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## Supplemental Data

# Mutations in the BAF-Complex Subunit *DPF2* Are Associated with Coffin-Siris Syndrome

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**Supplemental Note: Description of additional CNVs and SNVs**

Microarray analysis in individual 1 showed a *de novo* 126 kb microdeletion in 14q21.3 (chr14:48,787,824-48,913,473) encompassing no known genes. The deletion was also previously identified in an individual of our control cohort, we thus considered it as a likely rare benign CNV. In individuals 2 and 3 were identified paternally inherited copy number aberrations. In individual 2 we detected a 328 kb microdeletion within 7q11.21 (chr7:63,449,550-63,777,276) encompassing no OMIM genes. In the absence of clinical features of the father we interpreted this as a rare benign CNV. Individual 3 had a microduplication of approximately 105 kb within 3p23-p22.3 (chr3:32,119,982-32,224,839) including the *GPD1L* gene (MIM: 611778). While missense variants in this gene have been correlated with Brugada-Syndrome 2 (MIM: 611777),[1] the affected child, as well as the father, did not present with any cardiac anomalies or arrhythmias. Furthermore, no cases of syncope or sudden unexplained death had been reported in the family, we thus also characterized it as non-causative. In individual 5 a missense variant c.3078G>C (p.(Lys1026Asn)) in *ASXL3* (MIM: 615115) gene was found, inherited from the healthy father. This substitution has been observed in ExAC and gnomAD once (rs372219878) and was predicted *in silico* as potentially affecting function. *De novo* mutations in *ASXL3* gene have been described in patients with a novel syndrome with phenotypic similarities with Bohring-Opitz syndrome, characterized by severe psychomotor delay, feeding difficulties, small size at birth and microcephaly.[2] Recently the intellectual disability syndrome due to *ASXL3* mutations was recognized as a distinct clinical phenotype, called Bainbridge-Ropers syndrome (MIM: 615485).[3] Nevertheless, all pathogenic mutations reported until recently are truncating and are located within two evolutionarily conserved regions with phosphorylation sites and a serine-rich motif, located upstream of the identified substitution in individual 5.[2] Taking the aforementioned data together, the missense alteration c.3078G>C (p.(Lys1026Asn)) in *ASXL3* seems to be a rare variant not affecting function and we thus did not consider it as relevant to the phenotype.

Individual 6 further carried a maternally inherited *GDF1* (MIM: 602880) variant c.681C>A (p.(Cys227*)) (rs121434422). This nonsense variant has been described as pathogenic four times in ClinVar database (Variation ID: 6747) in patients with dextro-looped transposition of the great arteries with autosomal dominant inheritance (MIM: 613854), as well as in individuals with autosomal recessive right atrial isomerism (MIM: 208530), a heterotaxy syndrome, including asplenia.[4,5] Although the mother was healthy without any cardiac anomalies and the bicuspid aortic valve as well as the dilated ascending aorta diagnosed in

individual 6 are not the major cardiac manifestations of patients with this mutation, we cannot exclude a possible contribution to the described cardiac anomalies. Yet the remaining clinical features cannot be explained by this aberration. Moreover, microarray analysis revealed a *de novo* duplication of 652 kb (chrX:92,416,787-93,069,211) in Xq21.32, encompassing two genes; *FAM133A* without OMIM phenotype and *NAP1L3* (MIM: 300117), the latter gene being homologous with the genes of nucleosome assembly protein family (NAPs) encoding for brain specific proteins. *NAP1L3* is closely linked to a region (Xq21.3-q22) of genes responsible for X-linked intellectual disability, but to our knowledge neither *NAP1L3* nor other members of NAP family have been implicated in developmental-delay disorders.[6] We characterized this as a variant of unknown significance (VUS), but given that the aberration is a gain, we consider the variant unlikely to have clinical significance. In individual 7, a *de novo*, heterozygous missense variant c.4885C>T (p.(Arg1629Cys)) in the chromatin modifier *SETD1B* (MIM: 611055), a subunit of histone methylotransferase complex, was identified. Microdeletions in 12q24.31 encompassing *SETD1B* have been associated with syndromic intellectual disability. The major clinical phenotype of the patients reported includes moderate to severe intellectual disability, autism, epilepsy, behavioural problems and muscular hypotonia. Labonne et al. reported the smallest 360 kb microdeletion in this region including 6 candidate genes and proposed *SETD1B* as one of the two most compatible candidates to the clinical phenotype.[7,8] *SETD1B* seems not to be a conserved gene (ExAC pLI=0.02 and Z score=1.57), making a pathogenic relevance of the variant c.4885C>T (p.(Arg1629Cys)) unlikely. Also, variants in *SETD1B* or in the other candidate genes in this region have not been associated with disease in humans to date; consequently the underlying mechanism of deletions in this region behind the clinical phenotype remains elusive. Finally, individual 7 presented with borderline ID without evidence of seizures or autistic behaviour which is not consistent with the phenotype described in patients with 12q24.31 microdeletions (Table S2).

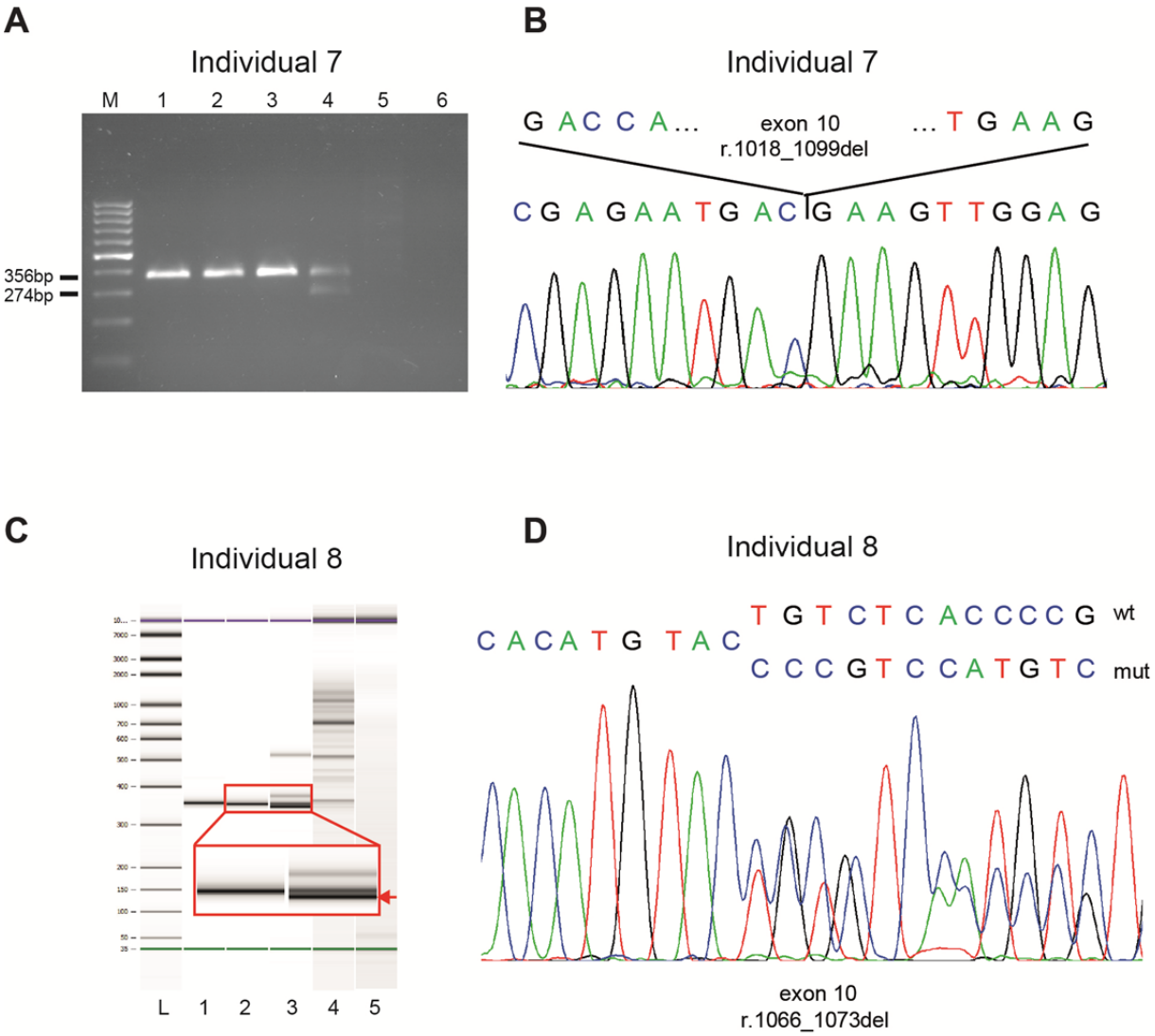


**Figure S1. RT-PCR analysis of *DPF2* in individuals 7 and 8.**

**(A)** Exon spanning RT-PCR on RNA from PAXgene stabilized lymphocytes of individual 7 using primers in exon 9 (DPF2_Exon9_RT_F) and 11 (DPF2_Exon11_RT_R) (Table S4) resulted in an additional aberrant product of 274 bp in individual 7 (lane 4), whereas controls (lanes 1–3) showed only the expected 356 bp fragment (lane 5, genomic DNA control; lane 6, no template control; M, size standard 100 bp).

**(B)** Sequencing electropherogram of RT-PCR amplified aberrant product verifies the skipping of exon 10 (r.1018_1099del).

**(C)** Agilent Bionalyzer electrophoresis analysis of exon spanning RT-PCR on RNA from PAXgene stabilized lymphocytes of individual 8 using primers described in Figure S1A. Note the presence of the additional aberrant transcript of 348 bp in individual 8 (lane 3). Controls (lanes 1–2) showed only the expected 356 bp transcript. Lane 4, genomic DNA control; lane 5, no template control; L, size standard). Enlargement of the two fragments (red outline); the aberrant product is indicated with the red arrow.

(D) Sequencing electropherogram of RT-PCR product from the affected Individual 8 confirms the 8 bp deletion (r.1066_1073del).

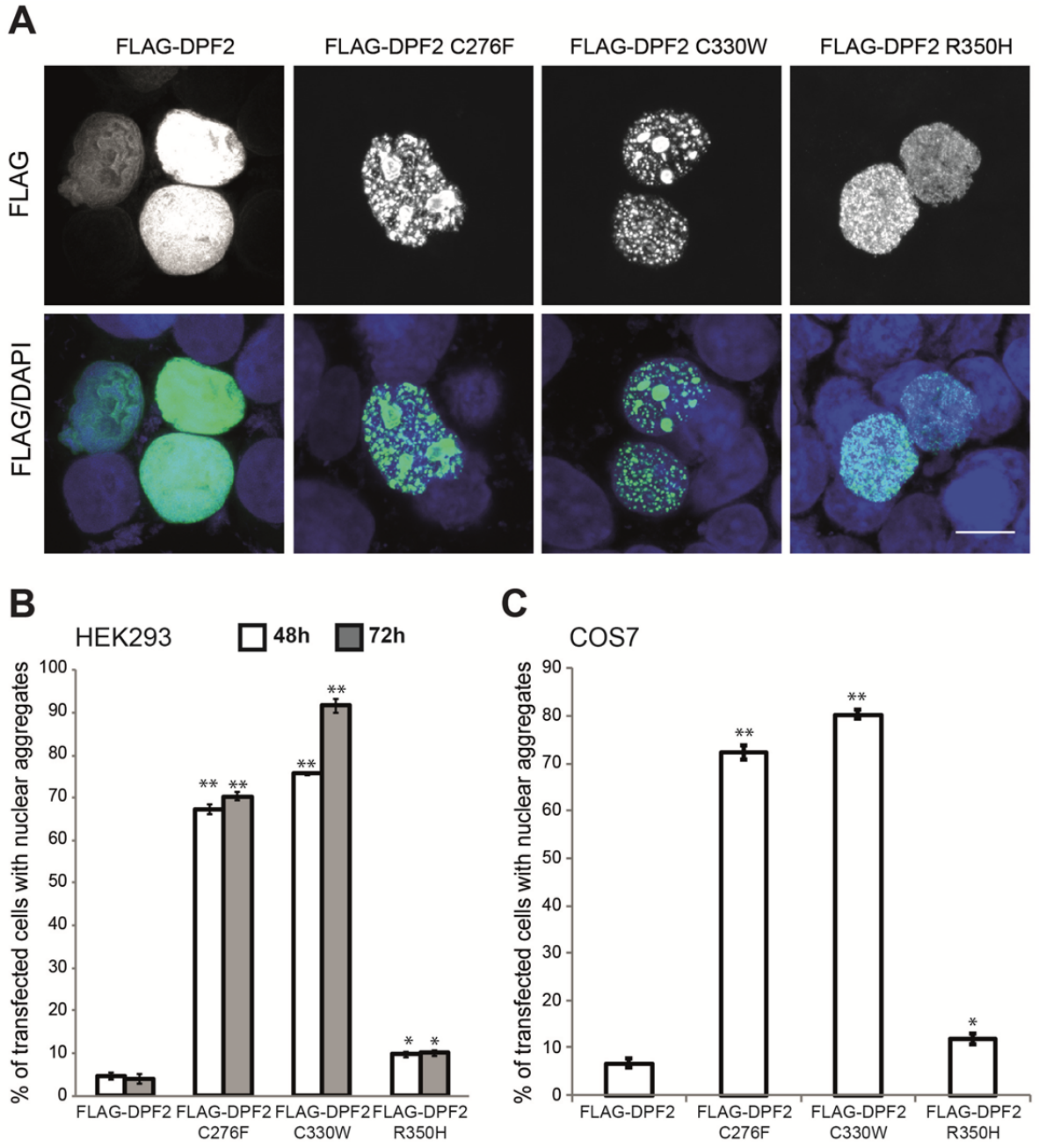


**Figure S2. Validation of aggregate formation in cells transfected with FLAG-tagged DPF2 mutants.**

**(A)** Representative confocal immunofluorescence microscopy images of HEK293 cells expressing FLAG-tagged DPF2 and mutants. Note the formation of nuclear aggregate-like structures in cells transfected with the indicated mutants (columns 2-4) as compared to the wild type DPF2 (column 1). Top panel shows staining with antibody against FLAG and lower panel merged pictures with DAPI. Scale bar 10 µm. FLAG-tagged proteins were generated by subcloning from the pGEX4T1 vector into a pCMV-Tag2b (FLAG) vector via EcoRI/SalI restriction sites. Primers used for cloning and mutagenesis are described in Table S4. Transfection with FLAG-tagged DPF2 plasmids and immunofluorescence were performed as described in Figure 3B, but using Lipofectamine (Invitrogen). Cells were incubated with mouse monoclonal anti-FLAG M2 primary antibody (Sigma-Aldrich) followed by incubation with goat anti-mouse conjugated to Alexa Fluor 488 (Life Technologies).

**(B)** HEK293 cells were examined after 48 and 72 hours post transfection and graphs indicate the percentage of cells exhibiting nuclear aggregates. Error bars represent mean ± S.E.M. and significant values are indicated with *p<0.05, **p<0.01 by unpaired student´s t-test. 100 random cells per experiment, n=3.

(C) Quantification of COS7 cells after 48 hours post transfection with indicated plasmids showing the percentage of cells exhibiting nuclear aggregates. 100 random cells per experiment, n=3.

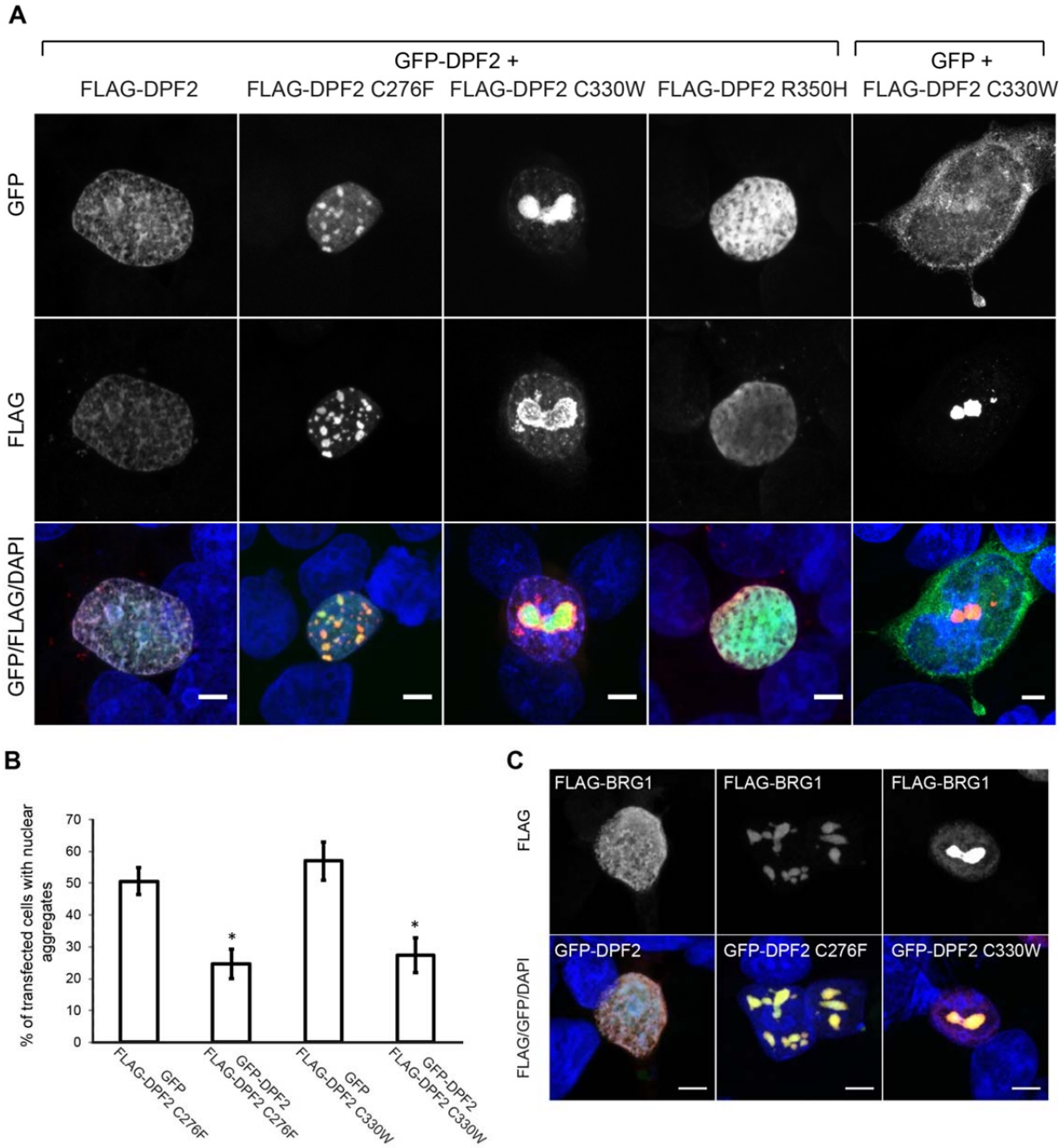


**Figure S3. C276F and C330W mutants recruit wild type DPF2 and BRG1 to the aggregates.**

**(A)** Representative confocal immunofluorescence microscopy images of HEK293 cells co-transfected with GFP-tagged DPF2 (columns 1-4) or empty GFP vector (column 5) together with FLAG-tagged DPF2 and mutants (columns 2-5). Note the recruitment of wild type GFP-DPF2 to the nuclear aggregate-like structures in cells co-transfected with FLAG-tagged mutants (columns 2 and 3). Empty GFP vector is not sequestered to the aggregates (column 5). Top panel shows GFP, middle panel staining with antibody against FLAG (FLAG M2 primary antibody; Sigma-Aldrich) and lower panel merged pictures with GFP/FLAG/DAPI. Scale bar 5 µm.

**(B)** Quantification of experiments conducted in (A) after 48 hours post transfection. Error bars represent mean ± S.E.M. and significant values are indicated with *$p<0.05$ by unpaired student's t-test. 100 random cells per experiment, n=3.

**(C)** Representative confocal immunofluorescence microscopy images of HEK293 cells co-expressing GFP-tagged DPF2 (column 1) and mutants (columns 2-3) together with FLAG-tagged BRG1 (Addgene).[9] Note the co-localization of BRG1 with the indicated mutants in the nuclear aggregates (columns 2-3). Top panel shows staining with antibody against FLAG and lower panel merged pictures with FLAG/GFP/DAPI. Scale bar 5 μm.

Cladogram PHD finger domains

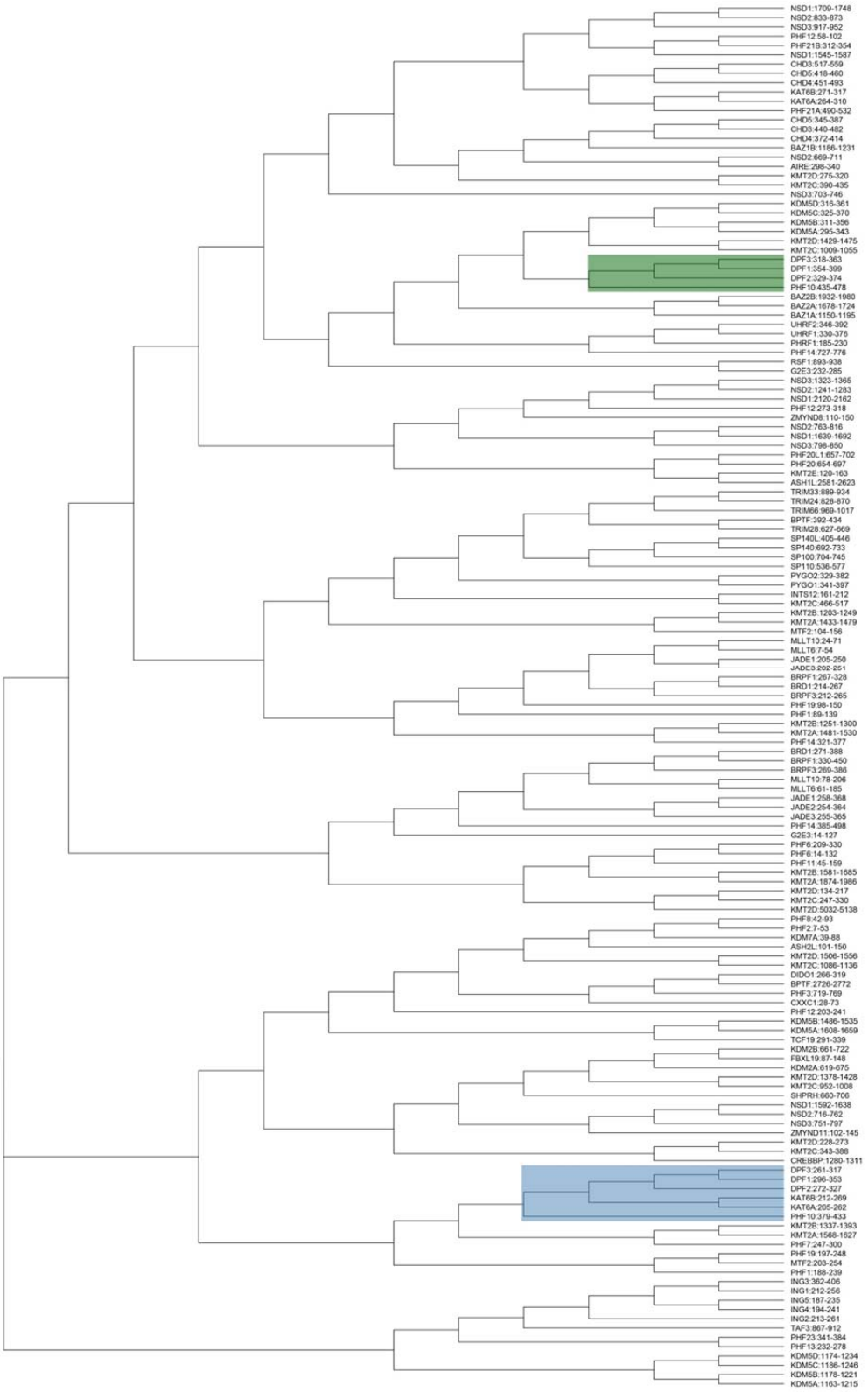

**Figure S4. Cladogram of PHD finger domains used to find DPF2 paralog proteins.**

To find putative human paralog proteins of DPF2 with similar tandem PHD fingers for the multiple amino-acid sequence alignment we compiled a list of known proteins with PHD finger domains (see also Data S1, sheet ´PHD_finger_proteins_(PHF)´) using different sources (see also Data S1, sheet ´data´) and extracted the amino acid sequence of their PHD finger domains based on the NCBI annotations using bedtools v2.26.0.[10] These sequences were then aligned using ClustalW[11] the msa package in R.[12] The cladogram was plotted using the ape package [13] and ggtree package in R.[14] The node for the first tandem PHD finger was marked in blue and the node for the second PHD finger was marked in green. Based on these results and the visual inspection of the protein domain structures the proteins DPF1 (NP_001128627.1), DPF3 (NP_001267471.1), KAT6A (NP_006757.2), KAT6B (NP_036462.2) and PHF10 (NP_060758.2) were selected as putative paralogs of DPF2 (NP_006259.1) and used for multiple sequence alignment (Figure 1D and Figure S5B).

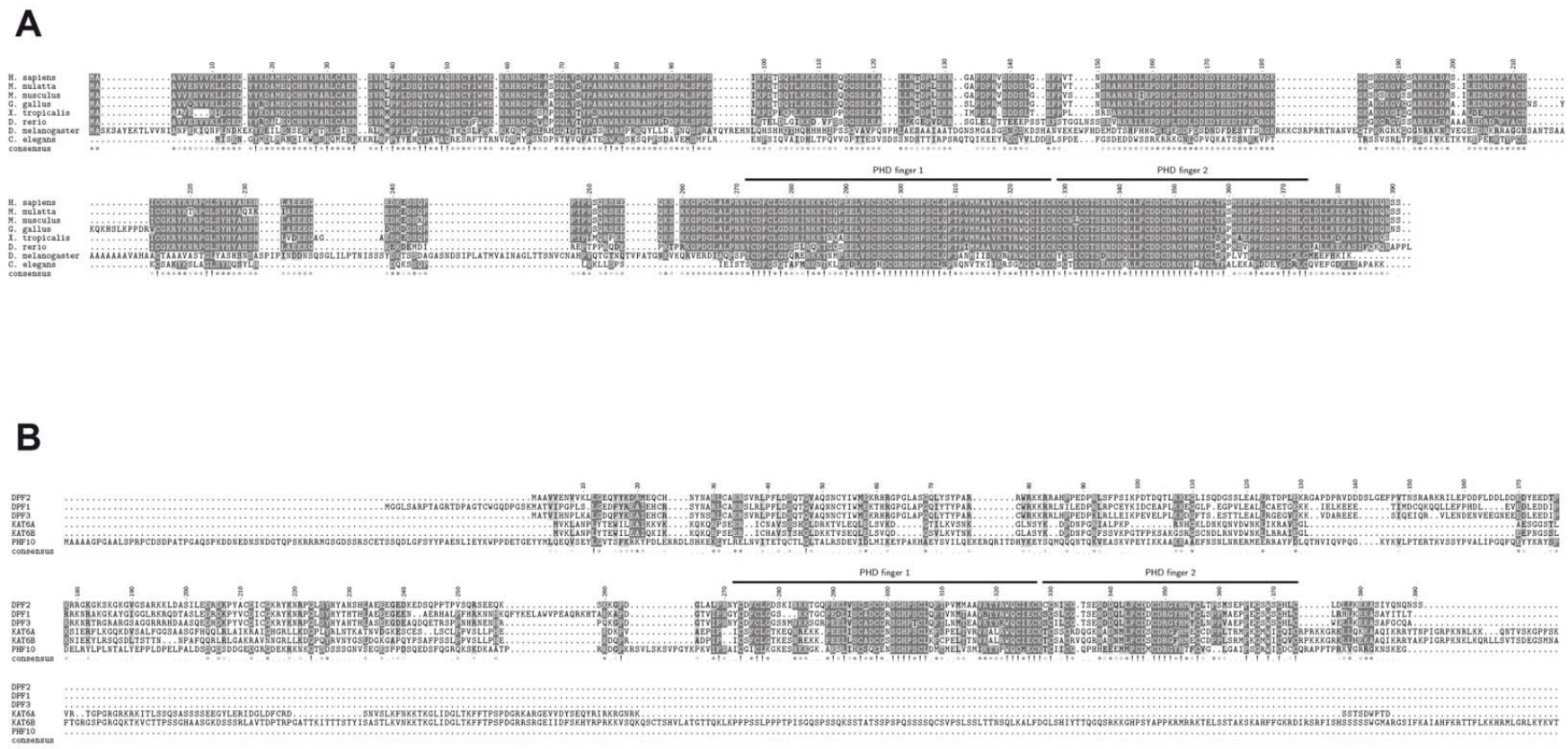


**Figure S5. Full multiple sequence alignment of DPF2 orthologs and paralogs.**

**(A)** Full multiple sequence alignment of DPF2 orthologs. Protein sequences were obtained from the NCBI (see Data S1, sheet ´DPF2_orthologs´) and ClustalW[11] from the msa package[12] in R was used for alignment. **(B)** Full multiple amino-acid sequence alignment of DPF2 and putative human paralog proteins with similar tandem PHD fingers. Protein sequences were obtained and alignment was performed as described above (see Data S1, sheet ´PHD_finger_proteins_(PHF)´)

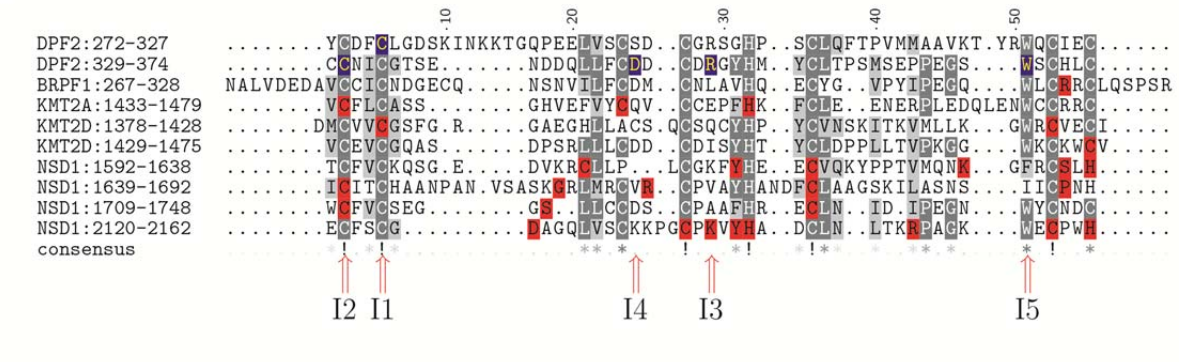


**Figure S6. Multiple sequence alignment of PHD finger domains from intellectual disability associated genes with (likely) pathogenic missense variants from ClinVar**

Protein sequences were obtained from the NCBI while missense variants of class 4 or 5 in genes associated with an intellectual disability phenotype in OMIM containing a PHD finger domain were obtained from ClinVar on 2017-11-26 (see Data S1, sheet ´PHD_finger_proteins_(PHF)´). Only missense variants in canonic PHD fingers (PHD finger 1-6 in sheet ´PHD_finger_proteins_(PHF)´) were considered. ClustalW[11] from the msa package in R[12] was used for alignment. The positions with de novo missense variants in *DPF2* in the 5 individuals (I1-I5) are indicated with a red arrow. The homologous amino acid position affected by the variant c.827G>T (p.(Cys276Phe)) in the first PHD finger (272..327) of DPF2 (I1) is also affected by the c.4148G>A (p.(Cys1383Tyr)) variant (ClinVar Variation ID: 419661) in the PHD finger 3 (1378..1428) of KMT2D. The homologous amino acid position affected by the variant c.990C>G (p.(Cys330Trp)) in the second PHD finger (329..374) of DPF2 (I2) is also affected by the c.4301G>C (p.(Cys1434Ser)) variant (ClinVar Variation ID: 429266) in the PHD finger 1 (1433..1479) of KMT2A and by the c.4918T>A (p.(Cys1640Ser)) and c.5129G>T (p.(Cys1710Phe)) variants (ClinVar Variation IDs: 235776, 373004) in the PHD finger 3 and PHD finger 4 (1639..1692; 1709..1748) of NSD1. The homologous amino acid position affected by the variant c.1049G>A (p.(Arg350His)) in the second PHD finger (329..374) of DPF2 (I3) is also affected by the c.6418A>G (p.(Lys2140Glu)) variant (ClinVar Variation ID: 159418) in the PHD finger 5 (2120..2162) of NSD1. Additionally other variants in these genes affect highly conserved amino acid positions in the Cys4-His-Cys3 (C4HC3) architecture.

## Table S1. Detailed description of clinical and genetic findings in individuals with *de novo DPF2* variants

| | Individual 1 | Individual 2 | Individual 3 | Individual 4 | Individual 5 | Individual 6 | Individual 7 | Individual 8 |
|---|---|---|---|---|---|---|---|---|
| ***DPF2* variant (NM_006268.4)** | c.827G>T p.(Cys276Phe) | c.990C>G p.(Cys330Trp) | c.1049G>A p.(Arg350His) | c.1037A>G p.(Asp346Gly) | c.1105T>C p.(Trp369Arg) | c.904+1G>T p.? | c.1099+1G>A p.Asp340Glufs*12 | c.1066_1073del p.Cys356Profs*5 |
| **Exon** | 8 | 9 | 10 | 10 | 11 | intron 8 | intron 10 | 10 |
| **Genomic position (hg19)** | chr11:g.65113452G>T | chr11:g.65113803C>G | chr11:g.65116352G>A | chr11:g.65116340A>G | chr11:g.65119159T>C | chr11:g.65113530G>T | chr11:g.65116403G>A | chr11:g.65116369_65116376del |
| **Localization** | PHD1 | PHD2 | PHD2 | PHD2 | PHD2 | PHD1 | PHD2 | PHD2 |
| **Inheritance** | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* | *de novo* |
| **Sex** | male | female | female | male | male | female | female | male |
| **Family history** | negative | negative | negative | negative | negative | negative | negative | younger maternal half-brother with *de novo* microdeletion 22q11 |
| **Abnormalities during pregnancy** | - | - | - | - | - | mild polyhydramnios | oligohydramnios, singular umbilical artery, elevated nuchal translucency | - |
| **Birth parameters (length, weight, OFC)** | 18th ct, 12th ct, 25th ct | 16th ct, 9th ct, 29th ct | NA, NA, NA | NA, NA, 47th ct | NA, 92th ct, NA | 3rd ct, 13th ct, large (precise size not known) | 11th ct, 9th ct, 7th ct | 10-25th ct, 10th ct, 50th ct |
| **Age at last clinical assessment** | 10 years | 16 years | 18 years, 6 months | NA | 15 years | 3 years, 2 months | 7 years, 5 months | 3 years, 9 months |
| Height | < 3rd ct | 50th ct (< 3rd ct until puberty) | 20-50th ct | < 3rd ct | < 0,4th ct | 11th ct | 15th ct | < 3 ct |
| Weight | < 3rd ct | < 3rd ct | 50-75th ct | < 3rd ct | < 0,4th ct | 52th ct | 39th ct | NA |
| OFC | 50th ct | 50th ct | - | >97th ct | 50th ct | >97th ct | 96th ct | 50th ct |
| **Brain MRI scan** | NA | right cerebellar hemisphere atrophy | NA | NA | NA | small pituitary gland | Arnold-Chiari malformation I | NA |
| **Development** | | | | | | | | |
| Developmental delay | global | moderate global | moderate global | mild | global | mild/moderate | mild global | global |
| Cognition status | mild ID | moderate ID | moderate ID | borderline ID | moderate ID | precise estimation not possible (the patient is too young) | borderline ID | mild ID |
| First words | 48 months | 36 months | 48 months | 36 months | 24 months | ~24 months | 33 months | 18 months (mama)- no other words for many months |
| First sentences | 84 months | 53 months | 60 months | NA | NA | not speaking in sentences yet | NA | 36 months |
| Age of walking | 17 months | 24 months | 23 months | 22 months | 18 months | 17 months | 23 months | 21 months |
| Behavioral anomalies | stereotypes | - | temper tantrums, hyperactivity, obsessive compulsive behavior. poor sleep pattern, stereotypic movements of hands | - | fixations, temper tantrums, competitive behavior, autism | - | - | - |
| **Feeding problems** | + | + | + | - | + | - | - | + |
| Beginning of feeding problems | at 6 months | infancy | NA | | infancy | | | infancy (due to bilateral cleft lip palate) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Duration of feeding problems | 1-2 years | ongoing due to gastro-esophageal reflux | ongoing | | until solid food introduced | | | ongoing |
| **Muscular hypotonia** | + | - | - | NA | - | + | + | + |
| **Hearing loss** | - | + (high frequency) | + ( congenital,severe left-sided) | + (mild conductive) | - | - | - | + |
| **Vision problems** | hypermetropia, photophobia | unilateral intermittent strabismus | - | - | strabismus, hypermetropia | - | myopia | - |
| **Craniofacial anomalies** | | | | | | | | |
| Prominent forehead | + | - | - | + | - | + | + | + |
| Thick eyebrows | + | - | + | + | - | NA | - | - |
| Long eyelashes | + | - | + | - | - | NA | + | + |
| Hypertelorism | - | - | - | + | - | - | + | - |
| Epicanthic fold | - | - | - | - | - | NA | + | + |
| Down-slanting palpebral fissures | + | + | + | + | + | NA | - | + |
| Flat nasal bringe | - | - | - | - | - | + | + | + |
| Broad nose | - | - | + | + | beaked nose | - | + | + |
| Upturned nasal tip | - | - | - | + | - | - | - | - |
| Thick alae nasi | + | - | + | + | small alae nasi | - | + | + |
| Broad philtrum | - | - | + | + | - | - | - | + |
| Short philtrum | + | + | - | - | - | + | - | + |
| Thin upper lip | - | + | - | + | + | - | - | + |
| Thin upper vermillion | - | - | - | + | - | - | - | - |
| Thick lower vermillion | + | - | + | - | - | - | + | + |
| Wide mouth | + | - | - | + | - | - | + | + |
| Downturned mouth | - | - | - | - | - | - | + | - |
| Macrotia | + | + | + | - | - | - | - | - |
| Other ear anomalies | - | low-set and/or posteriorly rotated ears | stenosis of external auditory canal | low-set and/or posteriorly rotated ears | prominent, cup-shaped, low-set ears and abnormality of the pinna | creased earlobes with overfolded helices | - | prominent, low-set and/or posteriorly rotated ears |
| **Skeletal anomalies** | | | | | | | | |
| Craniosynostosis | - | - | - | sagittal | sagittal | - | trigonocephaly (radiographic imaging was not performed) | - |
| Delayed bone age | NA | NA | NA | NA | - | + | + | NA |
| Scoliosis | - | - | - | + | - | - | - | - |
| Pectus excavatum | - | + | - | - | + | - | - | - |
| Brachydactyly | only of 5$^{th}$ finger | - | - | general | only of 5$^{th}$ finger | only of 5$^{th}$ finger | - | general |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Clinodactyly | - | only of 5th finger | - | - | only of 5th finger | - | - | only of 5th finger |
| Complete absent of 5th distal phalanx | - | - | - | possibly | - | - | - | - |
| Joint laxity | + | - | - | + | - | NA | NA | + |
| **Ectodermal anomalies** | | | | | | | | |
| Sparse scalp hair | + | in childhood | relatively | relatively | - | NA | + (and fine hair) | + |
| Hypertrichosis | - | - | + (upper lip, arms, legs and back) | - | - | - | - | - |
| Nail hypoplasia/Aplasia | 4th and 5th toenails | right 5th toenail, small left 5th toenail | right 5th toenail, 4th and 5th fingernails, dysplasia of all nails | all toenails, 5th and index fingernails | 5th toenails, small thickened toenails | all toenails, 5th fingernails | 5th toenails | all toenails, 5th fingernails |
| delayed primary dentition | NA | + | NA | - | - | NA | + | - |
| delayed permanent dentition | NA | - | NA | + | - | NA | + | + |
| Microdontia | + | - | - | - | - | NA | - | + |
| C**ongenital anomalies** | | | | | | | | |
| Intestinal anomalies | - | gastroesophageal reflux (gastrostomy), constipation | severe constipation since infancy | - | severe constipation (colostomy at 3 years and colectomy with ileostomy at 13 years) | constipation | constipation | constipation |
| hernia | - | - | umbilical | inguinal | - | - | - | - |
| Cardiac anomalies | - | mild pulmonary stenosis, PFO | - | VSD | - | bicuspid aortic valve, dilated ascending aorta | aortic valve dysplasia | - |
| Renal anomalies | - | - | - | large kidneys | - | - | - | - |
| Laryngo/Tracheomalacia | - | laryngomalacia | | - | - | - | laryngomalacia | - |
| **Dermatological anomalies** | Cutis marmorata | Cutis marmorata | - | multiple melanocytic naevi, dry skin palms of hands, supernumerary nipple, "bronzed" skin | capillary | capillary hemangioma on forehead, orbital creases | - | neurodermatitis |
| **Recurrent otitis** | + (otitis media) | - | + | NA | - | NA | + (otitis media) | +(otitis media) |
| **Other** | - | low hanging columella, bilateral single palmar creases | prominent fetal fingertip pads, low anterior and posterior hairlines, synophrys | very thick gums, broad thumbs | prognathism, broad thumbs, bilateral undescended testes IGF-1 deficiency | prominent fetal fingertip pads, short broad thumbs, pes planus, decrease of calf muscle mass | - | low anterior hairline, bilateral cleft lip palate, prominent fetal fingertip pads, widely spaced nipples, undescended testes, Perthes disease, iron deficiency, |

**Table S2. Additional CNVs and gene alterations identified in DPF2 individuals**

| | CNVs | Gene variants |
|---|---|---|
| Individual 1 | arr[hg19] 14q21.3(48,787,824-48,913,473)x1 | - |
| Individual 2 | arr[hg19]7q11.21(63,449,550-63,777,276)x1 | - |
| Individual 3 | arr[hg19] 3p23p22.3(32,119,982-32,224,839)x3 | - |
| Individual 4 | - | - |
| Individual 5 | - | NM_030632.2 (*ASXL3*): c.3078G>C (p.(Lys1026Asn)) |
| Individual 6 | arr[hg19] Xq21.32(92,416,787-93,069,211)x3 | NM_001492.5 (*GDF1*): c.681C>A (p.(Cys227*)) |
| Individual 7 | - | NM_015048.1(*SETD1B*): c.4885C>T (p.(Arg1629Cys)) |
| Individual 8 | - | - |

## Table S3: Computational analysis of DPF2 variants

| ID | Individual 1 | Individual 2 | Individual 3 | Individual 4 | Individual 5 | Individual 6 | Individual 7 | Individual 8 |
|---|---|---|---|---|---|---|---|---|
| **CHROM** | chr11:g.65113452 G>T | chr11:g.65113803 C>G | chr11:g.65116352 G>A | chr11:g.65116340 A>G | chr11:g.65119159 T>C | chr11:g.65113530 G>T | chr11:g.65116403 G>A | chr11:g.65116369_65116376del |
| **GENE** | *DPF2* | *DPF2* | *DPF2* | *DPF2* | *DPF2* | *DPF2* | *DPF2* | *DPF2* |
| **FEATUREID** | NM_006268.4 | NM_006268.4 | NM_006268.4 | NM_006268.4 | NM_006268.4 | NM_006268.4 | NM_006268.4 | NM_006268.4 |
| **EFFECT** | missense variant | missense_variant | missense_variant | missense_variant | missense_variant | splice_donor_variant&intron_variant | splice_donor_variant&intron_variant | frameshift_variant |
| **HGVS_C** | c.827G>T | c.990C>G | c.1049G>A | c.1037A>G | c.1105T>C | c.904+1G>T | c.1099+1G>A | c.1066_1073del |
| **HGVS_P** | p.(Cys276Phe) | p.(Cys330Trp) | p.(Arg350His) | p.(Asp346Gly) | p.(Trp369Arg) | p.? | p.Asp340Glufs*12 | p.Cys356Profs*5 |
| **gnomadr201_AC** | na | na | na | na | na | na | na | na |
| **Cosmicv81_CNT** | na | na | 1 | na | na | na | na | na |
| **CADDv1.3_phred** | 31.0 | 27.5 | 35.0 | 34.0 | 27.2 | 26.1 | 28.2 | 35.0 |
| **M_CAP_score** | 0.421 | 0.956 | 0.169 | 0.456 | 0.602 | na | na | na |
| **REVEL_score** | 0.525 | 0.876 | 0.736 | 0.853 | 0.83 | na | na | na |
| **SIFT_score** | 0.378 | 0.0 | 0.0 | 0.004 | 0.0 | na | na | na |
| **Polyphen2_HVAR_score** | 0.0 | 0.999 | 0.991 | 0.989 | 0.992 | na | na | na |
| **MutationTaster_score** | 1.0 | 1.0 | 1.0 | 1.0 | 0.999 | 1.0 | 1.0 | na |
| **spidex_dpsi_zscore** | 0.817 | 1.505 | -1.061 | -0.554 | na | -3.177 | -3.038 | na |
| **dbscSNV_ada_score** | na | na | na | na | na | 0.999 | 0.999 | na |
| **dbscSNV_rf_score** | na | na | na | na | na | 0.926 | 0.934 | na |

## Table S4. Cloning, mutagenesis and RT-PCR primers

| | |
|---|---|
| DPF2_pEGFP_EcoRI_F | 5´-CTCAAGCTTCGAATTGCGGCTGTGGTGGAGAAT -3´ |
| DPF2_pEGFP_EcoRI_R | 5´-GTCGACTGCAGAATTTCAAGAGGAGTTCTGGTTCTGGTA-3´ |
| DPF2_pGEX4T1_BglII_F | 5´-GGGCCCAGATCTTGGCGGCTGTGGTGGAGAATGTAG-3´ |
| DPF2_pGEX4T1_ pCMV-Tag2b_SalI_R | 5´-GGGCCCGTCGACTCAAGAGGAGTTCTGGTTCTG-3´ |
| DPF2_pCMV-Tag2b_EcoRI_F | 5´-GGGCCCGAATTCGCGGCTGTGGTGGAGAATGTAG-3´ |
| DPF2_pGEX4T1_ pCMV-Tag2b_SalI_R | 5´-GGGCCCGTCGACTCAAGAGGAGTTCTGGTTCTG-3´ |
| DPF2_C276F_F | 5´-CAACTACTGTGACTTCTTCCTGGGGGACTCAAAG-3´ |
| DPF2_C276F_R | 5´-CTTTGAGTCCCCCAGGAAGAAGTCACAGTAGTTG-3´ |
| DPF2_C330W_F | 5´-CGAGTGCAAATGTTGGAATATCTGCGGCAC-3' |
| DPF2_C330W_R | 5´-GTGCCGCAGATATTCCAACATTTGCACTCG-3' |
| DPF2_R350H_F | 5´-GTGATGACTGCGATCATGGCTACCACATGTAC-3´ |
| DPF2_R350H_R | 5´-GTACATGTGGTAGCCATGATCGCAGTCATCAC-3´ |
| DPF2_Exon9_RT_F | 5´-CAGTGCATCGAGTGCAAATGTTG-3´ |
| DPF2_Exon11_RT_R | 5´-CAGCTGCCAAAGGCAGGCTGGC-3´ |

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