for normal controls. This result was confirmed by a Rotterdam study using a slightly different method, showing 10-fold-increased sensitivity to UV, comparable to that in patients with severe XP who were from complementation group A (see fig. 2A). Since CS cells with a defect in transcription-coupled NER (TC-NER) normally show no greater than approximately



**Figure 2** DNA-repair characteristics of patient's fibroblasts (*black symbols*) and of normal C5RO cells (*white symbols*). Standard-error bars are shown wherever they exceed symbol size. *A*, UV-survival curves, compared to typical XP-A and XP-C strains measured in a separate experiment. *B*, GG-NER activity in mononuclear cells of patient (*black bars*) mononuclear cells from fusion partner (representative of XP group B, D, or G) (*white bars*), and heterokaryons in fusion experiments (*gray bars*), measured as UDS and represented as a percentage of that in normal C5RO cells tested in the same experiment. *C*, Residual rates of overall DNA synthesis 16 h after exposure to various UV doses. Typical responses of XP-A and CS-B cells are from a separate experiment.

fourfold-increased sensitivity, presence of the XP-CS complex—in which GG-NER, as well as TC-NER, is affected—was suspected.

**UDS.**—The overall rate of NER activity, measured autoradiographically in single cells (i.e., UDS), was severely impaired and reduced to 1%–2% (see fig. 2*B*), which is comparable to that in the most severe cases of XP, in complementation groups A, B, and G and which confirms a generalized defect in both the GG-NER and the TC-NER modes.

**Inhibition of DNA synthesis.**—UV-induced DNA lesions act as a block to RNA polymerase II-dependent transcription. Recovery from inhibition of RNA synthesis depends on an intact TC-NER process; consequently, the overall rate of replicative-DNA synthesis is temporarily depressed as well. Whereas normal cells exposed to 2–5 J/m<sup>2</sup> of UV regain most of their original DNA replication in <16 h, the patient's fibroblasts failed to do so, to an extent similar to that in typical patients with either CS or severe XP (see fig. 2*C*). In conclusion, the patient's cells suffered from an almost complete impairment of NER, in both GG-NER and TC-NER, a biochemical defect comparable to that usually found in patients with classic XP patients who have defective *XPA*, *XPB*, or *XPB* genes; however, since the clinical picture was clearly distinct from that of XP, a further genetic study was indicated.

#### Gene Assignment and Mutation Analysis

To identify the responsible gene, we performed complementation studies by cell fusion. Restoration of NER

activity occurred after fusion with cells from XP-B and XP-G but not after fusion with cells from XP-D (see fig. 2*B*). Involvement of the *XPD* gene was further evaluated by restriction-enzyme fingerprinting of four overlapping fragments of the cDNA. We found no evidence for changes in fragments 1–3, whereas at least three new bands appeared in fragment 4, with both enzyme digestions (see the example shown in fig. 3*A*), presumably suggesting the presence of two heterozygous mutations in the 3' quarter of the *XPD*-gene cDNA. Subsequent cDNA sequencing revealed two heterozygous missense base transitions in fragment 4 (see fig. 3*B*) and no mutations in fragment 3. On the protein level, one transition results in a change, R616W, that, in an earlier study (Lehmann 2001), had been reported to be a null mutation occurring in several patients in XP-D. The second mutation, presumably in the other allele, appears to be responsible for the patient's phenotype, and it causes a new and unique amino-acid substitution, D681N. This change is unlikely to represent a polymorphism, since the normal aspartic acid residue shows complete evolutionary conservation in all known *XPD* genes (see fig. 3*C*).

#### Prenatal Diagnosis

Prenatal diagnosis was requested in a subsequent triplet pregnancy, had which had been by in vitro fertilization. The triplets consisted of one singleton (fetus A) and two MZ twins (fetuses B and C). Cell cultures were grown from chorionic villus (CV) samples obtained, at 10 and 12 wk of gestation, from the singleton and from

the common placenta of twins B and C, and the cultures were shipped from New York to Rotterdam, for further analysis. Table 1 shows results of UDS studies of singleton A and of twins B and C. CV cells of singleton A showed a deficiency of DNA-repair synthesis (UDS 13%–18% of that in normal controls), indicating that the fetus was affected. The UDS results for the CV cells of twins B and C were complicated by the presence of two different cell populations in an ~1:1 ratio. Cells with very low grain numbers (i.e., 0–10 grains/nucleus) were mixed with cells with grain numbers in a normal range (i.e., 22–65 grains/nucleus). Recovery of DNA synthesis was normal (results not shown). These results could be interpreted in three ways. (1) twins B and C are not MZ; one is affected, and the other is unaffected. However, the presence of a shared placenta indicates that the twins definitely should be MZ. (2) Twins B and C are both affected, and the UDS-proficient cells originate from the mother. However, twins B and C (as well as singleton A) were male; FISH analysis of the CV culture used for the DNA-repair study demonstrated exclusively XY cells, excluding maternal cell admixture. (3) Twins B and C are both unaffected; the UDS-deficient CV cells originate from singleton A. Since explanations (1) and (2) had to be rejected, and since cross-contamination in CV cultures from multiple gestational products is not an uncommon event (Van den Berg et al. 1999), the final diagnosis was that singleton A was affected and that twins B and C were unaffected.

Selective termination of singleton A was performed at

**Table 1**

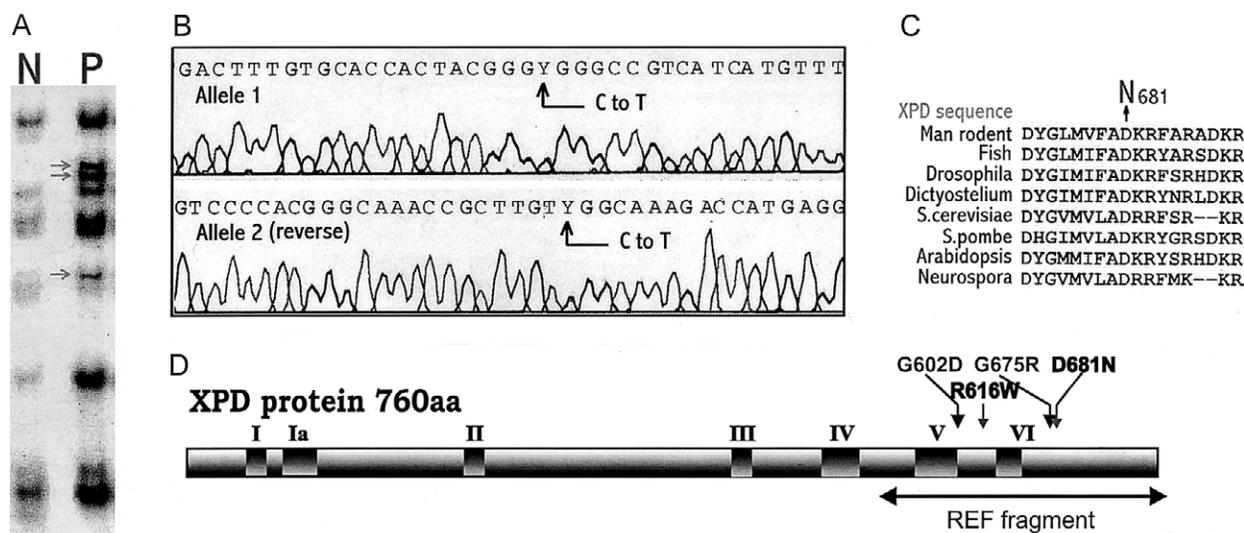
**Prenatal Diagnosis of XP-CS Complex in Two Pregnancies in One Family, after Birth of Proband: UV-induced UDS in CV Cells, Amniocytes, and Skin Fibroblasts**

SOURCE	RESULTS OF UDS <sup>a</sup> (average no. of grains/nucleus)	
	Fetus(es)	Normal Control(s)
Pregnancy 1:		
Singleton A, CV cells	9.2	50,70
Twin B/C, CV cells (two populations)	3.2, 35	65
Pregnancy 2:		
CV cells	54	36
Amniocytes	43	53
Skin fibroblasts:		
Index patient (COFS syndrome)	1.3	46
Fetus C (demise) <sup>b</sup>	32	48

<sup>a</sup> At UV exposure of 16 J/m<sup>2</sup>.

<sup>b</sup> Fetus C suffered intrauterine demise; no cells were available from either fetus A (which suffered selective reduction) or child B (who was healthy).

18 wk gestation, and a macerated male fetus was delivered at 22 wk gestation; thus, there was no possibility of cell culture for a confirmatory study. Twins B and C were delivered by cesarean section at 32 wk gestation, immediately after the intrauterine demise of one of the twins (i.e., fetus C). DNA-repair study (i.e., UDS; see table 1) and recovery of DNA synthesis (not shown) in cultured fibroblasts from twin C confirmed the presence



**Figure 3** Molecular genetic analysis of XPD gene. A, Restriction-enzyme fingerprinting of 3' cDNA fragment 4 (shown in panel D), digested with *RsaI-HhaI*. Red arrows indicate new bands appearing in patient's DNA (lane P), compared to normal sequence (lane N). B, Relevant parts of XPD sequence readout. Both mutations found were heterozygous. C, Conservation of XPD amino acids around mutation D681N. Rodent = *Mus musculus* and *Cricetulus griseus*; fish = *Xiphophorous maculatus*. D, Bar representation of XPD protein with seven helicase domains, mutations found in the proband (black) and in two other patients in XP-D who have symptoms of CS (gray) (from Lehmann 2001).

**Table 2**  
COFS Syndrome and Similar Syndromes

PARAMETER	STATUS <sup>a</sup>					
	COFS Syndrome	CAMFAK Syndrome	CS	MICRO Syndrome	CAHMR Syndrome	Martsolf Syndrome
General:						
Low birth weight	+/-	-	+/-	-	+/-	+/-
Failure to thrive	+	+	+	-	+	+
Feeding problems	+	+	+/-	-	+	+
Mental retardation	+	+	+	+	+	+
Early death	+	+	-	-	+/-	-
Seizures	-	-	-	-	-	+
Wide-set nipples	+	-	-	-	-	-
Hypertrichosis	+	-	-	+	+	+/-
Genital hypoplasia	-	-	+	+	-	+
Skeletal:						
Kyphoscoliosis	+	+	+/-	+	-	-
Camptodactyly	+	+	+/-	+	-	-
Flexion contractures	+	+	+/-	+	-	-
Abnormal feet	+	+	+/-	+	-	+
Craniofacial:						
Microcephaly	+	+	+	+	-	+
Cortical atrophy	+	+	+	-	+	+
Hypoplastic corpus callosum	+	-	+	+	-	-
Microphthalmia	+	-	-	+	-	-
Optic atrophy	+	?	+	+	-	-
Congenital cataracts	+	+	+	+	+	+
Deep-set eyes	+	+	+	-	-	-
Prominent nasal root	+	+	+	-	-	-
Low nasal bridge	-	-	-	-	+	+
Prominent upper lip	+	+	+	+	-	-
Maxillary retrusion	+	+	+	-	-	+
Micrognathia	+	+/-	+/-	-	+/-	+/-
Prognathism	-	-	-	-	-	+
Large ears	+	+	+	+	-	-
Microdontia	-	-	-	-	+	-

<sup>a</sup> A plus sign (+) denotes presence; a minus sign (-) denotes absence; a plus/minus sign (+/-) denotes that results were variable.

of normal NER in this fetus. Examination of the placenta of the twins revealed multiple anastomoses, which may have resulted in abnormal blood flow and, thereby, in the demise of twin C. When this article was accepted for publication, twin B was age 3 years and healthy. In a subsequent singleton pregnancy in the same family, normal UDS was demonstrated in CV cells, but, since the fetus was female, and since maternal-cell overgrowth could not be fully excluded, amniocentesis was also performed. Normal DNA-repair capability was confirmed in the cultured amniocytes (table 1). At the time of the present study, this girl was age 32 mo and healthy.

## Discussion

COFS syndrome is a rare, well-established birth-defect syndrome that was defined >20 years ago, as occurring with autosomal recessive inheritance in isolated Manitoba families (Pena and Shokeir 1974) characterized by frequent consanguineous marriages (Pena et al. 1978).

A number of other patients with similar findings have been reported, resulting in both a further nosological delineation of COFS syndrome and differentiation from other, similar clinical entities (Lowry et al. 1971; Scott-Emuakpor et al. 1977; Lowry 1982; Patton et al. 1989; Nance and Berry 1992). On the basis of these reports, significant clinical overlap with other, similar autosomal recessive eye/brain syndromes—such as CS, CAMFAK syndrome, MICRO syndrome, Martsolf syndrome, and CAHMR syndrome (for overview, see table 2)—has become apparent. Because COFS syndrome and CS are both associated with neurodegeneration and cataracts, they are usually considered to belong within the same differential diagnosis, but COFS-syndrome eye defects (i.e., microcornea with optic atrophy) are more severe than those usually associated with CS (i.e., pigmentary retinopathy), and cutaneous photosensitivity was not noted in the patients originally reported with COFS syndrome. The difficulty in delineating such rare progressive genetic disorders has been well illustrated and discussed

by various clinicians (Pena et al. 1978; Winter et al. 1981; Warburg et al. 1993), and it has been clear for some time that a specific biochemical or genetic marker would be of great benefit.

Key features of COFS syndrome include congenital microcephaly, with subsequent brain atrophy, reduced white matter, patchy gray matter, hypotonia, deep-set eyes with microphthalmia and cataracts, and camptodactyly with rocker-bottom feet. Movement is markedly decreased, leading to joint contractures, and life span is usually severely limited (Pena and Shokeir 1974; Preus and Fraser 1974; Pena et al. 1978). In children with COFS syndrome, failure to thrive is prevalent, because of feeding problems, aspiration, and repeated lower-respiratory-tract infections; and, in 8 of the 10 patients initially diagnosed with COFS syndrome, recurrent-aspiration pneumonia has led to death at age <30 mo. In 1978, Pena et al. reported longitudinal follow-up of some of their earlier-reported cases, the results of which emphasized the progressive nature of this disease and suggested that it might represent a primary neurodegenerative disorder associated both with progressive loss of subcortical white matter and myelin and with the development of intracranial calcifications. Linna et al. (1982) emphasized the diagnostic usefulness of finding the foci of intracranial calcifications via cranial computed-tomography scans of the lenticular nuclei and hemispheric white matter of affected siblings with COFS syndrome.

Neuropathology studies reported in the initial publications discussing COFS syndrome revealed generalized subcortical gliosis and decreased white matter with reduced myelin content, and Del Bigio et al. (1997) reported neuropathology in eight children with COFS syndrome, seven of whom were from the same Manitoba Aboriginal families. They noted progressive cortical neuronal loss with patchy absence of myelin and gliosis in white matter, as well as pericapillary and parenchymal mineralization in the globus pallidus, putamen, and cerebral cortex. In older children, there was severe cerebellar degeneration involving the internal granular layer and the Purkinje cell layer, and, in the younger cases, swollen ubiquitinated granular cells were found in the white matter shortly after birth. Such progressive demyelination with brain calcification is quite similar to what is seen in the severe infantile form of CS. In both conditions, intrauterine growth can be close to normal, but, after birth, growth deficiency is striking and unrelenting, and it has become increasingly more difficult to distinguish between early-onset CS and COFS syndrome in siblings who have congenital cataracts, microphthalmia, intracranial calcifications, and progressive demyelinating diseases similar to early-onset CS (Linna et al. 1982; Lowry 1982; Lerman-Sagie et al. 1987; Patton et al. 1989; Talwar and Smith 1989).

In 1992, Nance and Berry reviewed 140 cases of CS, in an effort to define diagnostic criteria for the condition. Requisite criteria included poor growth and neurologic abnormality, with other common manifestations including sensorineural hearing loss, cataracts, pigmentary retinopathy, cutaneous photosensitivity, and dental caries. The mean age at death was slightly >12 years, with some affected individuals living into their late teens. On the other hand, prenatal growth failure, severe neurologic dysfunction from birth, and development of cataracts during the first 3 years of life were predictors of severe disease and early death. This latter category was designated "type 2" CS, and the original case of CAMFAK syndrome (reported by the same institution) was included as a case of type 2 CS. This latter category of severe, early-onset CS clearly overlapped COFS syndrome, in symptoms.

Because of (a) dermatologic similarities between CS and XP and (b) the discovery that XP results from defects in DNA repair, various assays of UV sensitivity and DNA repair were performed on CS cells. Patients with CS were found to have increased sensitivity to killing from UV radiation, with delayed recovery of RNA (and DNA) synthesis after UV but with normal UDS. NER processes remove helix-distorting changes induced by some carcinogens as well as by UV exposure. XP, CS, and photosensitive forms of trichothiodystrophy (TTD [MIM 601675]) are caused by mutations in genes involved in NER. At least two NER subpathways can be differentiated: (1) rapid TC-NER, which eliminates lesions from the transcribed strand of active genes (thus permitting rapid resumption of transcription), and (2) GG-NER, which surveys the remainder of the genome and can be less rapid, depending on the type of lesion. Both processes are affected in most patients with either XP or UV-sensitive TTD (except in the case of XP-C, in which there is a defect in GG-NER only), whereas only TC-NER is impaired in CS. Since most NER genes are now cloned, it is possible to identify disease-causing mutations and to correlate genotypes with phenotypes (table 3).

Dry scaly skin, abnormal pigmentation on sun-exposed areas, photosensitivity, and predisposition to skin cancer are cardinal features of XP, and this autosomal recessive phenotype has been associated with mutations in one of a number of genes playing a role in NER (table 3). Mutations in some of these genes, particularly in the case of XPD, can also cause the related conditions: TTD (which is characterized by brittle, sulfur-deficient hair with or without short stature, mental retardation, ichthyosis, subtle dysmorphism, and photosensitivity) and CS (which is characterized by profound growth retardation, pigmentary retinopathy with optic atrophy, cataracts, mental retardation, wizened facies, and neurological deterioration). Although there

**Table 3**  
**Overview of Disorders Associated with NER Deficiency**

Mutated Gene	Sun-Sensitive Skin Only	XP with				TTD	UV-Sensitive COFS Syndrome
		XP	Neurological Complications	CS	XP+CS		
<i>XPA</i>		X	X				
<i>XPB</i>					X	X	
<i>XPC</i>		X					
<i>XPD</i>	X <sup>a</sup>	X	X	X <sup>a</sup>	X	X	X <sup>b</sup>
<i>XPE</i>	(x)	X					
<i>XPF</i>		X	(x)				
<i>XPG</i>		X	X		X		X
<i>XPV/RAD30A</i> <sup>c</sup>	(x)	X					
<i>CSA</i>				X			
<i>CSB</i>				X			X
<i>TTDA</i>						X	
<i>UVS</i>	X						

NOTE.—The clinical phenotype associated with a particular gene depends on either the type or the severity of the mutation involved. “X” denotes presence of the disorder; “(x)” denotes presence in only some of the patients.

<sup>a</sup> Source: unpublished observations of two authors (N.G.J.J. and A.R.) of the present article.

<sup>b</sup> Source: present study.

<sup>c</sup> Defect not in NER but, rather, in translesion DNA replication; added for completeness.

is some clinical overlap in phenotype, the latter two syndromes usually do not show either sun-damaged skin or predisposition to skin cancer. XP and TTD are associated with different types of mutations in the *XPD* gene, and mutation at any one given site is thought to be predictive of the specific phenotype.

CS is usually caused by mutations in either the *CSA* or *CSB* genes, which result in a selective defect of TC-NER. However, more rarely, CS may also involve defects in the *XPB*, *XPD*, or *XPG* genes, and in these cases there is an overall defect in both NER modes, which often results in symptoms of XP as well, a condition termed “XP-CS complex” (Moriwaki et al. 1996). The patient presented here fits this category and carries a mutation in the *XPD* gene, a situation that is unusual in a number of respects. First, association between COFS syndrome and XP-D has not been observed in earlier studies. Second, residual UDS is very low, entirely unlike other defects in the *XPD* gene that have been described thus far, in which significant residual UDS (i.e., 10%–60%) is commonly seen and in which even virtually normal levels may occur (N.G.J.J., A.R., and W.J.K., unpublished data). Third, the causative D681N mutation in this patient is unique and distinct from those in two other patients with mutations in the *XPD* gene and with XP-CS complex (see fig. 3D). Mutations in the *XPD* gene can be associated with distinct inherited conditions (Lehmann 2001), because of the dual involvement of the *XPD* protein in NER and basal transcription. Depending on the mutation, the balance between these two functions appears to be disturbed in distinct ways (Vermeulen et al. 2001). All disease-causing mutations found are subtle (usually they are mis-

sense point mutations), allowing for very substantial residual rates of basal transcription. Although the confounding influence of genetic background can never be fully dismissed, this importance of the mutation type is underlined by the finding that the causative mutations in the *XPD* gene in XP, TTD, or XP-CS complex show no overlap (Lehmann 2001). Furthermore, substitution D681N was also found in one compound heterozygote patient with XP (Lehmann 2001), together with a typical XP-associated mutation, suggesting that the latter allele is a less severely affected one.

Other patients diagnosed with COFS syndrome with concomitant cellular UV sensitivity have been reported as having mutations in the *XPG* gene (Hamel et al. 1996; Sigmundsson et al. 1998) and in the *CSB* gene (Meira et al. 2000; Powell et al. 2001). Since these genes overlap with those known to cause CS symptoms, we expect that future cases of COFS syndrome will be reported that have mutations in the *XPB* and *CSA* genes as well. We conclude that patients with UV-sensitive COFS syndrome should be added to the spectrum of individuals affected by the NER syndromes, which include XP, CS, and TTD.

Meira et al. (2000, p. 1227) have suggested that “COFS syndrome ... represents an allelic, clinically severe form of CS.” However, some patients with COFS syndrome have also been found to have normal UV sensitivity in vitro (N.G.J.J., A.R., and W.J.K., unpublished data). Given both the clinical overlap between COFS syndrome, Martsolf syndrome, and MICRO syndrome and the fact that no previously reported patient with the latter two syndromes has been studied for UV sensitivity, we suggest that the term “COFS syndrome”

be reserved for those patients with demonstrated UV sensitivity. Martsolf syndrome consists of microcephaly, mental retardation, cataracts, hypogonadism, and hypertrichosis (Martsolf et al. 1978; Sánchez et al. 1985; Hennekam et al. 1988; Strisciuglio et al. 1988; Harbord et al. 1989). MICRO syndrome was described in a consanguineous Pakistani family in which two sibs and a cousin were affected by microcephaly, microcornea, severe mental retardation, hypotonic spastic diplegia, optic atrophy, hypertrichosis, and hypogenitalism (Warburg et al. 1993). Gyral abnormalities were briefly mentioned by Warburg et al. (1993), along with hypoplasia of the corpus callosum, and Nassogne et al. (2000) reported a case of MICRO syndrome with progressive motor neuropathy and polymicrogyria. It is not known whether the siblings with MICRO syndrome who were reported by Warburg et al. had polymicrogyria or progressive motor neuropathy, but some previous cases of COFS syndrome were reported with cortical abnormalities before the pathogenesis of this condition became known (Lurie et al. 1976; Sakai et al. 1997). Those patients with a similar phenotype but with normal UV sensitivity should be temporarily considered as having MICRO syndrome or Martsolf syndrome (although further clinical and biochemical differentiation between these two syndromes is needed). These findings indicate that DNA-repair studies will not be useful for identifying all patients within this spectrum of autosomal recessive eye-brain disorders and that more study will be needed to determine whether all patients with COFS syndrome have a DNA-repair abnormality that could be regarded as a subtype of CS.

We conclude that UV-sensitivity tests in cases with suspected COFS syndrome are important as a means to refine the initial diagnosis. The finding of cellular UV sensitivity, although not evident in all patients who present with features suggestive of COFS syndrome, provides a biochemical marker, which allows for optimal genetic counseling and management of future pregnancies. Here we have presented the first example of prenatal diagnosis of COFS syndrome, on the basis of NER studies in two consecutive pregnancies in the same family, in which one of the prenatal diagnoses was complicated by the presence of multiple fetuses. We have shown here, with the caveat regarding the possibility of cross-contamination in CV sampling in multiple pregnancies, that biochemical repair parameters allow reliable prenatal testing of patients with UV-sensitive COFS syndrome, without the need for time-consuming and costly gene assignment and sequencing procedures as a prerequisite.

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## Electronic-Database Information

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