The American Journal of Human Genetics, Volume *98*

Supplemental Data

Pathogenic Variants in *PIGG* Cause Intellectual Disability with Seizures and Hypotonia

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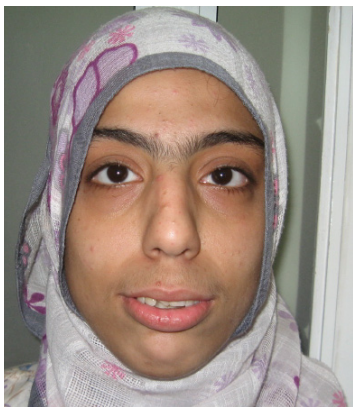
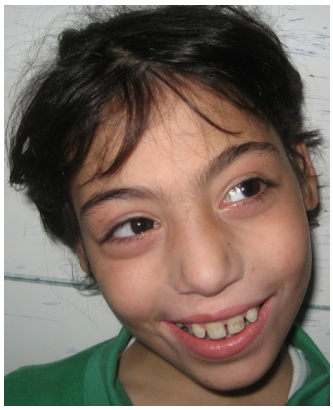
Supplemental Note

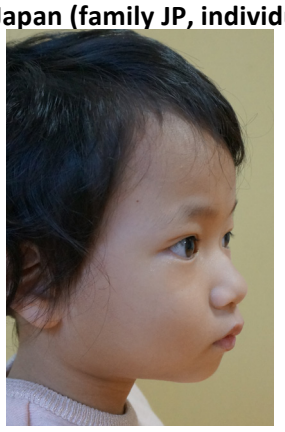
Case Reports

The affected individuals from Egypt (family EG, individuals EG01 and EG02)

We have initiated a project to identify known [1] and novel genes [2; 3] in consanguineous families of intellectual disability by whole exome sequencing. The affected individuals (V:1 and V:5, now aged 24 and 14 years respectively) are the offspring of a consanguineous marriage between first cousins, originating from Egypt, the father being himself offspring of a consanguineous marriage between first cousins (Figure 1). The presenting symptom was in both cases generalized seizures at the age of 4 months that were resistant to treatment. The older sister responded best to a combination of valproate and carbamazepine while the second to valproate and topiramate but they still have occasional seizures. EEG has shown in both cases that seizures were temporal with secondary generalization, while brain MRI as shown a thin corpus calosum, and asymmetry of the lateral ventricles (Figure 2A). Exact birth measurements are not available but have been reported to be within normal limits. The affected individuals presented since birth hypotonia with hyporeflexia and were also exhibiting marked joint laxity. They walked at 3 and 4 years respectively and they can currently walk only when supported. Intellectual disability is profound and uniform. The individuals show some dysmorphic features, but the fact that they differ between them doesn't allow drawing any conclusions. An extensive diagnostic workup was performed including: blood G-band karyotype, metabolic screening, liver, kidney and thyroid function, EMG, CPK dosage, hearing and ophthalmologic assessment and all were reported normal.

The affected individual from Japan (family JP, individual JP01)

The affected individual was recruited in a project screening individuals with infantile epilepsy by whole exome sequencing. She was born to non-consanguineous healthy Japanese parents (Figure. 1) as a first child after 34 weeks of gestation with intrauterine growth retardation. Birth was uneventful and birth weight was 1,536 g (-1.7 SD) while head circumference (HC) was 28.8 cm (-1.1 SD). She could roll over at the age of 6 months but was unable to sit at 12 months. Seizures were first noted at the age of 10 months. At 12 months of age, she developed vomiting followed by tonic seizures and lowering of consciousness. Video electroencephalograpy (EEG) revealed eye deviation to the right side and tonic-clonic seizures of left upper extremity or impairment of consciousness over 20 times per a day. Interictal EEG showed diffuse fast waves on awaking, and 1.5 to 2 Hz high-amplitude-slow-waves at bilateral frontal lobes during sleep at 1 year of age, and focal spikes at 3 years of age (Figure 2B, an upper panel). Ictal EEG showed a 9 Hz α wave at right parietotemporal lesions, which propagated to the right hemisphere (Figure 2B, a lower panel). Phenobarbital and carbamazepine were administered but were withdrawn after they induced skin eruption. Seizures were controlled after administration of clobazam at 15 months. She had neither dysmorphism nor hepatosplenomegaly. She developed severe psychomotor developmental delay with no speech development, autistic features, and growth retardation with poor appetite resulting in a temporary hospitalization for malnutrition and dehydration at 20 months. Her developmental quotient (Japanese Enjoji scale) was 10 at 3 years and 10 months of age. Brain magnetic resonance imaging (MRI) including diffusion-weighted images showed normal findings at 12 months (Figure 2B). Blood chemistry analysis showed no elevation of alkaline phosphatase (602 IU/L at 2 years of age). Her G-band karyotype was 46, XX.

Affected individuals from Pakistan (family PK, individuals PK01 and PK02)

The individuals were recruited during a project looking for pathogenic variants responsible for intellectual disability in consanguineous families. The affected individuals, presently aged 12 and 10 years old, are offspring of a mating between first cousins of Pakistani origin (Figure 1). Another girl (individual V:8), deceased at birth, was diagnosed with Fraser syndrome and they also have a non-affected brother. The individuals had normal birthweight (3200 and 3000g respectively) and both have had severe delay in their motor development; they sat at 12 and 15 months respectively and after they walked (at the age of 3 and 4 respectively) they were showing severe ataxia. The first individual has had a single episode of seizures while the second one had not any epileptic episode. Their height is on the 50^{th} and 25^{th} centile respectively and their head circumference at the 9^{th} and 5^{th} centile. None of them has shown any dysmorphic features. All diagnostic tests including G-band karyotype, lactate, urine and blood amino acids, urine organic acids and oligosaccharides, creatine kinase, white cell enzymes, transferrin electrophoresis were normal. Brain MRI revealed nonspecific cerebellar hypoplasia in both affected individuals and mild cerebral atrophy (Figure 2C).

Supplemental References

1. Makrythanasis, P., Nelis, M., Santoni, F.A., Guipponi, M., Vannier, A., Bena, F., Gimelli, S., Stathaki, E., Temtamy, S., Megarbane, A., et al. (2014). Diagnostic exome sequencing to elucidate the genetic basis of likely recessive disorders in consanguineous families. Hum Mutat 35, 1203-1210.
2. Hamamy, H., Makrythanasis, P., Al-Allawi, N., Muhsin, A.A., and Antonarakis, S.E. (2014). Recessive thrombocytopenia likely due to a homozygous pathogenic variant in the FYB gene: case report. BMC Med Genet 15, 135.
3. Makrythanasis, P., Temtamy, S., Aglan, M.S., Otaify, G.A., Hamamy, H., and Antonarakis, S.E. (2014). A novel homozygous mutation in FGFR3 causes tall stature, severe lateral tibial deviation, scoliosis, hearing impairment, camptodactyly, and arachnodactyly. Hum Mutat 35, 959-963.

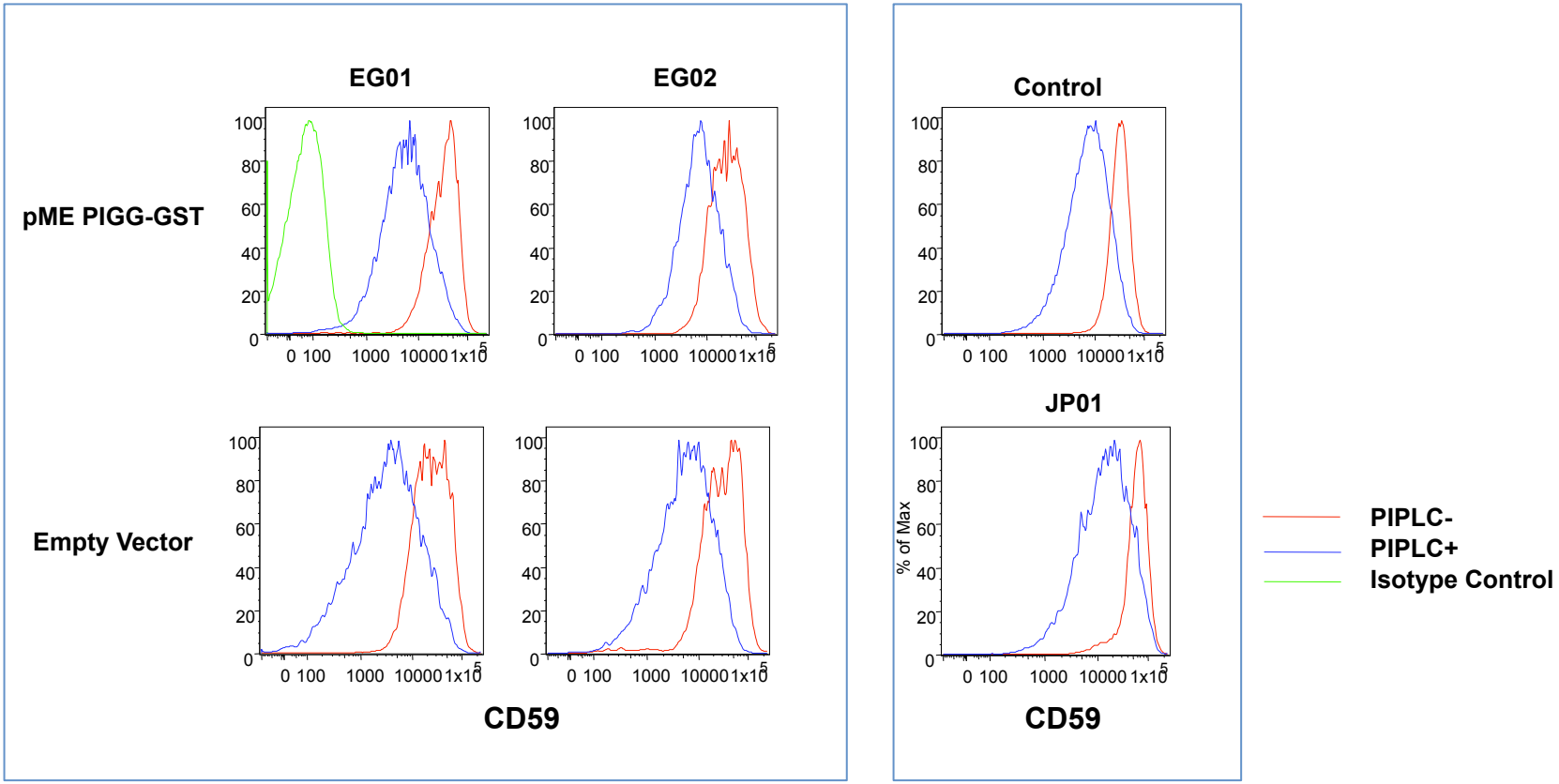


Figure S1. PIPLC sensitivity of PIGG deficient LCLs: Surface expression of CD59 on LCLs from individuals EG01 and EG02 transfected with PIGG or empty vector (left two lines) or LCLs from the individual JP01 and control (a right line) w/o PIPLC treatment. CD59 on PIGG deficient LCLs were similarly cleaved by PIPLC to wild type cells.

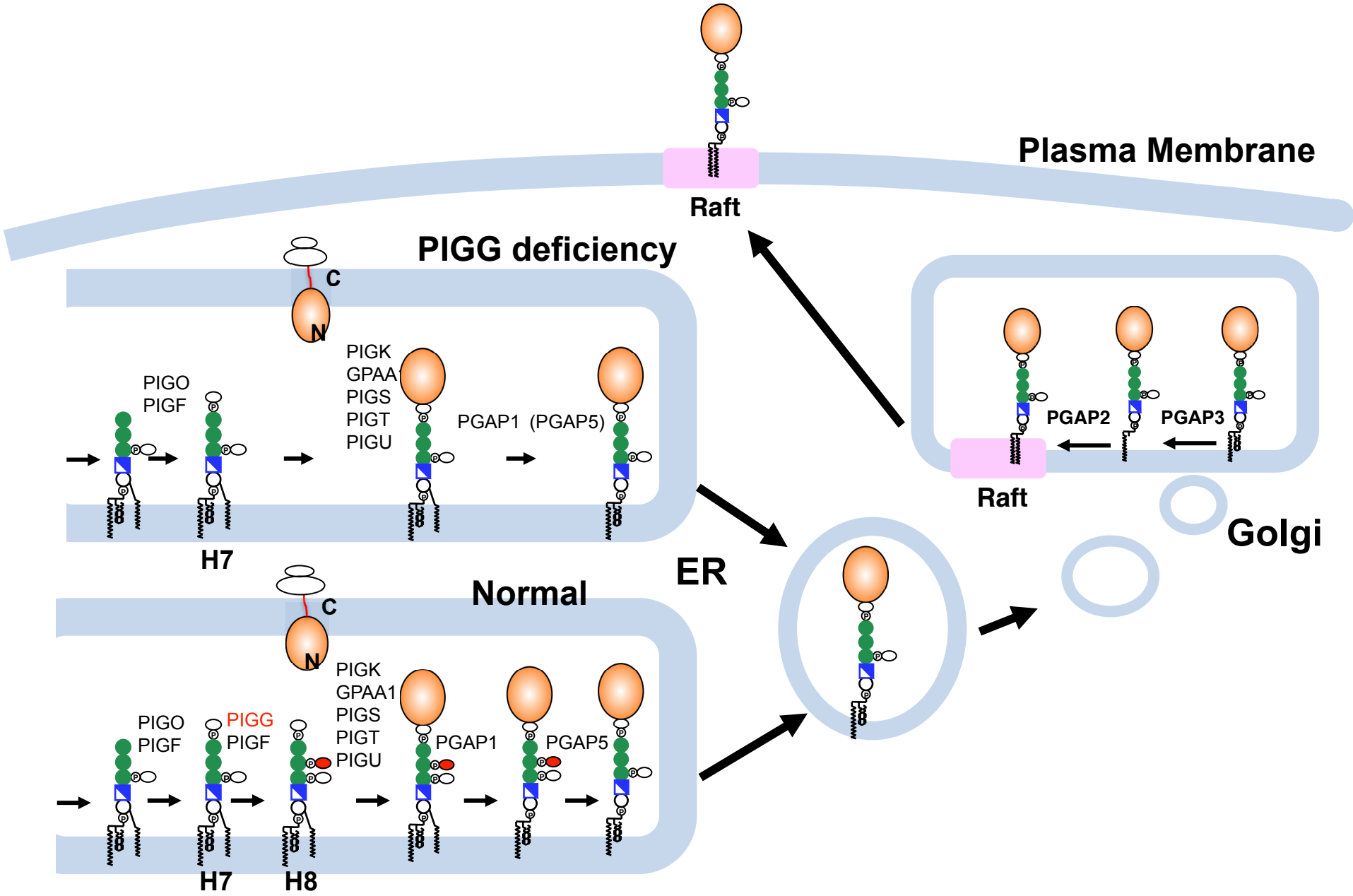


Figure S2. A part of GPI biosynthesis pathway in which PIGG participates: PIGG attaches ethanolaminephosphate, painted red, which is removed by PGAP5. H7 and H8 indicate each GPI intermediate. Precursor proteins are processed and attached to H7 even without PIGG, an acyl chain is further cleaved by PGAP1. Bypassing PGAP5 reaction, GPI-APs are transported to Golgi and are remodeled similar to wild type cells.

Variants found in *PIGG* in families 1 and 2 (NM_001127178.1)								
	Variant	dbSNP138	MAF (ExAC)	In-house database	SIFT	Polyphen2	MutationTaster	gerp++
EG01 (V:1) & EG02 (V:5)	c.928C>T p.(Gln310*)	novel	novel	novel	NA	NA	NA	5.1
JP01 (V:6)	c. c.2005C>T p. Arg669Cys	rs372392424	Cumulative: 0.0001801 East Asian: 0.0006605	4 / 575	0	0.991	0.999	4.69
PK01 (V:9) & PK02 (V:10)	c.2261+1G>C	novel	novel	novel	HSF: site broken	NNSplice: Site abolished	MaxEntScan: Site abolished	4.88
Additional variants in the affected individuals 1&2								
EG01 (V:1) & EG02 (V:5)	NM_032314.3(COQ5_v001) c.319G>A p.(Gly107Arg)	novel	novel	novel	0.001	0.973	0.999	5.91
EG01 (V:1) & EG02 (V:5)	NM_002926.3(RGS12_v001) c.188A>T p.(Gln63Leu)	rs537451677	Cumulative: 4.95e-05 African: 9.649e-05		0.58	0.936	0.991	2.58

Abbreviations are as follows: MAF, minimum allele frequency; ExAC, exome aggregation consortium; Gerp++, measurement of nucleotide conservation. Scores above 5 show a very high conservation among the species. HSF: http://www.umd.be/HSF/ , NNSplice: http://www.fruitfly.org/seq_tools/splice.html , MaxEntScan: http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html

Table S1. Variants identified in the affected individuals