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Supplemental Data

GTF2E2 Mutations Destabilize the General Transcription Factor Complex TFIIE in Individuals with DNA Repair-Proficient Trichothiodystrophy

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Supplemental Note: Case Reports:

Clinical descriptions of affected children

TTD379BE (IV-1 in Figure 1C)

TTD379BE a 10y/o Asian boy was diagnosed with TTD at age 1 year (Figure S1). He was born in India at term with low birth weight (2.4 kg - 10th percentile). As with other individuals with TTD [1-3] the pregnancy was complicated by mild intrauterine growth retardation (diagnosed at 34 weeks in gestation by ultrasound exam). At birth the skin was normal with no collodion membrane as seen in some TTD patients. He had an unremarkable neonatal period with no feeding problems, no sun sensitivity and no increase in infections during first year of life. However he was noted to have very dry skin and sparse slow growing hair. His gross motor development was delayed: attained head control at age 5 months, crawled at 8 months and walked at 3 years. At age 2 years he was found to be myopic and developed esotropia that was surgically corrected at age 5 years. He had cognitive delays at age 3 years. He has had no other hospitalizations, serious illnesses or additional surgery. At age 10 years he attended special education classes in public school and received physical, speech and occupational therapies. He is described as a very happy, overly friendly child who has a difficult time staying on task in class.

Clinical evaluations at age 10 years: well-developed well-nourished Indian male child who appeared younger than his chronologic age (Figure 1A). His height was 120 cm [<3rd percentile], weight 19.8 kg [<3rd percentile] and head circumference 45.9 [<3rd percentile]. He had mild micrognathia, low set ears and triangular head consistent with craniosynostosis. His scalp hair was short, thick, brittle and stuck out in several directions. His hair showed typical alternating dark and light “tiger tail” banding on polarized microscopy (Figure 1B). His eyebrows were short but his eyelashes were long and full. He had several small nevi on his face, lip and abdomen but did not have the large number of freckle-like pigmented lesions in sun exposed sites that are present in individuals with xeroderma pigmentosum (XP)[4] (Table 1 and Figure S1). There was thick coarse ichthyosiform scaling most prominently over the chest and abdomen and anterior lower legs. He

had hyperlinear palms and soles. The nail plates were within normal limits; they were not thin or peeling. His eyes had nystagmus and strabismus with residual small-angle esotropia following eye muscle surgery. He had micro-cornea with mild myopic astigmatism. There was no evidence of lenticular opacities.

Neurological evaluation showed abnormal long tract signs and cerebellar dysfunction. His deep tendon reflexes were 1-2+ with 2 beats of clonus elicited at the ankles. Fine motor skills were delayed for age. He ambulated with increased base and pronated his feet with spastic-ataxic gait. He had marked cognitive delays including a speech articulation disorder with features of attention deficit disorder. Formal IQ testing was not performed. Audiometric thresholds indicated slight hearing loss bilaterally which was suggested to be sensorineural because of normal tympanometry and absent DPOAEs.

His parents are first cousins. Both parents (III-3 and III-4 in Figure 1C) and his brother (IV-2 in Figure 1C) are clinically normal (Figure 1C).

Laboratory: Routine blood chemistries including electrolytes, liver and renal function tests were within normal limits as was a urinalysis and assessment for thyroid and parathyroid hormone levels. Hemoglobin and hematocrit were normal. However the MCV was reduced: MCV – 64.5 [normal 74.4-86.1] and his hemoglobin electrophoresis was abnormal: Hb A – 91.7 [normal 94.8-97.8] HgbA2 – 6.8 [normal 2.2-3.2] Hb F – 1.5 [normal 0.0-2.0] (Figure S1) as described in other individuals with TTD. [5]

X-ray exam showed no evidence of osteosclerosis, osteopenia or hip abnormality as seen in some individuals with TTD. [1, 2] However, he had probable craniosynostosis of coronal sutures. His bone age was normal [chronological age is 10 years and 3 months – the bone age was about 11 years] with interval maturation noted since a previous exam. CT exam showed a morphologically normal brain with no gross atrophy or calcifications. There was decreased attenuation throughout the white matter possibly representing a leukoencephalopathy. There was evidence of prior mastoiditis with persistent fluid in the left mastoid.

TTD28PV (V-1 in Figure 1E)

TTD28PV a 16y/o Moroccan female weighed 3110g at 38.5 weeks gestation (Table 1 and Figure S1). The pregnancy was reported to be without complications. There was no notion of a collodion membrane and she was not sun sensitive. She had a patent ductus arteriosus at birth that was closed by catheterization. She had brittle hair with tiger tail banding under polarized microscopy (Figure 1D). She has lamellar ichthyosis of her skin. She could sit at 1 year, walk independently at 35 months and spoke 1 word at 33 months. She suffered from chronic rhinosinusitis but did not need hospitalization for infections or other problems. She had surgery for nasal adenoid hyperplasia. She has bilateral pes cavus with Babinski in extension. She wears glasses (-0.5 D) but has no special problems (normal fundus).

At 16 years her height (152.6 cm) and weight (39.7 kg) were <<3rd percentile. She had growth hormone deficiency (Insulin-like growth factor I /Somatomedin C 72 µg/L [normal 207-859 µg/L]) and has been treated with growth hormone with good response (increase of 6.1 cm and 7.1 kg from 2011 to 2014). Hb A2 was elevated (4.8%), Hb F 7.8% and Hb A 87%. MCV was low (61.7 fL) indicating microcytosis as seen in other TTD affected individuals [1, 2] (Figure S1). She had an IQ of 40 and follows special education (type 2, which, in Belgium, is for children with moderate intellectual disability). She has not had any clinical seizures and EEG at age 10 years was normal. She is very friendly, always laughing with good social interaction.

The parents (IV-1 and IV-2 in Figure 1E) are from Morocco and are distantly related although their precise ancestry is not known. They have five other children who are phenotypically normal (V-2 to V-6 in Figure 1E).

Hearing studies were performed on TTD28PV Impedance revealed bilateral mobile tympanic membranes. The tone audiogram had a perceptive loss of 30-40 dB HL, but the results could be influenced by her intellectual disability causing less adequate responses. Acoustic oto-emissions (AOE's) for the right ear were present with decreased amplitude at 1 and 8 kHz, absent at 0.5, 2, 4 and6 kHz. For the left ear AOE's were present at 1 kHz, and showed decreased

amplitude at 4, 6 and 8 kHz and absence at 0.5 and 2 kHz. Overall, it was concluded that she had a mild hearing loss, but no specific treatment was commenced.

MRI of the brain at 2 years and at 10 years was normal. X ray of the pelvis at 21 months showed bilateral coxa valga and delayed bone age (10 years at 13y of age).

Supplemental Figures

ABNORMALITIES	TTD (*ERCC2, XPD*) Features	TTD379BE (*GTF2E2*) (c.448G>C; p.Ala150Pro)	TTD28PV (*GTF2E2*) (c.559G>T; p.Asp187Tyr)	XP (XP-D) Features	Normal
	repair + TRANSCRIPTION defect	TRANSCRIPTION defect	TRANSCRIPTION defect	REPAIR + transcription defect	Normal
ABNORMAL CLINICAL FEATURES		10 y/o Male	16 y/o Female		
Dry Skin	Yes	Yes	yes	Yes	No
Ichthyosis	Yes	Yes	Yes	No	No
Short, Brittle Hair	Yes	Yes	Yes	No	No
Tiger-Tail Banding of Hair	Yes	Yes	Yes	No	No
Sulfur Deficient Hair*	Yes	Yes	not known	No	No
Nystagmus/Strabismus	Yes	Yes	no	No	No
Short Stature	Yes	Yes	Yes <<3 centile	No	No
Microcephaly	Yes	Yes	Yes << - 2SD	No	No
Developmental Delay	Yes	Yes	Yes IQ 40	Yes/No	No
Happy Engaging Personality	Yes	Yes	Yes	No	No
Myopia	Yes	Yes	Yes	No	No
ADHD/Hyperactivitiy	Yes	Yes	Hyperactive	No	No
Ataxia	Yes	Yes	no	Yes/No	No
Craniosynostosis	Yes/No	Yes	no	No	No
Sensorineural Deafness	Yes/No	Slight	30-40 dB hearing loss	Yes	No
Acute Burning on Minimal Sun Exposure	Yes/No	No	No	Yes	No
Xeropthalmia (dry eyes)	Yes/No	No	No	Yes	No
Fever induced hair loss	Yes/No	No	No	No	No
Osteonecrosis of hips	Yes/No	No	bilat coxa valga (21 mo)	No	No
Delayed bone age	Yes/No	No	Yes (10 yr at 13 yr)	No	No
Undescended Testicles	Yes/No	No	N/A	No	No
Congenital Cataracts	Yes	No	No	No	No
Central Osteosclerosis	Yes	No	No	No	No
Peripheral Osteopenia - with nl Vit D	Yes	No	No	No	No
Chronic Diarrhea/Constipation	Yes	No	No	No	No
Recurrent Infections	Yes	No	No (only rhinosinusitis)	No	No
Increased Freckle-like Pigmentation	No	No	No	Yes	No
Skin Cancer	No	No	No	Yes	No
Pterygium	No	No	No	Yes	No
Dilated ventricles (CT or MRI)	No	No	No (MRI nl at10 yr)	Yes	No
Progressive Cognitive Impairment	No	No	IQ 40	Yes/No	No
Axonal Neuropathy/Neuronal Degeneration	No	Not tested	EMG (normal 10 yr, mildly diminished sensory amplitudes 13 yr)	Yes	No
Dysmyelination of the Brain (MRI)	Yes	Not tested	No (MRI nl at10 yr)	No	No
ABNORMAL CLINICAL LABORATORY TESTS					
Low MCV	Yes	Yes	Yes	No	No
RBC elevation	Yes	Yes (6.05x10*6/mm3)	Yes (5,8x10*6/mm3)	No	No
Neutropenia	Yes	Yes -21.2%	Yes - 27%	No	No
Elevation of Hgb A2	Yes	Yes	Yes	No	No
Reduced Hgb A	Yes	Yesm- 91.7%	Yes - 87%	No	No
Pregnancy Abnormalities	Yes	Yes (2.4 kg, IUGR)	No (birth wt nl)	No	No
ABNORMAL RESEARCH LABORATORY TESTS					
DNA REPAIR					
Reduced post-UV cell survival	Yes	No	No	Yes	No
Reduced post-UV host cell reactivation	Yes	No	No	Yes	No
Reduced repair of CPD - confocal	Yes	No	No	Yes	No
Reduced repair of 6-4PP - confocal	No	No	No	Yes	No
Nucleotide Excision Repair (NER) PROTEINS					
Reduced XPB protein - Western	Yes	No	No	Yes	No
XPB protein - Absent localization to UV damage	Yes	No	No	No	No
Reduced XPD protein - Western	Yes	No	No	Yes	No
XPD protein - Absent localization to UV damage	Yes	No	No	Yes	No
TRANSCRIPTION PROTEINS					
Reduced TFIIEβ protein - Western	No	Yes	Yes	No	No
Reduced TFIIEβ protein - Confocal	No	Yes	Yes	No	No
Reduced TFIIEβ protein - Confocal with UV	No	Yes	Yes	No	No
TFIIEβ protein - Confocal localization to UV damage	No	No	No	No	No
Reduced TFIIEα protein - Western	No	Yes	Yes	No	No
Reduced phosphorylated TFIIEα protein in confluent cells - Western	Yes	Yes	Yes	No	No
Reduced TFIIEα protein - Confocal with UV	not tested	Yes	Yes	No	No
TFIIEα protein - Confocal localization to UV damage	not tested	No	No	No	No

Figure S1. Clinical and laboratory abnormalities in individuals with TTD or XP having mutations in *ERCC2* or with TTD having *GTF2E2* mutations. Abnormal clinical features (purple shading), abnormal clinical laborotory tests (light blue), abnormal research laboratory DNA repair tests (tan shading) and abnormal research laboratory tests of transcription protiens (pink shading) are listed in the first column. Typical findings in TTD individuals with mutations in *ERCC2 (XPD)* with a predominant transcription defect are shown in the the second column. Features of TTD379BE and TTD28PV with mutations in *GTF2E2* are shown in columns 3 and 4. Typical finding in XP indivuduals with mutations in *ERCC2 (XPD)* with a predominant DNA repair defect are shown in column 5. Findings in normal people are shown in the last column. Features that are present are indicated by "yes" with dark orange shading. Features that are absent are indicated by "no" with white background. Features that are present in some but not all affected individuals are indicated by "yes/no" with light orange shading. Subjects TTD379BE and TTD28PV have many (but not all) of the same clinical and laboratory test abnormalities that are present in TTD patients but their cells do not have the same DNA repair defects. Cells from subjects TTD379BE and TTD28PV have abnormalties in transcription proteins that are not found in cells from patients with TTD or with XP.

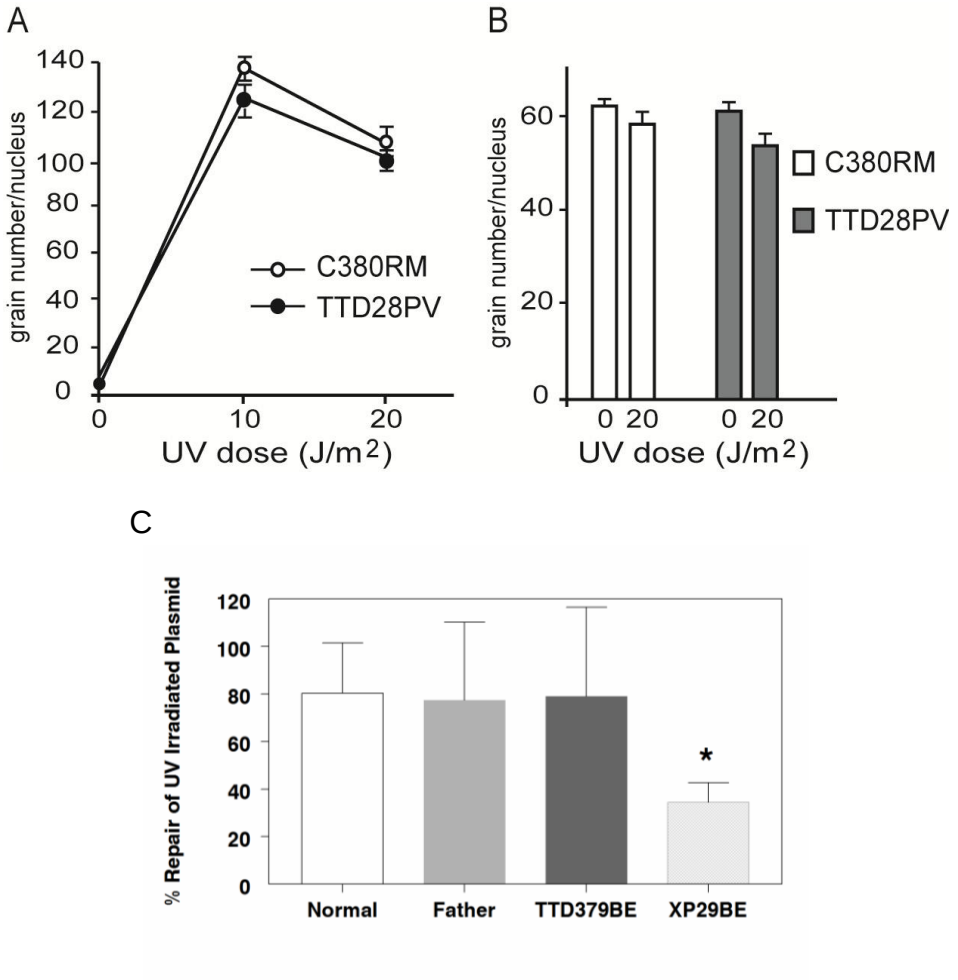

Figure S2. DNA repair responses to UV irradiation of the affected individuals TTD28PV and TTD379BE is similar to that of the normal controls.

A. UV-induced DNA repair synthesis (UDS) 3 hours after UV irradiation expressed as mean number of autoradiographic grains/nucleus. Bars indicate the SE. C380RM is a normal donor.

B. Recovery of RNA synthesis (RRS) 24 h after UV irradiation expressed as numbers of autoradiographic grains per nucleus. Bars indicate the SE.

C. Post-UV host cell reactivation in normal, father, TTD379BE and XP29BE [XP/XP-D] fibroblasts. Cells were transfected with a UV-C irradiated (500 J/m^2) luciferase reporter vector and incubated for 48 hours. Repair of the plasmid is expressed as induced light units of active luciferase compared to a non-irradiated luciferase plasmid. One experiment with each sample in triplicate was performed. Error bars indicate SEM. * indicates $p < 0.05$ vs all others.

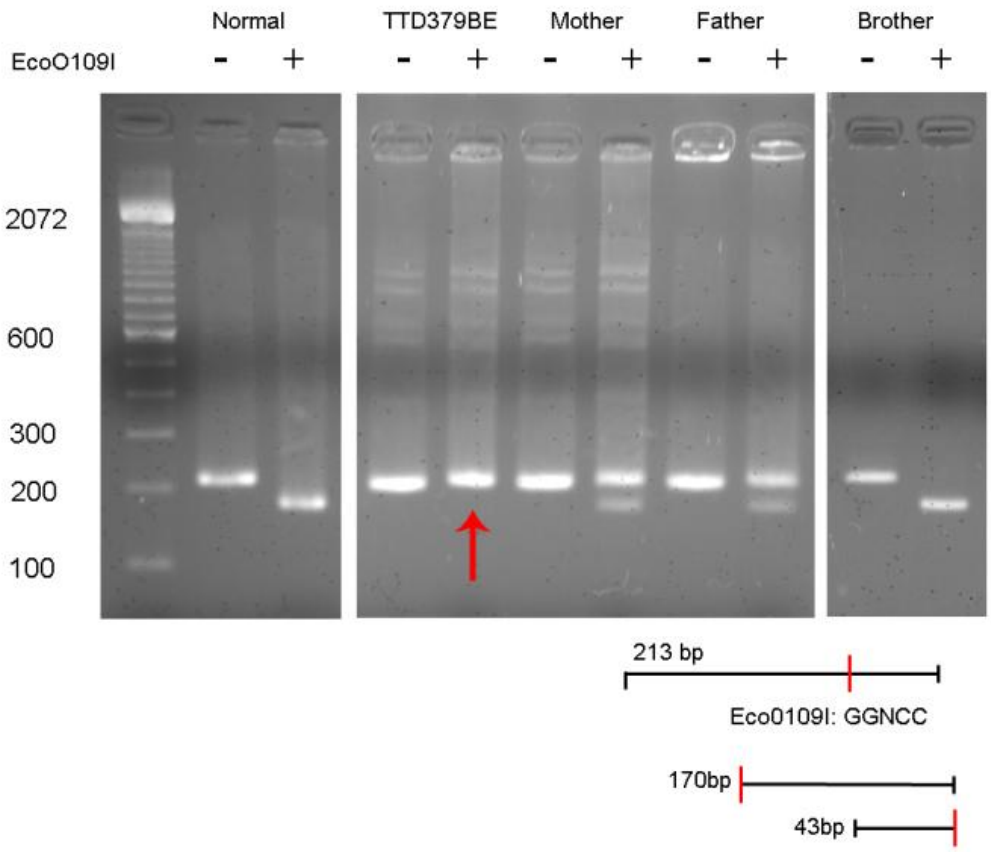

Figure S3. *GTF2E2* PCR and RFLP of genomic DNA from cells of affected boy TTD379BE and family members. The 213 bp region of *GTF2E2* DNA containing a G at cDNA position 448 can be digested with Exo01091, resulting in fragments of 170 bp and 43 bp. The TTD379BE cells (IV-1 in figure 1C) had a c.448G>C mutation and thus was resistant to Eco01091 digestion (arrow). DNA from the parents (III-3 and III-4 in Figure 1C) was heterozygous, resulting in digested and undigested amplifications while DNA from the brother (IV-2 in Figure 1C) was normal.

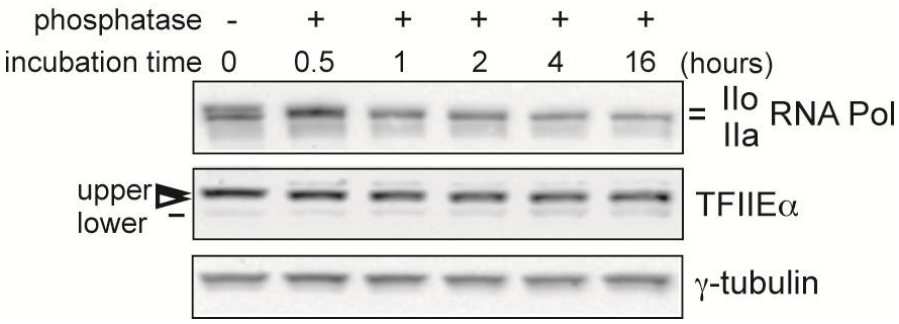

Figure S4. Immunoblot analysis of TFIIEα in the cell lysate from normal C3PV fibroblasts after phosphatase treatment. The lysate was incubated at 37°C with 3U/μl of calf intestinal phosphatase for the indicated time points. The phosphorylated (black arrowhead) and dephosphorylated (white arrowhead) upper TFIIEα bands are indicated. The phosphorylation status of RNA Pol IIo over the unphosphorylated RNA pol IIa was investigated in parallel. γ-tubulin is the loading control.

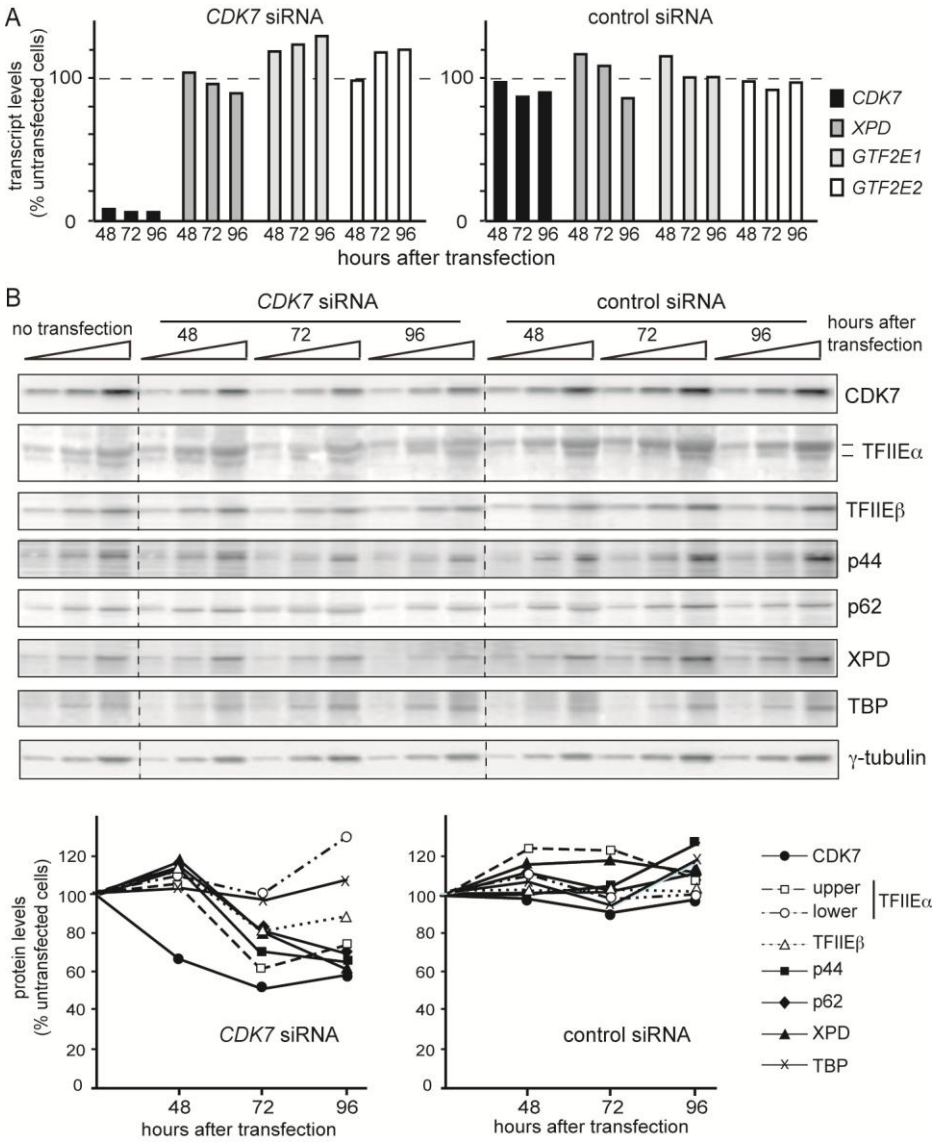

Figure S5. Reduced levels of TFIIE and TFIIH subunits in normal C3PV fibroblasts following *CDK7* silencing. C3PV fibroblasts were transfected with *CDK7* or control siRNA and processed for transcript (A) and protein (B) levels after 48, 72 and 96 hours.

A. *CDK7*, ERCC2 (*XPD)*, *GTF2E1*, and *GTF2E2* transcript levels were normalized to *GAPDH* transcript level and expressed as percentages of the corresponding value in untransfected C3PV fibroblasts. The mean levels of a single experiment done in triplicate are reported. Standard errors of the mean were lower than 10%.

B. Immunoblot analysis of whole cell lysates. The levels of the TFIIE subunits α and β, of the TFIIH subunits CDK7, p44, p62 and XPD and of TBP were normalized to the γ-tubulin content and expressed as

percentages of the corresponding value in untransfected C3PV cells. The mean levels of the three increasing concentrations are reported. Standard errors of the mean were lower than 10%. Statistically significant reductions in the amount of the TFIIEα upper band ($p < 0.005$) and in the amount of TFIIEβ ($p < 0.05$) were observed 72-96 hours after siRNA transfection.

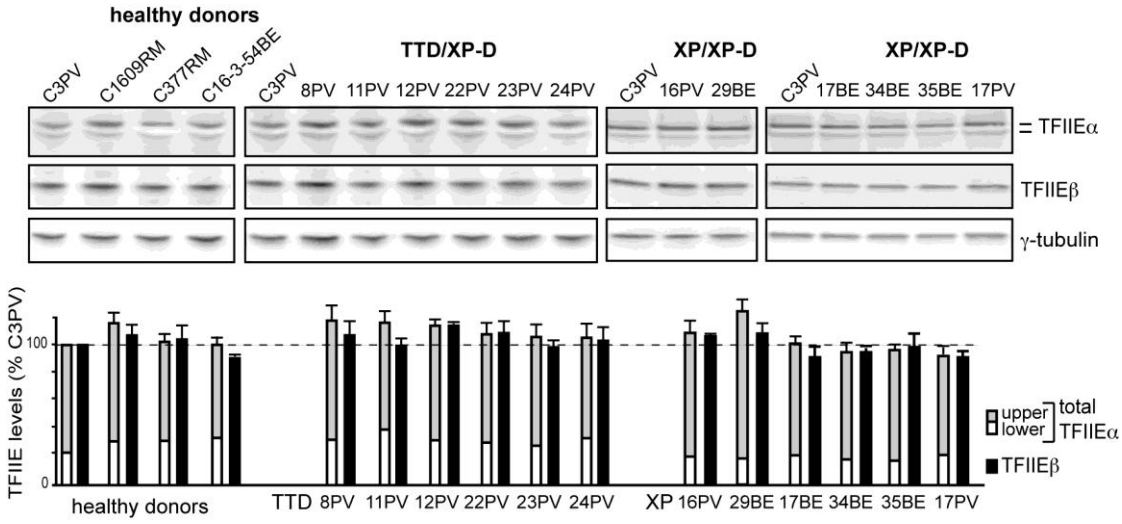

Figure S6. Normal TFIIEα phosphorylation in proliferating TTD/XP-D and XP/XP-D primary fibroblasts. Immunoblot analysis of lysates obtained by directly scraping 4 normal (C3PV, C1609RM, C377RM and C16354BE), 6 TTD/XP-D (TTD8PV, TTD11PV, TTD12PV, TTD22PV, TTD23PV, and TTD24PV) and 6 XP/XP-D (XP16PV, XP29BE, XP17BE, XP34BE, XP35BE, and XP17PV) fibroblast strains in Laemmli buffer. The levels of the upper (grey) and lower (white) forms of TFIIEα and of TFIIEβ (black) were normalized to the γ-tubulin content and expressed as percentages of the corresponding values in C3PV cells. The reported values are the means of at least two independent experiments. Bars indicate the SE.

Table S1. Molecular features of the TTD and XP cells mutated in *ERCC2* (*XPD)* analyzed in this study.

Donor	Phenotype[a]	Mutated alleles[b]	TFIIH levels[c]	References
TTD8PV	TTD	p.Arg112His (m) p.Arg112His (p)	34	6, 7
TTD11PV	TTD	p.Arg112His (m) p.Val121_Glu159del (p)	43	6, 7
TTD12PV	TTD	p.Arg722Trp (m) p.Cys259Tyr (p)	65	6, 7
TTD22PV	TTD	p.Gln662X [p.Glu731Val731fsX100, p.Glu731GlyfsX50, p.Gly731_Gly735delins42]	36	8
TTD23PV	TTD	p.Arg112His Not expressed	60	9, 10
TTD24PV	TTD	p.Arg722Trp (m) [p.Glu317AspfsX110, normal XPD] (p)	55	8
TTD351BE	TTD	p.Arg722Trp (m) p.Arg378His (p)	N.D.[e]	11, 12
XP16PV	XP	p.Arg683Gln (m) p.Arg683Gln (p)	108	7, 13
XP29BE	XP	p.Arg683Trp (m) p.Gln452X (p)	95	14, 15
XP17BE	XP	p.Arg683Trp p.Asp681Asn	80	11, 12
XP34BE[d]	XP	p.Arg683Trp (m) p.199insPP (p)	80	11, 14
XP35BE[d]	XP	p.Arg683Trp (m) p.199insPP (p)	74	11, 14
XP17PV	XP	p.Arg683Trp p.Arg616Pro	81	7, 16

[a]TTD: trichothiodystrophy, XP: xeroderma pigmentosum

[b] in parentheses inheritance of the mutated alleles (m: maternal, p: paternal)

[c] TFIIH levels measured by immunoblot analysis expressed as a percentage of that in normal cells analyzed in parallel

[d] siblings

[e]Not determined

Table S2. cDNA and genomic primers used in this study

Assay[a]	Gene	Position[b]	Primer	Sequence 5'-3'
cDNA PCR	*GTF2E2*	c.-21_-2	GTF2E2-F1	CTGCCCTTCTCACTCAGCAT
		c.492_473	GTF2E2-R1	TCCTAATCCTCGCTGGTCAT
	GTF2E2	c.251_270	GTF2E2-F2	ACATGAAGACACGGCATCAG
		c.+23_+4	GTF2E2-R2	CTGTTCCAGGGCAAAACTGT
	GTF2E1	c.-48_-28	GTF2E1-F1	GTTCCAGGATTCGCCTTGTA
		c.658_639	GTF2E1-R1	GGTCCTTGCTCTGTTTCAGG
	GTF2E1	c.521_540	GTF2E1-F2	GCACACTTTTGGCAAGGTTT
		c.+101_+82	GTF2E1-R2	ACATCAAGAGGGGCAGAAAG
Genomic DNA PCR	*GTF2E2*	50944_50963	GTF2E2-int5F	TGGAGGACCCTATGGTGGTA
		51300_51277	GTF2E2-int6R	TGGCTGTGTTCTAAGAAATGATA
qPCR	*GTF2E2*	c.675_694	GTF2E2-F3	AGATTCCATGGACGAGGAGA
		c.795_776	GTF2E2-R3	CTGTGAAGCAGGCTTTTTCC
		c.366-387	GTF2E2 -F4	GGCTTTAGTCAACAATCCCAAA
		c.532-513	GTF2E2 -R4	AATTGGGCAGTGCTTCTTCT
	GTF2E1	c.798_817	GTF2E1-F3	AGCCTCACTGGAAGGGAAAT
		c.967_948	GTF2E1-R3	GCATGACCTCTTCGTTGTCA
	CDK7	RT2 qPCR primer assay for human cdk7 PP00935E-200 (Qiagen Sciences, Maryland, USA)		
	XPD	c.2087_2108	XPD-F	GGATCCAGGAGCACCTCACAGA
		c.2176_2155	XPD-R	GTGCCATCTGCCGCAGGAAGTA
	GAPDH	c.20_39	GAPDH-F2	GAGTCAACGGATTTGGTCGT
		c.204_185	GAPDH-R2	GACAAGCTTCCCGTTCTCAG
		c.453-473	GAPDH-F	TGCACCACCAACTGCTTAGC
		c.540-519	GAPDH-R	GGCATGGACTGTGGTCATGAG

[a] PCR amplified cDNA and genomic DNA fragments were sequenced using the corresponding F and R PCR primers.

[b] Positions of cDNA PCR primers and qPCR primers refer to the GenBank reference mRNA sequences NM_002095.4 (*GTF2E2*), NM_005513.2 (*GTF2E1*), NM_001799.3 (*CDK7*), NM_000400.3 (*XPD*), NM_002046.3 (*GAPDH*). For cDNA numbering, +1 corresponds to the A of the ATG translation initiation codon in the reference sequence.
Positions of genomic DNA primers refer to the *GTF2E2* GenBank reference genomic sequence NC_000008.10 nt 30436031-30515738.

Table S3. Predicted effect of mutations in GTF2E2 protein

Mutation	PolyPhen[17]	Sift[18]	MutationTaster[19]
A150P	0.835 possibly damaging	0.22 tolerated	0.999 disease causing
D187Y	0.997 probably damaging	0.01 damaging	0.999 disease causing

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