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Supplemental Data

Mutations in *PIGO*, a Member of the GPI-Anchor-Synthesis Pathway, Cause Hyperphosphatasia with Mental Retardation

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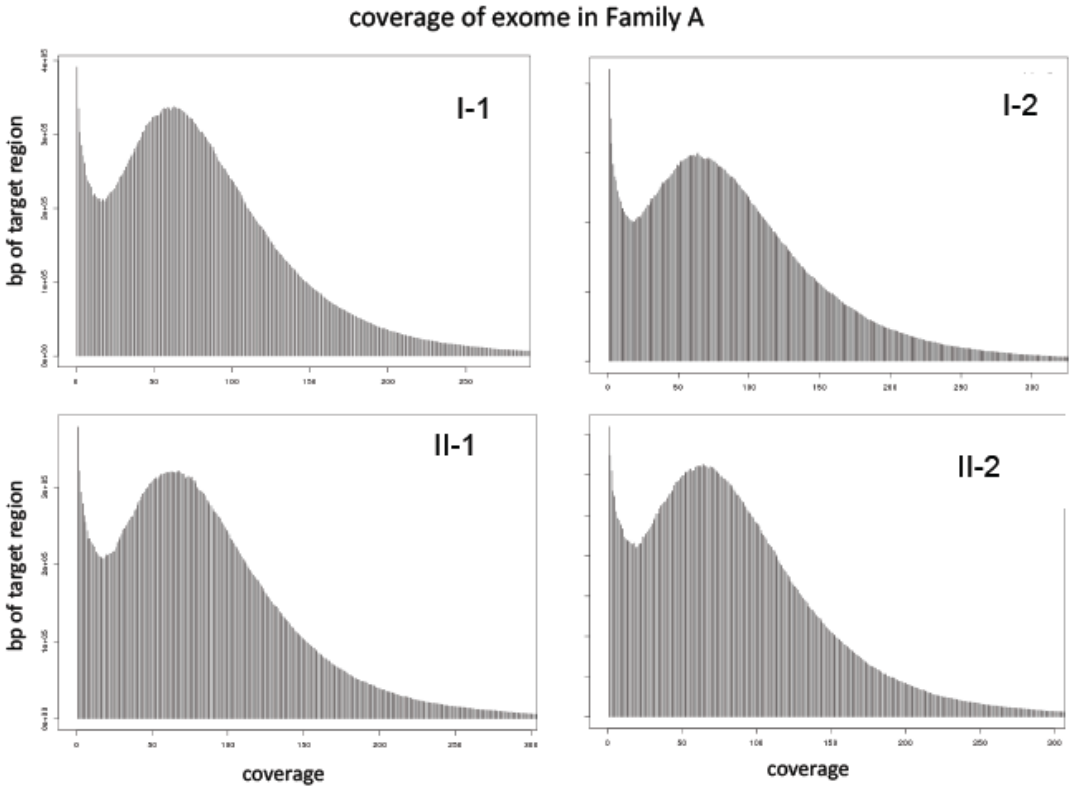

Figure S1. Coverage of Exome Target Region

Over 90% of all CCDS exons were covered by more than 10 reads allowing highly specific variant detection.

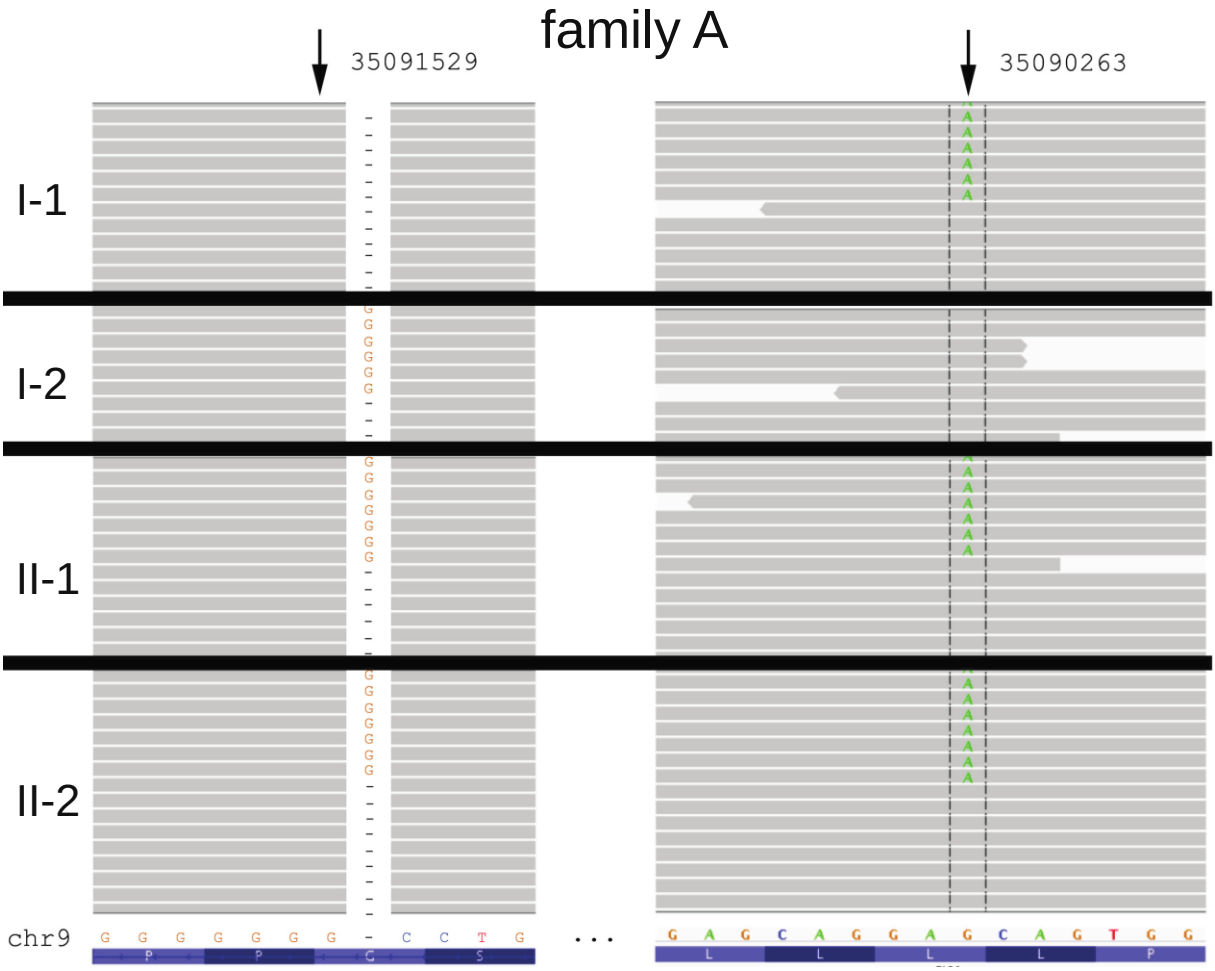

Figure S2. Compound Heterozygous Mutations c.2361dup (left) and c.2869C>T (right) of Family A

A snapshot at the respective position in the alignment was taken from the integrative genome viewer.

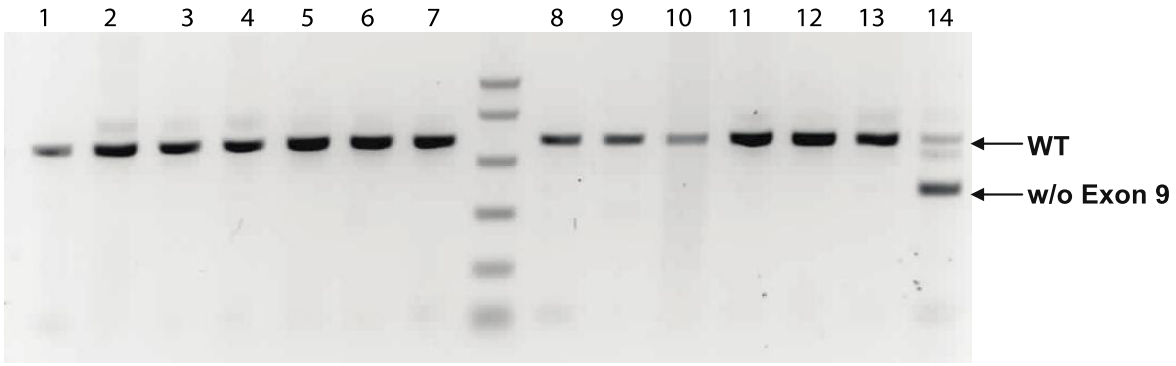

Figure S3. cDNA Controls in Comparison with an Aberrant Splicing Product Due to Skipping of Exon 9

cDNA controls (lane 1-13) in comparison with an aberrant splicing product due to skipping of exon 9 (lane 14 from heterozygous carrier B.I.2). The following primers were used for cDNA-PCR amplification: PIGO_F7_FW: 5'- TCCTACATCTGCTTGCTGCTGGG -3' (located in PIGO exon 6) and PIGO_F7_RV: 5'-GGAGAAGTCCCACGCTGCTCAC-3' (located in PIGO exon 10). PCR was performed in a total volume of 20 µl containing 0.25 µM primers (MWG Biotech AG), 300 µM dNTPs (Bioline), 2.0 µl of 10x PCR Gold buffer (Applied Biosystems), 2.0 mM MgCl2 (Applied Biosystems), 1,5 U AmpliTaq Gold Polymerase (Applied Biosystems) and 3.0 µl cDNA. The GeneAmp PCR System 9700 (Applied Biosystems) was utilized for thermal cycling applying the following thermal conditions: 11 min at 95°C, 35 cycles of 94°C for 30 s, 67,6°C for 30 s and 72°C for 2 min as well as a final elongation step of 72°C for 10 min. Finally, all samples were cooled down to 10°C and thereafter analyzed by agarose gel electrophoresis.

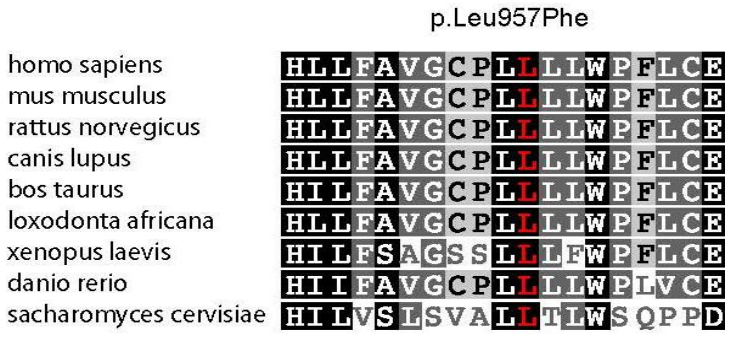

Figure S4. Alignment of PIGO Sequences from 9 Species

The missense mutation p.L957F affects a highly conserved residue within a transmembrane segment.

Table S1. Summary of Clinical Findings in Patients Carrying *PIGO* and *PIGV* Mutations

	HPO ID[a]	Family A II-1	Family A II-2	Family B II-1	Manifestations of Patients with *PIGV* Mutations (n = 14)[b]
Sex		Female	Female	Female	9 females, 5 males
Age of last assessment		15 years	12 years	20 months	7 months - 17 years
Origin		White British	White British	White	German, Maroccan, Dutch, Polish, British, Caucasian-American
Height (SD)		-4.2	-1.4	-2.5	Normal in 13/14
Weight (SD)		-1.5	+0.6	-3.3	Normal in 13/14
OFC (SD)		-0.2	+0.7	-5.5	Normal in 12/14
Hyperphoshatasia[c]	HP:0003155	+	+	+	14/14
Global developmental delay	HP:0001263	+	+	+	14/14
Age at walking		5 years	2 years	No walking	Delayed
Delayed speech and language development	HP:0000750	+	+	Absent speech (HP:0001344)	14/14
Muscular hypotonia	HP:0001252	+	Mild	+	11/12
Seizures	HP:0001250	-	-	+	9/12
Facial gestalt[a]					
Apparent hypertelorism	HP:0000316	+	+	+	+
Long palpebral fissures	HP:0000637	+	+	+	+
Broad nasal bridge	HP: 0000431	+	+	+	+
Broad nasal tip	HP:0000455	+	+	+	+
Tented upper lip vermilion	HP:0010804	+	+	+	+
Brachytelephalangy[a]	HP:0009882	+	+	+	14/14
Analrectal abnormalites/ constipation	HP:0002025 (anal stenosis)	+	+	+	6/12
Aganglionic megacolon	HP:0002251	-	+	-	2/14
Atrial septal defect	HP:0001631	-	-	+	1/14
Vesicoureteral reflux (requiring reimplantation of ureters)	HP:0000076	+	-	-	-
Atonic bladder	-	-	+	-	-
Peripheral pulmonary stenosis	HP:0004957	-	-	+	-
Left coronal synostosis	-	-	-	+	-

[a]Human Phenotype Ontology with term identification (ID).

[b]Not all features were documented in the reported patients.

[c]Consistent features in bold.

Table S2. Summary of Computational Variant Filtering in Family A

Filter	II1	II2	I1	I2
Sequence variants in CCDS	22395	22137	22299	22078
Rare variants (not in dbSNP 132)	853	879	887	842
Missense, nonsense, frame shifting or splice site affecting	427	440	434	416
Candidate genes with homozygous mutations	0			
Candidate genes with compound heterozygous mutations	1			

The total number of single-nucleotide variants (SNV) and short indels (<20bp). These were further filtered to rare variants not present in dbSNP 132 or in the 1000 genomes project, and finally to variants that are nonsynonymous. Candidate genes were filtered assuming a recessive mode of inheritance. Genes were analyzed for variants that were homozygous in both patients and heterozygous in the parents or for genes with compound heterozygous variants in both patients whereby each of the parents was heterozygous for a different variant.

Table S3. Coordinates of Detected Mutations with Respect to Genome Sequence, Coding Sequence, and Protein Sequence

Genomic (hg19)	Transcript (NM_032634)	Protein (NP_116023)
chr9:35090058C>T	c.3069+5G>A	–
chr9:35090263G>A	c.2869C>T	p.Phe957Leu
chr9:35091522T>TG	c.2361dupC	p.Thr788Hisfs*