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Supplemental Data

Identification of Mutations in TMEM5 and ISPD

as a Cause of Severe Cobblestone Lissencephaly

Sandrine Vuillaumier-Barrot, Céline Bouchet-Séraphin, Malika Chelbi, Louise Devisme, Samuel Quentin, Steven Gazal, Annie Laquerrière, Catherine Fallet-Bianco, Philippe Loget Sylvie Odent, Dominique Carles, Anne Bazin, Jacqueline Aziza, Alix Clemenson, Fabien Guimiot, Maryse Bonnière, Sophie Monnot, Christine Bole-Feysot, Jean-Pierre Bernard, Laurence Loeuillet, Marie Gonzales, Koryna Socha, Bernard Grandchamp, Tania Attié-Bitach, Férechté Encha-Razavi, and Nathalie Seta

Figure S1: Pedigrees of our two multiplex families: **A:** Family 1 and **B:** Family 2, in which the first *ISPD* and *TMEM5* mutations were detected. Black diamonds indicate fetuses with cobblestone-LIS.

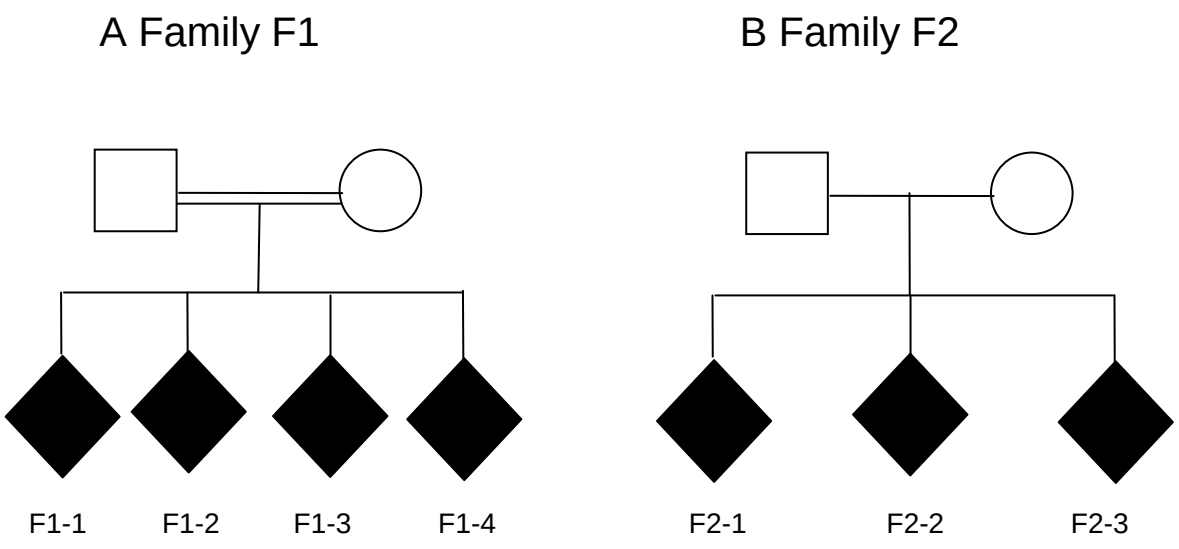

Figure S2: Alignment of the amino acid sequences of TMEM5 with EXT1 (BlastP) showing 19 % of identities between TMEM5 amino acid 218 to 353. Amino acids concerned by substitutions are in bold. DXD motifs (putative glycosyltransferase catalytic site motifs) are underlined for each gene.

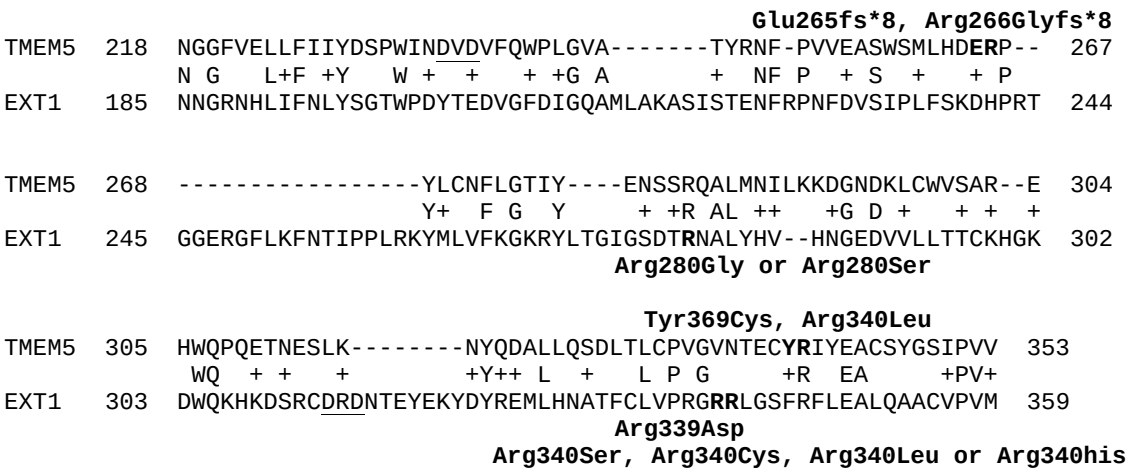

Table S1: ROH of the consanguineous family F1, and linkage regions of more than 2 Mb with an LOD score >1 for the multiplex family F2. A homozygote mutation was found in the *TMEM5* gene on chr12:64 173 637-64 202 887, and hemizygosity was found in the *ISPD* gene on chr7:16,127,152-16,460,947 (hg19).

ROH in consanguineous Family 1				
Chromosome	**Start (hg19)**	**End (hg19)**	**size Mb**	**Number of genes**
4	157174388	163243244	6.06	20
4	171298350	182716439	11.42	37
12	**63212175**	**64379443**	**1.17**	**10**
15	85493132	87388101	1.89	15
20	41107656	41182902	1.07	5

Linkage regions of more than 2 Mb with LOD score >1 for multiplex family F2				
Chromosome	**Start (hg19)**	**End (hg19)**	**size Mb**	**Number of genes**
1	60748394	68618199	7.86	64
6	114705622	130898269	16.19	111
7	**792020**	**25345110**	**24.55**	**239**
7	25364592	36700216	11.30	132
10	15926632	36961506	21.03	176
11	7835752	15144280	7.30	88
17	15082587	23019858	7.93	198
20	15734480	26170699	10.43	132
20	29314247	46819863	17.50	328
21	15821015	41172018	25.35	270

Table S2: Exome sequencing. DNA (5 µg) of two fetuses in each family was subjected to exome sequencing using the Sure Selecthuman All exon 50MB kit from Agilent ; captured exons were subsequently sequenced using the GAIIX Illumina (2x72 bp reads) technologies. The sequences were aligned to the human genome reference sequence (hg19 assembly). All calls with read coverage ≤ 2 and a Phred-scaled SNP quality of ≤ 20 were filtered out. Filtering of the data by removing the known variants, non coding and synonymous coding sequence variations, combined with mapping data identified a unique gene within targeted homozygous regions in F1 or linkage regions in F2. The mutated gene was found in indel data for F1 and SNV data for F2.

Indel	**Number of variations F1-1**	**Number of variations F1-2**
Total variants	27 506	28 444
Coding disrupted frameshift	831	1 019
Unknown variants (dbSNP132/1K genome/EVS and in-house database filtering)	538	720
Quality filtering (Coverage ≥ 5 reads)	455	557
Homozygosity (% reads variation/ref >0.7)	75	57
Mapping analysis	1	1

SNV	**Number of variations F2-1**	**Number of variations F2-2**	**Number of common variations (F2-1 & F2-2)**
Total variants	203 604	206 683	
Non-synonymous + Essential Splice-sites variants	27 548	28 005	
Unknown variants (dbSNP132/1K genome/EVS and in-house database filtering)	1 028	950	
Quality filtering (Coverage ≥ 5 reads)	970	915	
Mapping analysis	46	52	35
Mutated Genes	18	16	12
Gene(s) with two mutated alleles	1	1	1

Table S3: Primers for for *TMEM5* and *ISPD* amplification and direct sequencing.

Mutational screening of TMEM5 and ISPD was performed by direct sequencing of PCR products of the 6 and 10 coding exons and the adjacent intronic junctions respectively. Size of amplification products are indicated. Supplemented primers were used for sequence only. PCR primers were selected with ExonPrimer according to reference sequence NM_014254.1 and NM_001101426.3 PCR products were purified with the Exo-SAP cleanup kit (USB) and sequenced with BigDye chemistry and ABIPRISM 3130XL sequencer (Applied Biosystems). Sequences were analyzed with SeqScape® software.

GENE	Oligo name	Sequence	Size (bp)
TMEM5	1S_TMEM5	CTG-GGG-ACT-GCC-TGG-AAA-C	395
	1R_TMEM5	TCA-GGC-CCT-GCG-GGT-AAT-C	
	2S_TMEM5	TTT-TGT-TTT-CAT-TGT-GTA-TTA-CCA-G	472
	2R2_TMEM5	TGT-CTA-ATT-GGA-AGG-AGA-AAG-G	
	3S_TMEM5	TGA-TTT-GGA-GCT-GTT-GCT-TG	490
	3R_TMEM5	TGA-TGC-CAA-AAA-GGA-GTA-TTC	
	4S_TMEM5	TGT-GGA-TTA-AAT-CTC-ATT-GTA-G	501
	4R_TMEM5	AAT-TTT-GAA-TAA-GTC-TTC-CTG-G	
	5S_TMEM5	GGA-GTT-TTC-CAA-AGT-ATT-CAT-GG	453
	5R_TMEM5	ATC-TTC-TGG-GGA-AAG-ATT-GG	
	6S_TMEM5	ATC-ATC-TCT-ATA-AAG-TTT-CAC-C	636
	6R_TMEM5	CCT-TAA-AGA-ACA-TCT-ATA-AAT-AC	
	6R2_TMEM5	GTG-GTG-CAC-AGA-TGT-ATT-CC	
	6S2_TMEM5	TGA-GGC-TTG-CTC-CTA-TGG-C	
ISPD	ISPD_1S	GTC-CCT-CTC-CGT-GGT-CTG	487
	ISPD_1R	TGA-ATA-ACT-GAG-CGC-GGC	
	ISPD_2S	TTA-ACT-TGC-AGT-TTT-TAA-TCT-CC	498
	ISPD_2R	TGA-CAT-TTA-AAC-AGA-ATT-GAA-TC	
	ISPD_3S	TTT CTT AAG GTA GCT TGT TGA AGG	368
	ISPD_3R	ACG CTC AGT CCC ATC AGT TC	
	ISPD 3S2	ATC-TGT-CAA-AAG-TGA-GAT-ACT-G	
	ISPD_4S	CCC-TTC-CTT-TGT-TTG-CAA-TG	312
	ISPD_4R	CCG-TGA-GAC-TCC-CTT-AAC-TCC	
	ISPD_5S	ATT-TTA-TGT-GAA-TTT-GCT-GAT-GC	332
	ISPD_5R	AAT-CAG-AGA-CCT-TGG-CCT-AC	
	ISPD_6S	TGG-CCT-GAA-ATC-AAA-ACC-TC	375
	ISPD_6R	CTG-GCA-GAC-CAA-AGG-ATC-TC	
	ISPD_6S2	ATT-TCA-GAG-AGA-ATT-TCC-CAA-G	
	ISPD_7S	ATG-GTC-CTT-GGC-TTT-ATG-GG	395
	ISPD_7R	TTG-TCC-AAA-ATA-CCA-CTC-TTC-TC	
	ISPD_8S	GGC-AGG-TTT-GAC-TCA-ATT-AGT-AG	400
	ISPD_8R	TTG-AGT-ATG-GGT-CAA-TGC-TCT-C	
	ISPD_9S	AAT-CAT-ATG-GGT-TTT-GAG-CTT-C	360
	ISPD_9R	GCA-CAC-ACA-TAG-ATG-AGT-AAC-TTT-CC	
	ISPD_10S	TCC-TCT-TTA-TTC-GGG-GAA-CC	482
	ISPD_10R	GTT-CAG-GCA-ATT-AAT-ATT-TGT-CAT-AC	