The American Journal of Human Genetics, Volume *105*

Supplemental Data

Mutations in *PIGB* Cause an Inherited GPI Biosynthesis Defect with an Axonal Neuropathy and Metabolic Abnormality in Severe Cases

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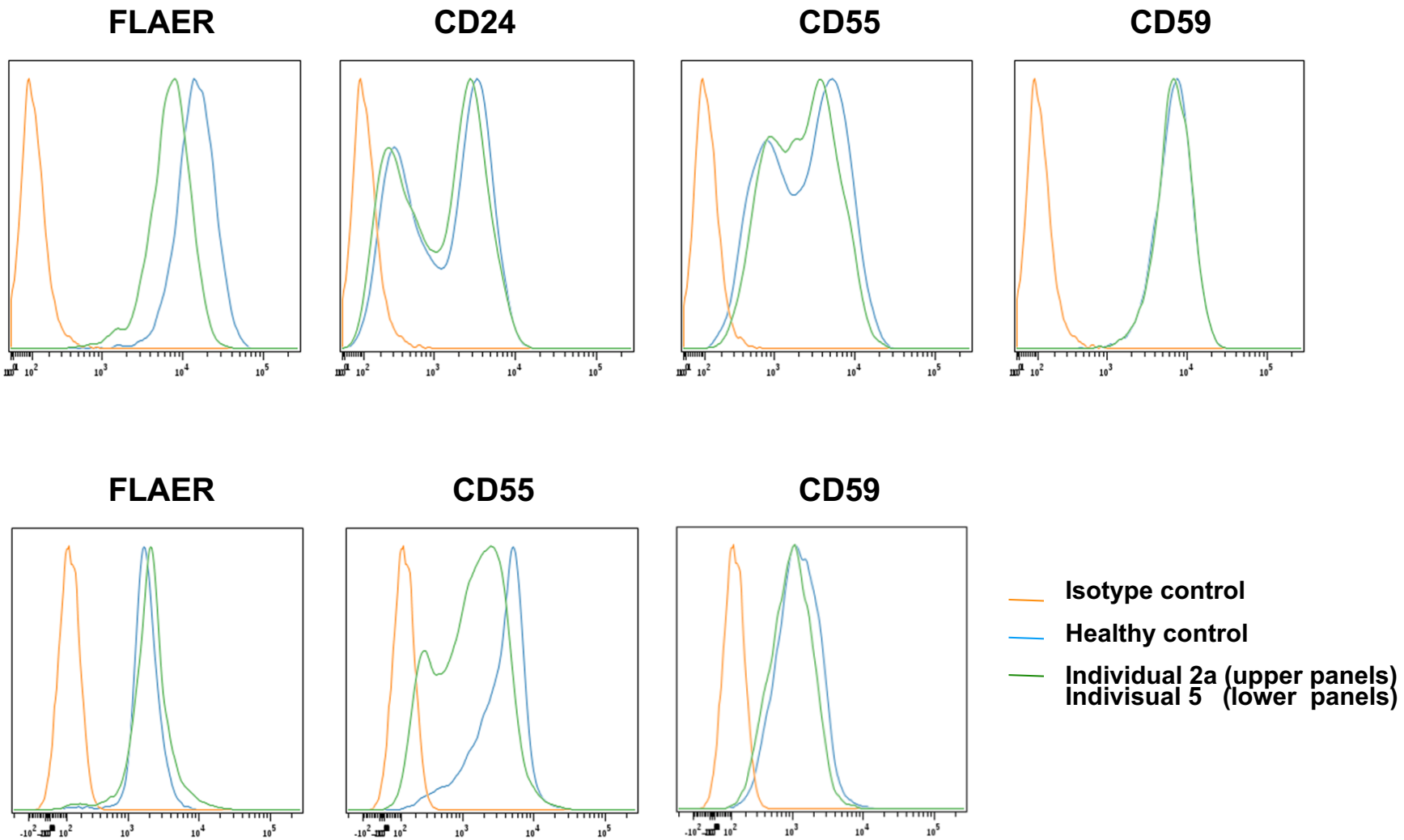

Figure S1. Flow cytometry analysis of cell surface GPI-APs of lymphoblastoids and lymphocytes of individual 2a and 5, respectively. : Upper panels, LCLs of individual 2a established by Epstein-Barr virus immortalization of peripheral blood mononuclear cells (PMBC) were stained with GPI-AP markers (FLAER, CD24, CD55 and CD59). Lower panels, lymphocyte population from blood sample of individual 5 in the same experiments shown in Figure 5B was gated to analyze using the Cytobank software. The figure shows representative results from experiments done in triplicate.

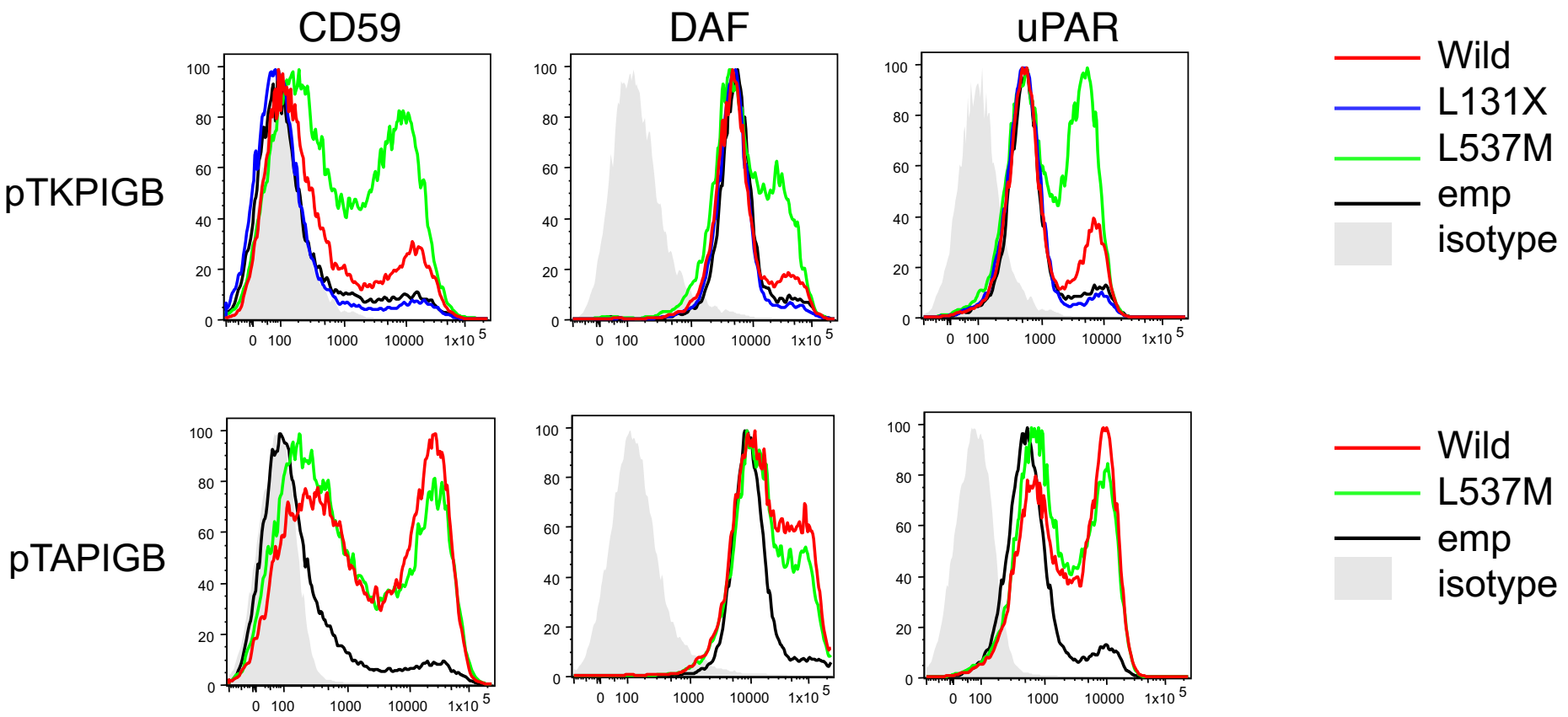

Figure S2. Functional analysis of the mutant *PIGB* cDNAs found in family 5 using weak promoters:
PIGB-deficient CHO cells were transiently transfected with wild-type and mutant *PIGB* cDNAs subcloned in pTK (medium strong promoter) (upper panels) and in pTA (weak promotor) (lower panels). Restoration of the surface expression of CD59, CD55 (DAF) and uPAR was assessed 2 days later by flow cytometry. Black lines, empty vector; green and blue lines, various mutant PIGB; red line, wild-type PIGB; light-gray shadows, isotype controls.

Table S1. Detailed phenotypes

Family	1		2		3	4	5	6		7	8	9	10	
Individual	1a	1b	2a	2b	3	4	5	6a	6b	7	8a	9b	10a	10b
Consanguinity	N	N	N	N	Y,	N	N	Y	Y	Y	Y	Y	N	N
Gender	M	F	F	M	F	F	F	F	F	F	M	M	M	M
Age at last examination	5y	3.8y	13y	11m	6m	4y	19y	4m	8m	8m	2d (deceased)	18m	6 m	4 m
Height, cm	NA	NA		76			158	54	66	65		83	63	-
Weight, kg	NA	NA		9.5			75	5.7	8.66	7.2		11.5	4.5	5.8
OFC, cm	NA	NA		44.5			55.5	38.5	43	41.5	large	46.2	40	-
DD/ID	Y,	Y,	Y, +++	Y, +++	Y	Y	Y	Y, +++	Y,+++	Y			Y	Y
Hypotonia	Y	Y	NA	Y	Y	N	Y	Y	Y	Y	NA	Y	Y	Y
Seizures (and start)	Y, Myoclonic 3w	Y, GTCS 3m	Y, 6m	Y, GTCS, 1m	Y, with Hypsarrythmia	Y, 1y	Y, infantile	Y, 3d	Y, 3m	Y, 3m	Y, 1d (on EEG)	Y, infantile	Y, 2 w	Y, 1 m
Seizure responsiveness	NA	Refractory	Sodium valproate	Refractory	NA	Potassium bromide, Levetiracetam, Sodium valproate	Carbamazepine	Refractory	Sodium valproate, Phenytoin, Phenobarbital, Levetiracetam	Refractory	Phenobarbital Midazolam	Phenobarbital	Levitiracetam	Refractory
Other neurological abnormalities	Axonal degenerative polyneuropathy	Axonal degenerative polyneuropathy	Absent reflexes	Mixed axonal loss Demyelinating sensorimotor polyneuropathy. Absent reflexes	Poor feeding, absent otoacoustic emissions	Epileptic encephalopathy	Hypohydrosis	NA	NA	Weak reflexes, Episodes of apnea	NA		Poor feeding, Hyperthermia, Sensorimotor axonal polyneuropathy	Poor suck, Mixed axonal and demyelinating polyneuropathy
MRI	Normal posterior fossa Thin corpus callosum.	Hyperdensities in the pons cerebri	Normal	Normal at 5 m (Calcifications in left thalamus on ultrasound at 1 m)	Mild dilatation of ventricules, Poor myelination. thinning of the white matter, diffusion restriction in the central tegmental tracts, hyperintensity of the globus pallidus scalloping of the calvarium related to delayed synostosis repair	Normal	Normal	Normal	Normal	Dilatation of ventricles	Post-mortem MRI: Dandy walker malformation Vermis hypoplasia, Thin corpus callosum, Pontine hypoplasia. Possibly diffuse polymicrogyria, Possibly intracranial vessel tortuosity.	(9a)F Polymicrogyria along the bilateral occipital lobes. Diffuse cerebral volume loss. Hypomyelination in peri-ventricular and subcortical white matter more pronounced at the bilateral occipital and frontal lobes.	16 days Multiple foci of Altered signal intensity bilateral periventricular and subcortical region	Thin corpus callosum with hypoplastic splenium, Mild prominence of lateral ventricles, Symmetric areas of restricted diffusivity in posterior pons and medulla
Dysmorphisms	Dysplastic ears, upslanting palpebral fissures, full cheeks, micrognathia, tented mouth, and high palate.	Metopic ridge, biparietal narrowing, Dysplastic ears, full cheeks, micrognathia		Broad nasal bridge, Large mouth with protruding tongue Overfolded helix, frontal lobule of ear, bilaterally	Hypertelorism, Coarse facial features, Wide protruding eyes (proptosis), Low set ears, High arched palate, Retromicrognathia, Craniosynostosis (Coronal, Sagittal, metopic) with Scaphocephaly, mildly overfold superior helixes bilaterally, low set and posteriorly rotated ears, hypoplastic nasal alae	Normal	Coarse facial features, large tongue, slightly upturned earlobes	Broad nasal bridge, Long smooth philtrum, Tented upper lip, Full cheeks, Large upturned earlobes	Broad nasal bridge, Long smooth philtrum, Tented upper lip, Full cheeks, Large upturned earlobes	Low-set, posteriorly rotated ears, Wide nasal bridge Bulbous tip, Retrognathia	Coarse facial features, Hypertelorism , Long palpebral fissures, Epicanthus inversus, Broad eyebrows, Broad nose, Short columella, Long philtrum, Short chin,. Coarse, large earlobes, Overfolded helix.	NA	Facial hypertricjosis. Coarse facies, Long smooth philtrum, Prominent nasal tip, Pointed chin with horizontal crease, Uplifted ear lobules	Facial hypertrichosis, Gum hypertrophy, Large ears with uplifted ear lobules, Small neck, Coarse facies, Long smooth philtrum, Pointed chin with horizontal crease

Hearing	BAEP and OAE showed little or no response bilaterally	Unilat hearing loss.	50-60 db		Impaired	Normal	Normal			TEOAE showed no response bilaterally	NA	NA	Impaired BAEP at birth - normal	Impaired BAEP – not done
Vision	At age 2 months he had a normal fundus, VEP showed a weak response, and ERG was normal	'Short fixation, abnormal eye movements, and intermittent strabismus. VEP and ERG show decreased response	Impaired Bilateral, myopia gravis ou, ou retinal depigmentation and initial dystrophic changes	Impaired, Pale optic disc on fundoscopy	left corneal opacity. Exposure keratopathy, corneal ulcers, increased ICP, gaze evoked nystagmus. Diffuse epitheliad effects bilaterally, poor vision. Inability to close eyelids since birth	Normal	Normal	Impaired. Unable to fix and follow	Impaired. Unable to fix and follow	VEP showed no response bilaterally	NA	NA	NA	NA
Digital anomalies	Triphalangeal thumb, Brachytelephalangy and hypoplastic nails hands and feet	Triphalangeal thumb, Brachytelephalangy and hypoplastic nails hands and feet	N	Transverse palmar crease	Flexion contractures of fingers, suspicion of triphalangeal thumbs, Shortening of distal phalanges with tapering fingers, Hypoplastic nails, Bilateral single palmar creases	N	Y	N	N	Hypoplastic nails on hands and feet	Brachydactyly, Absent nails on digit 5 of both hands and on digits 3-5 of the feet. Aberrant dermatoglyphic pattern	NA	Bilateral toes hypoplasia with hypoplastic / absent terminal phalanges, Nail hypoplasia, 5th finger hypoplasia and absent distal phalanx	Bilateral toes hypoplasia with hypoplastic / absent terminal phalanges, Nail hypoplasia, 5th finger hypoplasia and absent distal phalanx
Plasma alkaline phosphatase (U/L)	NA	NA	Normal (356 U/L, normal <1000)	NA	Elevated	Elevated	Elevated	Elevated (1000-1500 U/L)	Elevated (1000-1500 U/L)	Elevated	NA	NA	Elevated (1272) [normal 150 – 420]	Elevated (1546)
Other clinical features	2-oxoglutaric aciduria	2-oxoglutaric aciduria	Born at 34 weeks, Pes equinovarus, hyperactive bladder, Severe constipation, achillotomy, Frequent respiratory infection, transient hypothyroidism	exocrine pancreatic insufficiency. Severe constipation. Pyelectasis. Hepatomegaly	Premature birth, hypoplasia of labia majora and minora, Small chest, Cardiac: ASD, PFO and aneurysm of the interatrial septum, moderate pulmonary hypertension		Hypohydrosis, very dry, scaly skin elevated CK	Anal stenosis, polyhydramnios	Polyhydramnios, Hydronephrosis	Severe failure to thrive Necessitated PEG feeding tube Pulmonary hypertension, Left ventricular hypertrophy	Micropenis, Abnormal heart axis. Similarly affected cousin, also homozygous for variant.	NA	Born at 37 weeks. Poor suck and feeding since birth, persistent lethargy, constipation, EEG – burst suppression pattern, Rough, wrinkled skin	Antenatal – increased nuchal fold thickness, 30 wks gestation – polyhydramnios, protuberant abdomen and upper lip Rough, wrinkled skin
Outcome	Lost to follow-up	Died at 3.8y		Died	Died	Seizures decreased at age 4	Seizures resolved at age 4 years	Died at 6 months		Recurrent respiratory insufficiency and pneumonia, palliative care started at 8 months of age	Died day 2	infantile onset focal motor seizure with preserved consciousness. Similarly affected sibling passed away at age 4.	Died at 6 months	Died at 6 months

TEOAE：Transiently Evoked otoacoustic Emission, BAEP: brainstem auditory evoked potentials, VEP: Visual evoked potentials, ERG: Electroretinogram

Table S2. Common symptoms between IGDs and DOORS syndrome

Syndrome name	IGD													DOORS
Mutated gene	PIGA,C,P,H,Q	PIGL	PIGW	PIGM	PIGV	PIGN	PIGB	PIGO	PIGG	PIGT,S,GPAA1	PGAP1	PGAP2	PGAP3	TBC1D24
Deafness	Y	Y	NR	NR	Y	Y	Y	Y	NR	Y	NR	Y	NR	Y
Nail anomalies	Y	NR	NR	NR	Y	Y	Y	Y	NR	Y	NR	Y	NR	Y
Short fingers or hands	Y	Y	NR	NR	Y	Y	Y	Y	NR	Y	NR	Y	NR	Y
DD/ID	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Seizures	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
MRI anomalies	Y	Y	Y	NR	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Craniosynostosis	Y	NR	NR	NR	NR	NR	N	Y	NR	Y	NR	NR	NR	Y
Cranial shape anomalies	Y	Y	NR	NR	NR	Y	Y	NR	NR	Y	NR	NR	NR	Y
Ophthalmological anomalies	Y	Y	NR	NR	NR	Y	Y	Y	NR	Y	NR	NR	NR	Y
Cardiac anomalies	Y	Y	NR	NR	Y	Y	Y	Y	NR	Y	NR	Y	NR	Y
GU malformation	Y	Y	NR	NR	Y	Y	N	Y	NR	Y	NR	Y	NR	Y
Nephrocalcinosis	NR	NR	NR	NR	NR	NR	N	NR	NR	Y	NR	NR	NR	Y
Teeth anomalies	Y	Y	NR	NR	NR	NR	N	NR	NR	Y	NR	NR	NR	Y
Hirschsprung disease	NR	NR	NR	NR	Y	NR	Y	Y	NR	NR	NR	Y	NR	NR
anal atresia	Y	NR	NR	NR	Y	NR	Y	Y	NR	NR	NR	Y	NR	NR
diaphragmatic hernia	NR	NR	NR	NR	Y	Y	NR	NR	NR	NR	NR	NR	NR	NR
Serum alkaline phosphatase	mild↑	↑	↑	NR	↑	NR	↑	↑	NR	↓	NR	↑	↑	NR
2-oxoglutaric aciduria	NR	NR	NR	NR	NR	NR	↑	NR	NR	NR	NR	NR	NR	↑
Decreased expression of CD16 on the granulocytes	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	slightly	N

IGDs with orange color fit diagnostic criteria of DOORS syndrome

Supplemental Materials and Methods

Whole exome sequencing (WES)

For individual 1a, exome sequencing and analysis was performed as described[1]. For individuals 2a and 3, clinical WES was performed at Baylor Genetics as described previously[2]. For individual 4, WES using the DNA derived from the blood leukocytes of the proband was carried out as described previously[3]. In brief, genomic DNA was captured using the SureSelect Human All Exon V5 kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on a Illumina HiSeq2500 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Image analysis and base calling were performed using sequence control software with real-time analysis and CASAVA software (Illumina). Reads were aligned to GRCh37 using Novoalign (http://www.novocraft.com/). Marking PCR duplicates, indel realignment, and base-quality-score recalibration were performed using Picard (http://picard.sourceforge.net/) and Genome Analysis ToolKit (GATK) (https://www.broadinstitute.org/gatk/index.php). Variants were called by the GATK UnifiedGenotyper (http://www.broadinstitute.org/gatk/) and annotated using ANNOVAR (http://www.openbioinformatics.org/annovar/) after excluding the common variants registered in the dbSNP135 database (minor allele frequency≥0.01). Detected variants were confirmed by Sanger sequencing. For individual 5, exome was performed as described previously.[4] For individual 6, exome was performed as described previously.[5] As for WES for individual 7, SureSelect Human All Exon V6 Kit was used, sequenced on an Illumina HiSeq4000 with 2x75bp PE reads. Basecalling and variant annotation were performed on the Cologne Center for Genomics in-house varbank pipeline on the GRCh37 reference genome. For individual 8a, exome was performed as previously described.[6] For individual 9b, exome was performed as described previously.[7] For individual 10b, whole genome sequencing was performed at Centogene on HiSeqX platform (Illumina, San Diego, CA, USA) with an avarage coverage of ~30x and a read length of 150 base pair paired-end reads. Fastq reads were aligned

against GRCh 37 human genome assembly using BWA aligner (http://bio-bwa.sourceforge.net/). Next, PICARD tool set was used to remove PCR duplicates and Genome analysis Toolkit (GATK) (https://software.broadinstitute.org/gatk/) was applied for base quality score recalibration. Variants were called using GATK HaplotypeCaller and annotation was performed using ANNOVAR (http://www.openbioinformatics.org/annovar/). Variants with Minor allele frequency (MAF) ≥ 1% in gnomAD database were considered. In addition, family history and clinical indications were put together to identify causative variants.

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