

Supplemental information

**A DNA repair disorder caused by *de novo*
monoallelic *DDB1* variants is associated with
a neurodevelopmental syndrome**

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SUPPLEMENTAL NOTES

CASE REPORTS

Individual 1

Individual 1 is the oldest of three full siblings born to healthy, non-consanguineous parents of Northern European ancestry. Following a pregnancy that was complicated by fetal growth restriction, she was born at 38 weeks gestation via Cesarean section due to breech positioning, birth weight 2040g (<3rd centile). She was admitted to the neonatal intensive care unit for 10 days due to feeding issues requiring nasogastric tube feeding. A small rectovaginal fistula was treated with rectal dilation. Mild micrognathia was noted.

Brain MRI revealed a structurally unremarkable brain structure with mild dilatation of the third and lateral ventricles. A renal ultrasound was normal. Normal molecular testing has included a peripheral blood karyotype, methylation studies for Prader-Willi syndrome, Fragile-X analysis, subtelomeric FISH analysis, and FISH 22q11 microdeletion. Oligo-based chromosome microarray was normal in 2008. A multi-gene NextGeneration Panel for diagnoses (178 genes) associated with intellectual disability was non-diagnostic in 2016.

Developmental milestones were delayed: she sat at 11 months, began crawling at two years and walked with assistance at 2 years 10 months of age. She used signs and approximately 10 words by age 4 years 6 months. Her motor development was always ahead of her language/speech development. She had ongoing issues with feeding, with sensitivity to textures. She received Early Intervention Services and Specialized Education (functional life skills programming in public school).

Her medical issues have included hypothyroidism; initial myopia with strabismus now with normal vision; constipation; recurrent urinary tract infections; gastroesophageal reflux;

increased cholesterol (diet controlled); recurring otitis media; ADHD, anxiety; overweight/obesity and short stature.

Her length/height were at the 10th percentile until 24 months of age when her height crossed percentiles. It then remained consistently at the 2nd centile. Her weight was less than the 50th centile in infancy, and between the 50-75th centile in childhood until puberty when her weight continued to increase and her height remained stable.

At her most recent evaluation, she was 18 years old and in overall good health. She continued to be treated for hypothyroidism and easily develops middle ear fluid. She attended school in a functional life skills classroom with occupational therapy, physical therapy, and speech therapy. She had a behavioural support staff for 12 hours per week. Height was 149.4 cm (2nd centile) and weight 87.2.kg (97th centile), BMI 39.2 (95-97th centile, obese range).

Examination was notable for being very interactive and expressive, but with few words. She had very full cheeks and a small midface, round facies, deeply set eyes with bilateral epicanthus. The neck was short with a prominent posterior fullness. Brachydactyly of the upper and lower extremities was present with short fourth metacarpals bilaterally. She had a very fair complexion and short, thin and upturned toe nails.

Individual 2

Individual 2 is the youngest of three children to healthy non-consanguineous parents of Ashkenazi Jewish ancestry. She was born at 40 weeks by a spontaneous vaginal delivery. She had neonatal hypoglycaemia and tachypnea and required neonatal intensive care admission for one month, at which time mild hypotonia, dysgenesis of the corpus callosum, mild ventriculomegaly, horseshoe kidney and anterior ectopic anus. She developed significant

hearing loss requiring hearing aides. Development was globally delayed and she did not begin communicating with words until approximately age 3. She began walking independently at age 2.

When assessed aged 6 ½ years, she had ~30 words, nasal speech but excellent receptive language in Russian and English. She was able to dress and undress independently. She had ongoing issues with balance, did not climb structures, but was able to climb stairs with alternate feet with support. She was on prophylactic antibiotics to prevent urinary tract infections.

At 6 ½ years, her head circumference measured 53.5cm (50-98th centile), weight 36.6kg (>98th centile), and height 120cm (50th centile). At age nine years, weight was 52.7kg (>97th centile) and height 137cm (75th centile), BMI 28.2 (95-97th centile, obese range).

She had fair blond hair with noticeably darker eyebrows with synophrys. Her eyes were a striking blue/cobalt color and displayed epicanthus inversus. She had a short nose with small nares and underdeveloped alae nasi, a narrow base and a prominent nasal bridge. The ears were mildly low-set. She had a short neck, a normally shaped chest and slightly prominent fibro-fatty deposition on the lateral dorsal aspect of the torso. The skin overlying this area was noticeably denser. She had a hirsute back with fine vellous hairs. There was significant partial cutaneous syndactyly of the 2nd-3rd toes bilaterally. She had short fingers, clinodactyly of the 5th fingers, proximally placed thumbs and fetal finger pads. She had mild hypotonia but brisk reflexes and hypermobility of the joints.

Individual 3

Individual 3 is the second of two children to healthy unrelated parents of Turkish origin. He was born after an uneventful pregnancy per Cesarean section at 42 weeks' gestation, birth

weight 4.04kg (70th centile), length 54cm (60th centile) and head circumference 37cm (74th centile). Feeding and muscle tone were normal. He was not able to cry loudly. Sitting without support was possible at twelve months and walking at 19 months. Speech was not delayed with first words at ten months. He had recurrent otitis media, he was a quiet and happy baby.

Assessment at age 10 ½ years showed mild motor and mild speech delay with good social skills. He was able to read simple children books, he was able to write his name and can calculate similar to a six-year-old child. He spoke complex sentences in Turkish and German. Weight was > 97th centile, height on the 95th centile and head circumference on the 79th centile, BMI 27.8 (95-97th centile, obese range).

He had a low frontal hairline, thick and slightly bowed eyebrows with synophrys and large ears with large and forward-facing earlobes. His hands were large with tapering fingers.

Individual 4

Individual 4 is the second of three children to healthy unrelated parents of Anglo-Australian background. She was born after an uneventful pregnancy at 42 weeks' gestation, birth weight 3.48kg (70th centile), length 52cm (93rd centile) and head circumference 34.5cm (60th centile). She had significant hypotonia in infancy, sitting at fourteen months and walking at 2 years, three months. Speech was mildly delayed with first words at fifteen months. She had a strabismus for which she wears glasses and audiology showed conductive hearing loss treated with tympanostomy tubes. She had an adenotonsillectomy for obstructive sleep apnoea.

Assessment at age three years showed moderate motor and mild speech delay with good social skills. Weight was on the 14th centile, height on the 15th centile and head circumference on the 10th centile. She had marked facial hypotonia with an open-mouthed expression, fair scalp hair with dark eyebrows which were horizontal, thick and had a medial flare and lateral extension. She had long eyelashes and her palpebral fissures had a lateral extension. She had large fleshy ears and earlobes. She had short toes. She had soft skin. Clinically, a diagnosis of Congenital Disorders of Glycosylation was considered because of the marked hypotonia. Renal ultrasound and echocardiogram were normal. Brain MRI aged 13 months was normal.

Individual 5

Individual 5 is the second child of Armenian non-consanguineous parents. At the age of 9 years she and her family arrived in the Netherlands. Not much information is available on her early development but she was born at a gestational age of 36 weeks with a birth weight of 2900 grams (25-50th centile) and her birth length was 47 cm (50th centile). Neonatal feeding difficulties and jaundice were reported. Assessment at age 13 showed a friendly girl with a mild to moderate psychomotor retardation, truncal obesity, a low anterior hairline, horizontal thick eyebrows, hirsutism, large fleshy ears and earlobes, small hands and feet and her height was below her target height (height -3.6 SDS, target height - 1.55 SD). She was diagnosed with a horseshoe kidney and uterine septum. She underwent nephrectomy of the left side of her horseshoe kidney because of reflux mediated nephropathy, recurrent urinary infections and hypertension. A correction of the urethral meatus and a resection of a vaginal septum were performed. A congenital bicornuate uterus was identified. Clinically, a diagnosis of Cornelia de Lange syndrome was considered based

on her features. Previously performed karyotyping showed a normal karyotype, DNA-diagnostics by Sanger sequencing in Cornelia de Lange syndrome associated genes (*NIPBL*, *SMC1A* and *HDAC8*) showed no abnormalities and a SNP-array showed a normal female profile.

Individual 6

Individual 6 was conceived via IVF and is the first liveborn child to healthy Caucasian parents. Oligohydramnios was noted at 38.3 weeks and he was born via scheduled Cesarean section. He had a NICU stay for 10 days, mainly for temperature instability and glucose instability. He was diagnosed with torticollis while in the NICU. Within a month he had failure to thrive, but growth recovered after being switched to formula. He had gross developmental delay, mild intellectual disability and hypotonia. Mild dysmorphism included low set ears, epicanthal folds with long eyelashes, mild strabismus, small narrow nose, and narrow/tented upper vermillion. Brain MRI showed FLAIR hyperintensities in the pons.

Individual 7

This individual is the first child in a family of Slavic origin. He was born at 41 weeks of gestation by caesarean section due to breech position, birth weight 3180g (-1 SD), length 51 cm (-1 SD), head circumference 35 cm (-0.5 SD) and Apgar score 9/9. First adaptation was normal. He was first time evaluated at 4 ½ months due to hypotonia and suspicion of seizures. Additionally, he had gastro-esophageal reflux and poor weight gain. Abnormal facial phenotype was noticed. He had convergent strabismus, nystagmus. His psychomotor development was delayed. He started to walk at 21 months.

Assessment at 2 ½ years showed moderate developmental and speech delay, and normal growth. His speech corresponded to the age of eleven months. He has remarkably dysmorphic face with deeply set eyes, inner canthal folds, hypotelorism, convergent strabismus, nystagmus, short and upturned nose, hypoplastic alae nasi and full cheeks. His ears were large with simple morphology. His hair and skin were fair. He has 2-3 toe partial syndactyly. Brain MRI at age 5 months revealed mild dilation of lateral ventricles and thin corpus callosum. Renal ultrasound showed mild hydronephrosis in left side. Chromosomal microarray, genomic sequencing panel and extensive metabolic investigations (amino acids, organic acids, very long chain fatty acids, transferrin isoforms, glycosaminoglycans, oligosaccharide, and creatine/guanidinino-acetate) were normal.

Individual 8

Individual 8 was born to healthy unrelated parents of Mexican background. She was seen at 9 months of age at an outreach clinic due to hypotonia, dysmorphic facial features, and developmental delay. There had been an initial concern for Down syndrome, but a postnatal karyotype was 46 XX. On examination she was found to have metopic craniosynostosis, a flat midface, depressed nasal bridge, apparent telecanthus with proptosis and epicanthus inversus, a medial eyebrow flare, and moderate hypotonia. After the initial visit she was lost to follow up.

Figure S1.

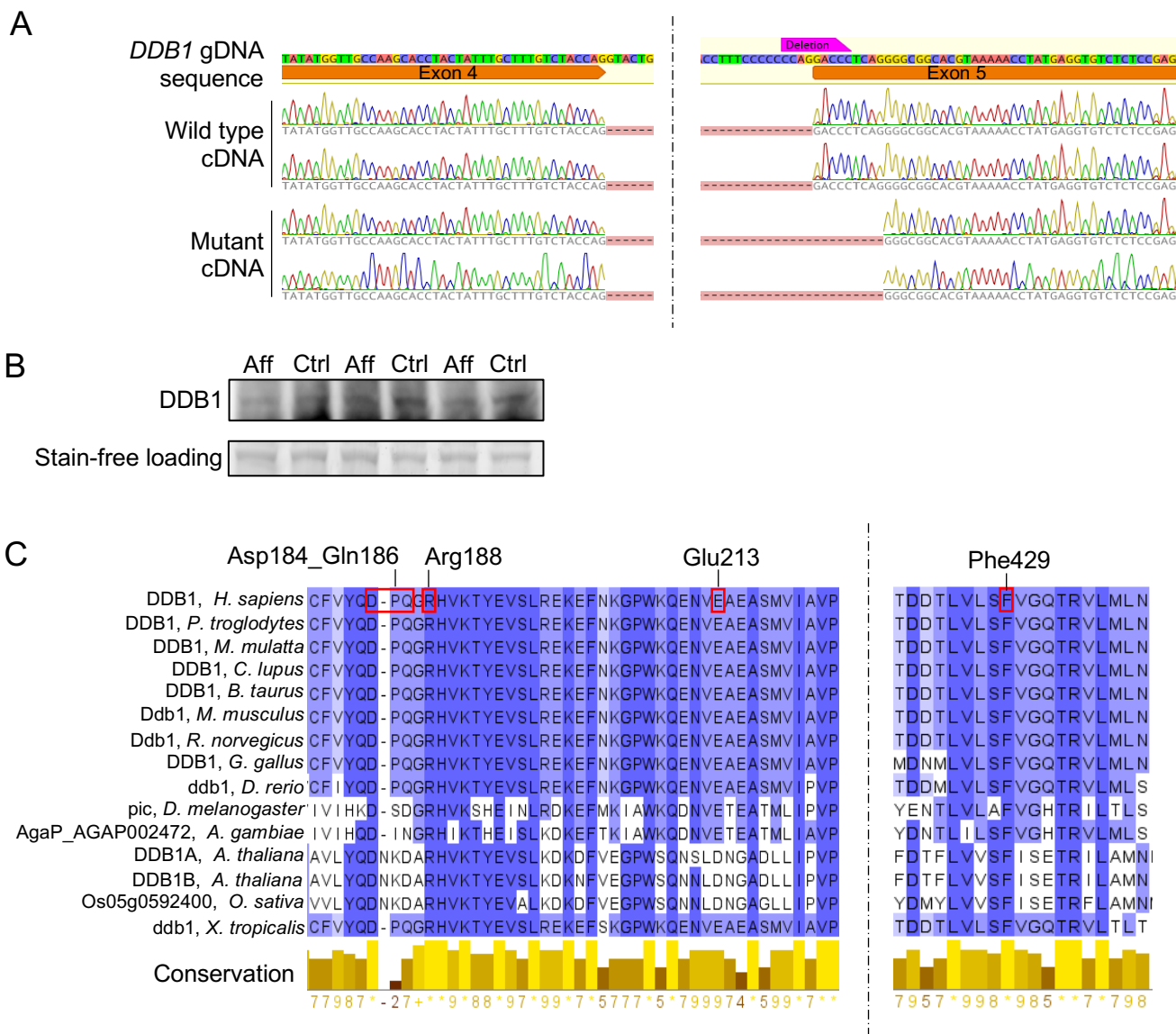


Figure S1. Effect of the DNA deletion in individual 1 on *DDB1* expression and protein sequence conservation of *DDB1* determined by homologous sequence alignment. (A) Sanger sequencing of cloned cDNA from individual 1 revealed a deletion of the first 9 nucleotides of exon 5 within the mutant allele, labelled “Mutant cDNA”. Individual 1 had one allele carrying g.61094361_61094369del, corresponding to 9 nucleotides spanning the intron 4-exon 5 boundary, depicted in the diagram by the pink arrow above the *DDB1* gDNA sequence. (B) Western blot analysis on total extracts from affected individual 1 (“Aff”) and control (“ctrl”) lymphoblast cells to assess total *DDB1* levels, which appear unchanged. (C) Protein alignment of *DDB1* homologues was performed to determine protein sequence conservation and regions of interest are shown. Residues impacted by variants within affected individuals in this study are highlighted in the *H. sapiens* track by red boxes and are labelled above accordingly. Higher scores in the conservation track indicate higher sequence similarity.

Figure S2

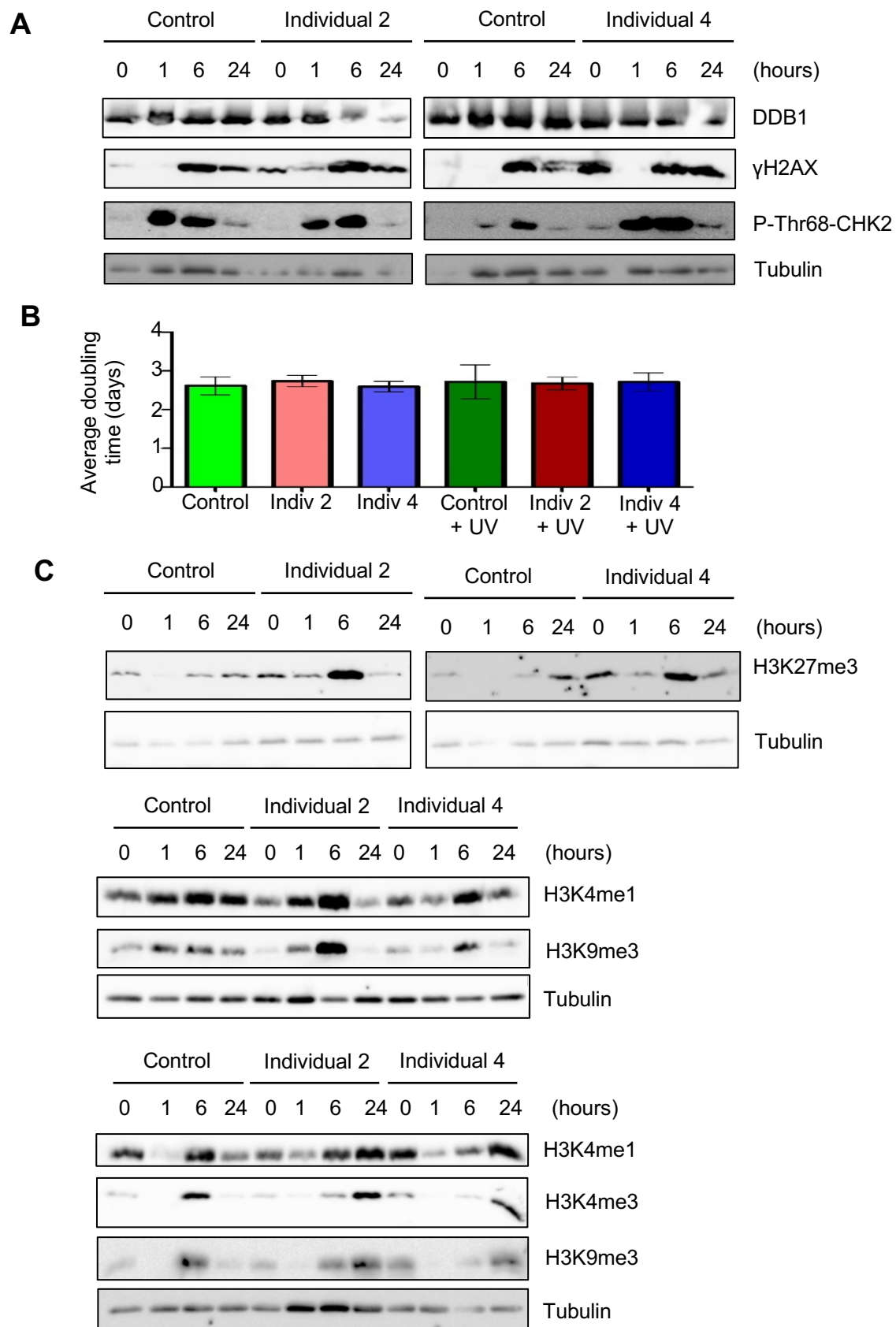


Figure S2. DNA damage signatures, proliferation rate, and histone methylation of lymphoblast cells following UV damage. (A) Another replicate of western blot analysis on total extracts from lymphoblast cells from control and affected individuals, from Fig. 3A. Untreated cells are shown at 0 h, whereas the other time intervals indicate the number of hours following UV exposure. Total DDB1 and the levels of γ H2AX and p-Thr68-CHK2 phosphorylation were assessed: again, DDB1 was found to be unchanged, γ H2AX and p-Thr68-CHK2 levels were induced as expected, p-Thr68-CHK2 generally to a higher level than controls, and γ H2AX increased to a similar level as controls but not elevated for as long after damage. (B) Cell proliferation of control and affected lymphoblast cells was measured by harvesting and counting cells on various days after initial plating, either with or without UV exposure, as depicted in Fig. 3C. For each of the three biological replicates, doubling time of the different cell lines with or without treatment was determined and these values were not determined to be statistically different using one-way ANOVA. Depicted error bars are standard error of the mean. (C) Additional replicates of western blot analysis from Fig. 3D using total extracts from control and affected lymphoblast cells. Untreated cells are shown at the 0 h time point, whereas the other time intervals indicate the number of hours following UV exposure. Levels of various histone H3 methylations were assessed and found to be abnormal in affected cells.

Table S1. Details of genomic sequencing for *DDB1* cohort

| Individual | Capture Kit | Coverage | Sequencer and chemistry |
|--------------|---|--|----------------------------|
| Individual 1 | Proprietary GeneDx | Mean depth of coverage 106X 98.4% sequence reads covered at least 10x | Illumina sequencer |
| Individual 2 | Agilent CRE V1.0 | Average coverage: 121, 154, 158x 96% of bases covered at least 20x | HiSeq 2500 2x100 chemistry |
| Individual 3 | Exome BGI Exome kit 59M | Average coverage: 177, 169 and 181x 97% of bases covered at least 20x | BGISEQ |
| Individual 4 | Illumina TruSeq Customized exome capture (37.5Mb) | Average coverage: 121x, 153x, 122x >95% of bases covered at 20x | Illumina HiSeq X |
| Individual 5 | Agilent exome V6 | Average coverage: 145X, 105X, 133X | Illumina NextSeq 550 |

| | | | |
|--------------|--|---|---|
| | | >96% of bases covered at 20X | |
| Individual 6 | Whole-exome captured with NimbleGen SeqCap EZ v.3.0 rapid or v.4 | Average coverage: 140.15 >97% of bases covered at 20x | Exome sequenced on a HiSeq 2500 or NovaSeq 6000 with the Kapa Biosystem's Library Preparation Kit |
| Individual 7 | SureSelect V7 | Average coverage: Proband: 90x Mother: 119x Father: 103x | NovaSeq 6000, 2x150 paired-end reads |
| Individual 8 | SureSelectV5 | Average coverage: 103x | HiSeq2500 |

Table S2. Additional variants from genomic sequencing considered for each individual. See separate excel file.

Table S3. In-silico predictions for *DDB1* variants

| Variant g. (GRCh37/hg19;chr11) Variant c. (NM_001923.4) Variant p. (NP_001914.3) | Individual | gnomAD v.2.1.1 (allele frequency) | Conservation of residue (considering 100 species) | CADD PHRED score ¹ | PolyPhen-2 score ² | SIFT score ³ |
|--|------------------|--|--|----------------------------------|----------------------------------|-------------------------|
| c.551_559del p.(Asp184_Gln186del) | P1 | absent | yes | N/A | N/A | N/A |
| g.61094353G>A c.562C>T p.(Arg188Trp) | P2 | absent | yes | 34 | 1 | 0 |
| g.61094352C>T c.563G>A p.(Arg188Gln) | P3 | absent | yes | 35 | 1 | 0.005 |
| g.61094278C>T c.637G>A p.(Glu213Lys) | P4, P5, P6 P7 | absent | yes | 34 | 1 | 0.008 |
| g.61083980A>C | P8 | absent | yes | 28.8 | 0.999 | 0.001 |

| | | | | | | | |
|----------------------------|--|--|--|--|--|--|--|
| c.1285T>G p.(Phe429Val) | | | | | | | |
|----------------------------|--|--|--|--|--|--|--|

Table S4. Detailed phenotype information for individuals with *DDB1* variants.

| Individual | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
|--|--|--|--------------------------------|---|---|--------------------------------|--------------------------------|----------------------------|
| Ethnicity | Caucasian | Ashkenazi Jewish | Turkish | Caucasian | Armenian | Caucasian | Caucasian | Mexican |
| Gender | Female | Female | Male | Female | Female | Male | Male | Female |
| Age at presentation | 12 months | 3y 10m | 10y 8m | 9 m | 9y 9m | 6m | 4.5 months | 1y |
| Age at last assessment | 17 years | 9y 2m | 10y 8m | 3y | 13y | 22m | 2 years 11 months | 1y |
| MOLECULAR DATA | | | | | | | | |
| DDB1 variant (g) Hg19 | chr11: 61094361_61094369del | chr11: 61094353G>A | chr11: 61094352C>T | chr11: 61094278C>T c.637G>A p.(Glu213Lys) | | | | chr11:g. 61083980A>C |
| DDB1 variant (c) (NM001923.4) Variant (p) (NP_001914.3) | c.551_559del p.(Asp184_Gln186del) | c.562C>T p.(Arg188Trp) | c.563G>A p.(Arg188Gln) | | | | | c.1285T>G p.(Phe429Val) |
| BIRTH DETAILS | | | | | | | | |
| Birth weight (g, gestation, centile) | 2040 (38/40) <3 rd centile | 2500 (40/40) (3-10 th centile) | 4040 (42/40) (70th centile) | 3480 (42/40) (70th centile) | 2900 (36/40) (25-50 th centile) | 2960 (38/40) (23rd centile) | 3180 (41/40) (12th centile) | NR |
| Birth length (cm, centile) | 47 (10 th centile) | 49 (42nd centile) | 54 (60th centile) | 52 (93rd centile) | 47 (50 th centile) | NR | 51 (72 nd centile) | NR |

| | | | | | | | | |
|--|---|--|-------------------------------|------------------------------|---|---|--|---------------------------|
| | | | | | | | | |
| Birth HC (cm, centile) | | NR | 37 (74th centile) | 34.5 (60th centile) | NR | NR | 35 (31st centile) | NR |
| Neonatal complications | Jaundice. Nasogastric tube feeding for one week | Hypoglycemia & tachypnea at birth, admitted to NICU for 1 month | Nil | Nil | Neonatal feeding difficulties and jaundice | Hypotonia, temperature instability, hypoglycemia, torticollis | nil | Hypotonia |
| Current weight (kg, age of measurement, centile) | 80.3 kg; > 95th percentile (at age 16 years, 9 months) 88 kg @18y2m (97 th centile) | 52.7 @9y 2m (>97th centile) | 52.8 @ 10y 8m (>97th centile) | 12.35 @3y (4th centile) | 45.5 @13y 9m (25-50 th centile) BMI 23,55 (+1.85 SD) | 13.05 @ 22 mo (82nd centile) | 12.9 @2y 5m (36th centile) | NR |
| Current height (cm, centile, age of measurement) | 150.4 cm @ 18y 2m (2nd percentile) | 137 @9y 2m (75th centile) | 138 @ 10y 8m (95th centile) | 90 @ 3y (14th centile) | 139 @13y 9m (<1 st centile) | 87.6 @ 18m (98th centile) | 86.2 @2y 5m (11th centile) | NR |
| Current HC (cm, centile, age of measurement) | NR | 51 @9y 2m (50th centile) | 53 @ 10y 8m (79th centile) | 47 @3y (5th centile) | 52.4 @13y 9m (10-25 th centile) | 48.2 @18m (73rd centile) | 49.7 @2y 5m (25 th centile) | NR |
| Malformations | Anterior anus with rectovaginal fistula | Accessory band across left ventricle of heart Horseshoe kidney Anterior ectopic anus | nil | nil | Horseshoe kidney with left vesico-ureteric reflux, pelvicalyceal dilatation kidney and megaureter | nil | Mild left hydronephrosis | Metopic cranio-synostosis |
| NEUROLOGICAL FEATURES | | | | | | | | |
| Hypotonia | Moderate | Mild | nil | Moderate | Moderate | Moderate | Moderate | Moderate |
| Intellectual disability | Moderate | Moderate DD | Mild (IQ69) | Mild | Mild-moderate | Mild | Moderate DD | Moderate DD |
| Vision | Normal vision | Normal | Normal | Hypermetropia and strabismus | Esotropia, hypermetropia nystagmus | Strabismus | Convergent strabismus and nystagmus | Normal |

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|-----------------------|---|--|---------------|---|--------------------------------|--|--|---|
| Hearing | Normal | Bilateral hearing loss - wears aids | Normal | Conductive hearing loss requiring tympanostomy tubes | Normal | Normal | Normal | Normal |
| Seizures | No | No | No | No | No | Episodes of eye rolling & head movement; no confirmed seizures | No | No |
| Brain MRI | Mild ventriculomegaly | Thinning of posterior body of corpus callosum Non-specific abnormal signal in the genu of corpus callosum Ventriculomegaly | Not performed | Mild delayed myelination | Not performed | Bilateral linear T2 Flair hyperintensities in the central segmental tract of the pons | Mild dilation of lateral ventricles, thin corpus callosum | Not performed |
| CRANIOFACIAL FEATURES | | | | | | | | |
| Eyebrows | Thick, light blonde | Prominent, straight and dark eyebrows with synophrys | Synophrys | Straight, dark, eyebrows with medial flare, lateral extension | Dark, straight, heavy eyebrows | Medial flare | Unremarkable | Medial flare |
| Eyes | Deep-set, upslanting palpebral fissures, epicanthic folds | Long palpebral fissures with lateral extension Epicanthus inversus Long dark eyelashes Striking blue irides | Small eyes | Lateral extension to palpebral fissures | Unremarkable | telecanthus, lateral extension to palpebral fissures epicanthal folds with long eyelashes | Deeply set eyes, inner canthal folds, hypotelorism, convergent strabismus, nystagmus | Apparent telecanthus with proptosis and epicanthus inversus |
| Nose | Short nose in early childhood | Short nose with small nares | Unremarkable | Short | Unremarkable | Small narrow nose with | Small and upturned nose, | Depressed nasal bridge |

| | | | | | | | | |
|---------------------------|---|---|----------------|------------------------------|---|---|-----------------------------------|---------------------|
| | | Hypoplastic alae nasi Narrow base High nasal bridge | | | | narrow alae nasi | hypoplastic alae nasi | |
| Midface | Very round cheeks | Mid-face hypoplasia | Unremarkable | Mid-face hypoplasia | Round face; mild midface hypoplasia; full cheeks | Unremarkable | full cheeks | Mid-face hypoplasia |
| Mouth | Thin upper vermillion border, wide mouth | Unremarkable | Unremarkable | Thin upper vermillion border | Protruding upper lip, thin vermillion border, maxillary overbite, asymmetric occlusion of teeth | Ankyloglossia (underwent frenotomy); retrognathia; narrow, tented lip | Unremarkable | Unremarkable |
| Ears | Large ears with large and long ear lobes | Low-set & large ears with long fleshy lobes | Large earlobes | Large ears with fleshy lobes | Large fleshy ears and earlobes | Low set and fleshy ears | Large ears with simple morphology | Unremarkable |
| Neck | Short neck with excess skin at base of neck (posterior thickening) | Short | Unremarkable | Short | Short | Torticollis | Unremarkable | Unremarkable |
| OTHER PHENOTYPIC FEATURES | | | | | | | | |
| Skin | Fair | Hirsutism on back. Fibro-fatty deposition on the lateral dorsal aspect of the torso with thickened overlying skin | Unremarkable | Soft skin | Hirsutism | Integumentary papules on chin | Mild cutis laxa | Unremarkable |
| Joints | Joint laxity | Hirsutism on back. Fibro-fatty deposition on the lateral dorsal | Unremarkable | Soft skin | Hirsutism | Integumentary papules on chin | Mild cutis laxa | Unremarkable |

| | | | | | | | | |
|----------------|--|---|-----------------------|--|------------|----|----------------------------|----|
| | | aspect of the torso with thickened overlying skin | | | | | | |
| Hands and feet | Very small hands and feet Fourth metacarpal shortening bilaterally, brachydactyly | Significant cutaneous syndactyly of 2-3 toes Brachydactyly Tapering fingers | Short toes | Short toes | Short toes | NR | 2-3 toe partial syndactyly | NR |
| Other | ADHD and anxiety requiring medication Hypothyroidism Recurrent otitis media | Nasal voice with some articulation difficulties Marked truncal obesity | Frequent otitis media | Obstructive sleep apnoea requiring adenotonsillectomy @ 2 y 9m | NR | NR | Gastro-esophageal reflux | NR |

Materials and Methods

Clinical recruitment

Eight independent families presented to Medical Genetics or Child Neurology Services (Portland, USA; Ontario, Canada; Düsseldorf, Germany; Melbourne, Australia; Utrecht, Netherlands; New York, USA; Tartu, Estonia; and Philadelphia, USA) for evaluation of children with apparent syndromic intellectual disability. Each underwent exome or genome sequencing to identify the molecular etiology of their condition. Research protocols were approved in each country via institutional research boards and regional ethics committees, in keeping with national guidelines and the principles laid out in the Declaration of Helsinki and informed consent was obtained from all participants.

Exome sequencing and variant validation

Affected individuals underwent exome sequencing in eight different genomic sequencing laboratories. Genomic DNA was extracted from whole blood from the affected children and their parents. Exome sequencing was performed with a variety of standard capture kits according to manufacturer's instructions. For each family, parentage was confirmed by analysis of inherited variants in the sequencing data. Sanger sequencing was used to validate *DDB1* variants identified by sequencing.

Protein sequence conservation analysis

Homologues of human *DDB1* were identified using the NCBI database HomoloGene.⁴ JalView was then used to generate multiple sequence alignments of these protein

sequences using the MUSCLE algorithm, as well as to determine conservation for each position of the alignment using AMAS scoring.⁵⁻⁷

Real-time PCR

Immortalized lymphoblast cell lines from affected individuals were established from blood samples at The Centre for Applied Genomics (Toronto, Canada). Total RNA was obtained from affected and control lymphoblast cell lines with the RNeasyMinikit (QIAGEN) and reverse transcribed into complementary DNA (cDNA) with iScript kit (BioRad Laboratories) according to manufacturer's instructions. Control reactions without reverse transcriptase were prepared in parallel. cDNA was amplified with gene-specific primers and iQ SYBR Green mastermix under the following conditions: 35 cycles of 95°C for 10 s, 55°C for 20 s, 72°C for 30 s, and a final melting curve generated in increments of 0.5°C per plate read on a CFX96 Touch Real-time PCR Detection System (BioRad Laboratories). Gene expression was quantified using the standard Ct method with CFX software (BioRad Laboratories), and all data corrected against *GAPDH* as an internal control. Primer sequences available upon request.

Splicing analysis

Total RNA was obtained from the affected lymphoblast cell line of interest and reverse transcribed into cDNA as above. cDNA was amplified with GoTaq DNA Polymerase and primers to span specific exons. PCR reactions were then purified using the QIAquick PCR Purification Kit (QIAGEN) before being used for TOPO TA cloning (Thermo Fisher Scientific). Transformed clones were isolated and cultured. Plasmid DNA was isolated using the

QIAprep Spin Miniprep Kit (QIAGEN), which was then used for Sanger sequencing to determine the inserted cDNA sequence.

Western blot analysis

Western blot analysis was conducted to assess protein levels in lymphoblast cells from affected individuals. Cells were lysed in radioimmunoprecipitation assay buffer containing 10 mg/mL each of aprotinin, phenylmethanesulfonyl fluoride, and leupeptin (all from Sigma) for 20 min at 4°C, followed by centrifugation at 13,000xg for 15 min and retrieval of supernatants. Total protein concentrations were determined by Bradford protein assay (BioRad Laboratories). Protein samples were resolved by SDS-PAGE, transferred onto nitrocellulose membrane and incubated in blocking solution [tris-buffered saline (TBS), 5% non-fat milk, 0.05% Tween-20] for 1 h at room temperature followed by overnight incubation with primary antibody at 4°C (DDB1, Abcam ab97522; phospho-histone H2AX (Ser139; γ H2AX), Sigma-Aldrich 05-636; phospho-Chk2 (Thr68), Cell Signaling 2197; β -Tubulin, Abcam ab6046; H3K4me1, Abcam ab8895; H3K4me3, Abcam ab8580; H3K9me3, Abcam ab195497; H3K27me3, Sigma-Aldrich 07-449). Membranes were washed with TBS and 0.1% Tween-20 three times followed by incubation with secondary antibody (HRP conjugated anti-rabbit or anti-mouse; BioRad Laboratories) for 1 h at room temperature. Blots were visualized by autoradiography using the Clarity Western ECL substrate (BioRad Laboratories). When necessary, membranes were stripped using Restore PLUS Western Stripping Buffer (Thermo Scientific) according to the manufacturer's instructions. Control protein was extracted from control cell lines from healthy, unrelated, age-matched individuals. All western blots were conducted in multiple biological replicates and representative images are displayed.

Proliferation assay

Lymphoblast cells were plated at a density of 10^5 cells/mL/well in 24 well dishes. At the appropriate time interval after plating and applicable treatment, cells were harvested and counted using the Countess Automated Cell Counter (Invitrogen). For each of the three biological replicates, the exponential growth nonlinear regression equation was used to determine the doubling time of the different cell lines, which were then compared using one-way ANOVA.

UV treatment

Lymphoblast cells were treated with 75 J/m^2 UV using the FB-UVXL-1000 UV Crosslinker (Fisher Scientific). After the appropriate time intervals following treatment, cells were harvested and used for subsequent analysis.

Facial analysis

An average face was generated while allowing for asymmetry preservation and equal representation by individuals using previously published methods.⁸

Supplemental References

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