Supplemental materials for

XP-J, a ninth xeroderma pigmentosum complementation group, results from mutations in GTF2H4, encoding TFIIH-p52 subunit

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Supplemental Table 1. Known XP-complementation groups and XP-genes.

XP	HUGO gene name	aliases	TFIIH	relevant references
A	*XPA* (1)	XPA	-	De Weerd-Kastelein *et al.*, *Nat New Biol* (1972) (2)
B (CS/TTD)	*ERCC3* (3)	XPB	XPB	Robbins *et al.*, *Ann Int Med* (1974) (4)
C	*XPC* (5)	XPC	-	De Weerd-Kastelein *et al.*, *Nat New Biol* (1972) (2)
D (CS/TTD)	*ERCC2* (6)	XPD	XPD	Robbins *et al.*, *Ann Int Med* (1974) (4)
E	*DDB2* (7)	XPE	-	De Weerd-Kastelein *et al.*, *Mutation Res* (1974) (8)
F (CS/FA/XFE/XP-CS-FA)	*ERCC4* (9)	XPF	-	Arase *et al.*, *Mutation Res* (1979) (10)
G	*ERCC5* (11)	XPG	(XPG)	Keijzer *et al.*, *Mutation Res* (1979) (12)
H/I	-	-	-	Withdrawn (Vermeulen *et al.*, 1991 (13); Bootsma *et al.* 1989(14))
J	*GTF2H4*	XPJ	p52	**this study**
(XP-CS/COFS/others)	*ERCC1*	ERCC1	-	Kashiyama *et al.*, *AmJHG* (2013) (15)
variant	*POLH* (16)	polη	-	Burk *et al.*, J Lab Clin Med (1971) (17)
TTD-A	*GTF2H5*	TTDA	p8	Giglia-Mari *et al.*, *Nature Genetics* (2004) (18)

Case Report

XP140BR: We report a 6-year-old girl diagnosed with XP, presenting with severe photosensitivity, progressive exposed-site lentigines, microcephaly and mild developmental delay (**Figures 1**). The pregnancy was complicated by intrauterine growth restriction and she was born at 37 weeks via caesarean section with low birth weight of 2.43 kg. Her mother had experienced two prior miscarriages.

Her skin was normal at birth. At three months of age, the patient experienced severe and exaggerated sunburn on a cloudy day in November whilst on holiday in Spain. This took about a week to resolve. She had further episodes of severe sunburn in the UK affecting all exposed sites, following 10 minutes of sun exposure, despite wearing a hat and sunscreen SPF50. On one occasion, she blistered and was admitted to hospital. From about 2 years of age, subtle lentigines were noted at exposed sites. There is no history of skin cancers. Before a diagnosis of XP was made, phototesting revealed a reduced minimum erythema dose (MED) at 305 and 320 nm, with a narrowband UVB MED of < 0.03 J/cm^2, resulting in erythema and blistering (XP sunburn severity score of 3/3 (19)).

Dermatological examination revealed lentigines on her face, upper chest and posterior neck, and to a lesser degree on the dorsal aspect of hands, forearms, and lower legs (**Figures 1a-d**). Evidence of scarring after blistering sunburn on the lower back at the UVB MED site was present 6 months after testing (**Figure 1e**). There was no xerosis or truncal ichthyosis. Her hair and nails

were clinically unremarkable. Examination of multiple thin blonde hair shafts, sampled from various locations on the scalp, did not demonstrate any structural abnormalities and polariscopic examination was unremarkable. Her teeth were normal.

As far as her ophthalmological findings, she denied any photophobia or redness. She was hypermetropic. On examination, there was no enophthalmos and the ocular surfaces were quiet with no interpalpebral pigmentation, corkscrew vessels, pterygia, or pingueculae (XP ophthalmology severity score of 0/12 (20)).

Developmentally, she experienced delays in motor and speech milestones, walking at 20 months and first words at 24 months. She was microcephalic with an occipitofrontal circumference (OFC) under the 0.4th centile. Her weight was on the 83rd centile and her height on the 32nd centile. There were reports of coordination issues, with frequent falls, and she struggled to walk or stand with her feet in tandem. Her IQ fell into the ‘intellectual disability’ range (FSIQ <70; XP neurology severity score of 2/8 (20)).

She had a normal full blood count with no evidence of anaemia or leukopenia. She has not suffered with recurrent infections. The MCV (80 fL) and MCH (27 pg/cell) were also normal (21). An X-ray of the left arm following a fall did not show evidence of delayed bone maturation. Her clinical features were in keeping with XP. She lacked any of the defining features of TTD, in particular tiger-tailed banding under polarizing light microscopy (22). There were no findings in keeping with CS. Further investigations confirmed abnormal

unscheduled DNA synthesis (UDS), indicating defective UV-induced NER. She will be monitored every 6-12 months going forward.

Methods

Sex as a biological variable

We examined one female case with a very rare autosomal recessive genodermatosis in this study. Sex was not considered as a biological variable.

Human studies

The XP-J patient and the parents samples were obtained with a local ethical approval from the Ethics Committee for Human Genome Studies at the Research Institute of Environmental Medicine (RIeM), Nagoya University. See below for details in **Study approval**.

Cell lines and culture

The following cell lines were used in this study: 48BR and 1BR (normal human primary fibroblasts), XP140BR (primary fibroblasts from the XP-J patient), and XP15BR (primary fibroblasts from an XP-A patient) (23-26). All cells were maintained in DMEM (WAKO) supplemented with 10% FCS (Invitrogen) and antibiotics, unless otherwise noted.

Unscheduled DNA synthesis (UDS) assay

Details have been described previously (23, 26). UDS was measured using a fluorescence-based ethynyldeoxyuridine (EdU) incorporation assay. Cells were plated in plastic 96-well plates 24h prior to the experiments. UV-irradiated (20J/m^2 at 254nm UVC) cells were incubated for 4h in medium supplemented with 5 μM 5-ethynyl-2'-deoxyuridine (EdU). Incorporated EdU was detected through fluorescent-azide conjugation (Click-chemistry), and nuclear fluorescence imaging and data processing was automated using the CX7 Imaging System (Thermo Scientific).

Recovery of RNA synthesis (RRS) assay

Details have been described previously (24-26). Cells were plated in plastic 96-well plates 24h prior to the experiments. Cells were UV irradiated (15J/m^2 UVC) and incubated for 18h for RNA synthesis recovery. RRS was measured using a fluorescence-based ethynyluridine (EU) incorporation assay. Recovered cells were incubated for 2h in medium supplemented with 100 μM 5-ethynyluridine. Incorporated EU was detected through fluorescent-azide conjugation and measured using the CX7 Imaging System.

Lentivirus complementation assay

Details have been described previously (25, 26). Virus infection was performed 48h before UDS assays. Human *GTF2H4* cDNA was cloned in-frame with a sequence encoding a C-terminal V5 tag into the pLenti6 vector (Invitrogen) to generate pLenti6/*GTF2H4*-V5. Lentivirus particles were produced in

HEK293FT cells transfected with the *GTF2H4*-encoding plasmid along with ViraPower Packaging Mix (Invitrogen), which includes pLP1, pLP2, and pLP/VSVG, using Lipofectamine 2000 (Invitrogen). Viral supernatants were collected 48h after transfection and concentrated using PEG-it Virus Precipitation Solution (System Biosciences).

Whole genome sequencing

Whole genome sequencing (WGS) was performed in-house as previously described (15, 27). Genomic DNA from the patient was sequenced on the MGI T7 sequencer (MGI, Beijing, China) using a paired-end (PE) flow cell, generating 150 base pair PE reads at 30X coverage. The NGS data were analysed using our standard WGS pipeline. Briefly, raw FASTQ reads underwent quality control and adapter trimming using fastp (28). Cleaned reads were aligned to the human reference genome (GRCh37/hg19) using BWA (v0.7.12-r1039) [https://arxiv.org/abs/1303.3997]. PCR duplicates were removed with Biobambam2 (v2.0.72) [doi:10.1186/1751-0473-9-13]. Local realignment and base quality recalibration were performed using GATK (v3.5) tools: IndelRealigner and BaseRecalibrator. Variants were detected using HaplotypeCaller and annotated with ANNOVAR (29) based on GENCODE release 19 (GRCh37.p13). Variants with MAF > 0.01 were excluded using public databases. Under an autosomal recessive model, candidate genes carrying homozygous or compound heterozygous variants were identified. We evaluated potential causative genes in the patient and identified *GTF2H4* as a candidate

pathogenic gene based on its functional involvement in NER.

Immunoblotting and antibodies

Whole-cell lysates were resolved by SDS-PAGE (5–20% gradient gels), and proteins were transferred to PVDF membranes for immunodetection. The following antibodies were used in this study: mouse monoclonal antibody to p52 (A10, Santa Cruz Biotechnology), mouse monoclonal antibody to XPB/p89 (G10, Santa Cruz Biotechnology), rabbit polyclonal antibody to XPD (A303-658A, Bethyl Laboratories), mouse monoclonal antibody to p62 (G10, Santa Cruz Biotechnology), mouse monoclonal antibody to XPG (8H7, Santa Cruz Biotechnology), rabbit polyclonal antibody to SMC3 (A300-060A, Bethyl Laboratories).

Statistics

All experiments were performed at least in triplicate. Standard errors of the mean (SEM) were calculated for UDS and RRS experiments.

Study approval

This study was approved by the Ethics Committee for Human Genome Studies at the Research Institute of Environmental Medicine (RIeM), Nagoya University (approval no. 337-42). Written informed consent was obtained prior to participation. Photographs of the patient were taken with separate consent for their use, and records of informed consent have been retained.

Data availability

All data and materials used in this study are available from the corresponding author upon reasonable request. Supporting data values for **Figures 1f-1h** are available in the Supplementary Data File (Microsoft Excel format).

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