Supplemental Data

Mutations in *SPINT2* Cause a Syndromic Form of Congenital Sodium Diarrhea

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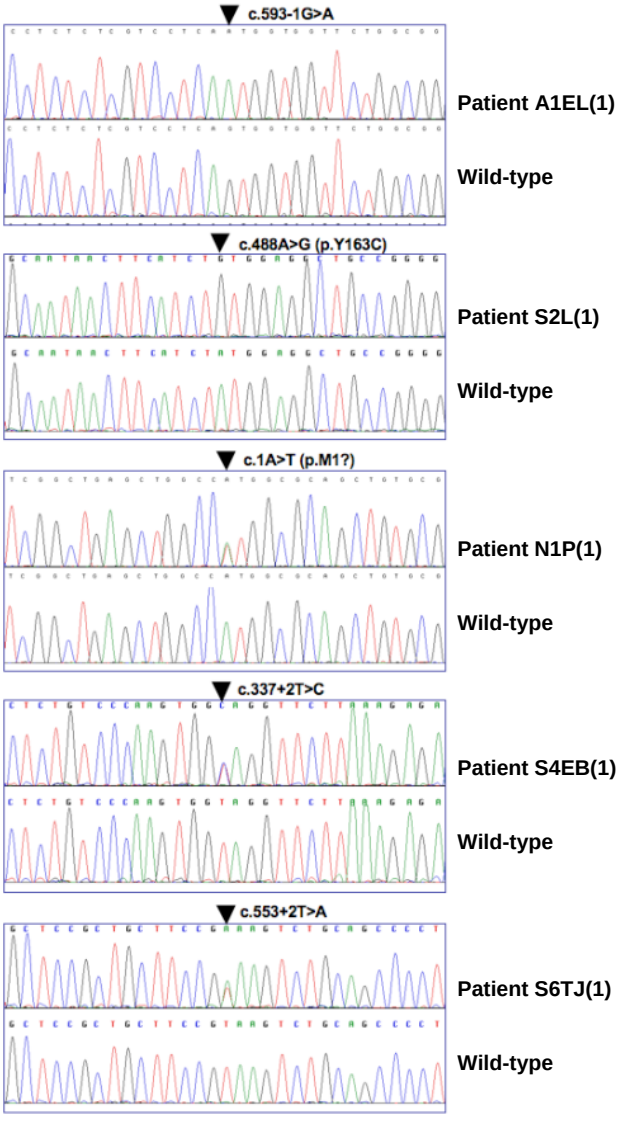
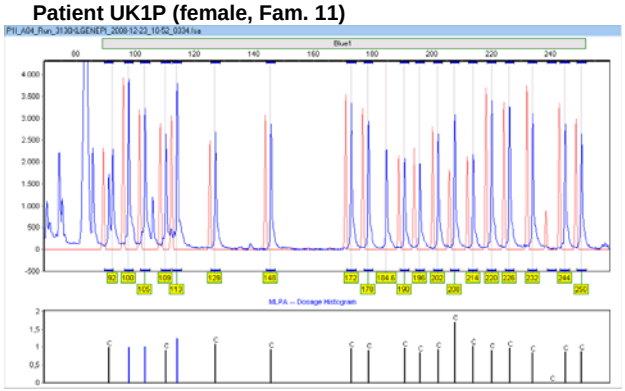
Figure S1. Detection of *SPINT2* Mutations in Syndromic CSD



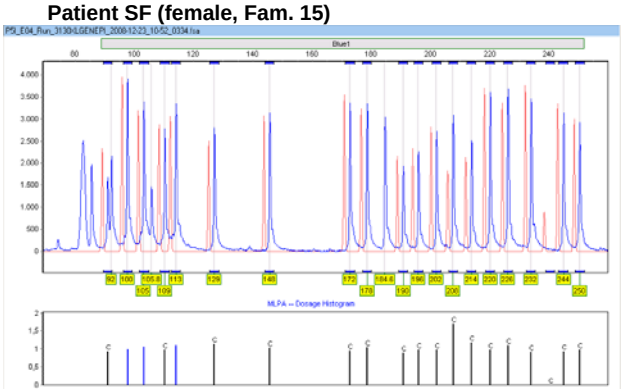
Sequence chromatograms revealing 5 distinct *SPINT2* mutations (arrow heads) identified in patients with syndromic CSD.

Figure S2. MLPA Analysis Does Not Reveal *SPINT2* Copy Number Changes in CSD Patients

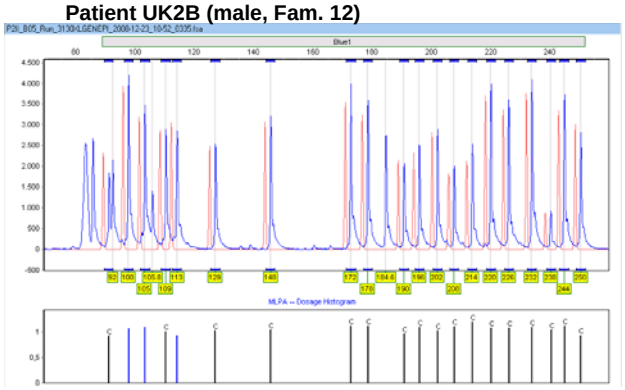
Patient UK1P (female, Fam. 11)



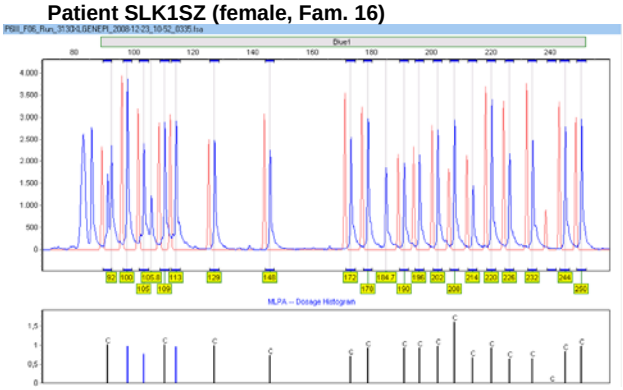
Patient SF (female, Fam. 15)



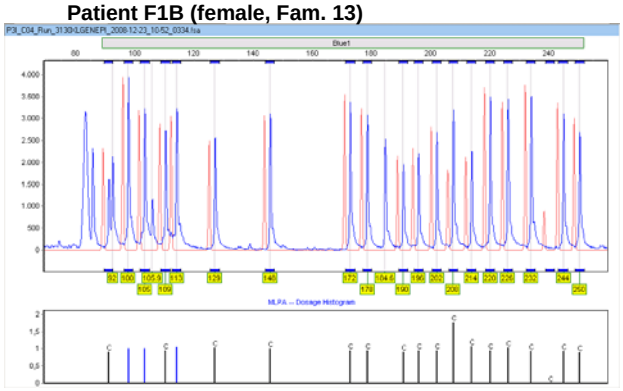
Patient UK2B (male, Fam. 12)



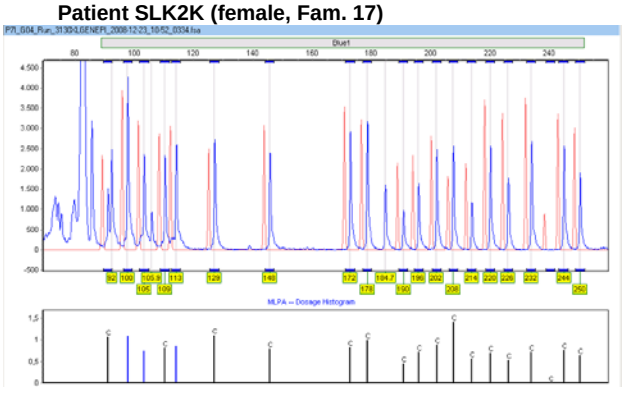
Patient SLK1SZ (female, Fam. 16)



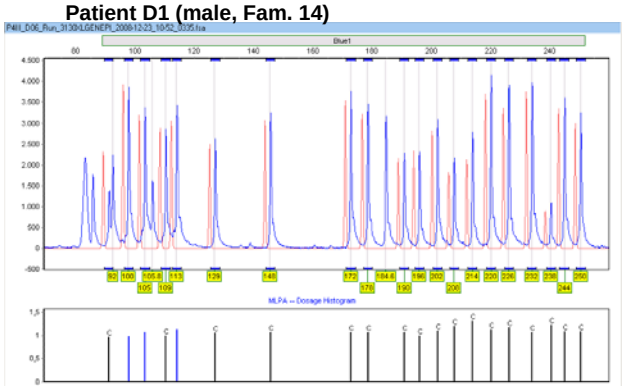
Patient F1B (female, Fam. 13)



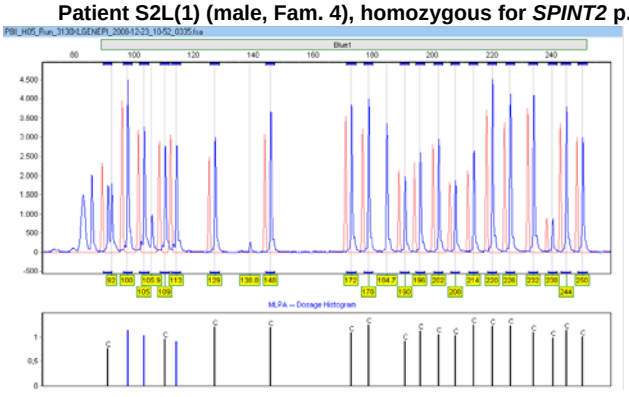
Patient SLK2K (female, Fam. 17)



Patient D1 (male, Fam. 14)



Patient S2L(1) (male, Fam. 4), homozygous for *SPINT2* p.Y163C



Multiplex ligation-dependent probe amplification (MLPA) analysis was conducted for each CSD patient without *SPINT2* mutations identified upon genomic sequencing and for patient S2L(1) who was identified with a homozygous p.Y163C mutation, and compared with the results of 8 control samples. Two panels are shown for each patient. Each upper panel displays the raw data of the fragment analysis including 3 probes corresponding to *SPINT2* exons 5, 2, and 7, respectively, and 17 control probes from different chromosomes contained in the MRC-Holland SALSA MLPA P-300-A1 kit. Fragment traces of patient samples are shown in blue and are each compared with the average of 8 control samples (in red). The size of the fragments is given in bases on the X-axis, and the amount of the fragments is displayed on the Y-axis. The results of the MLPA analyses are shown in the lower panels, and the relative copy numbers for each probe are scaled on the Y-axis. Blue bars correspond to *SPINT2* exons 5, 2, and 7, displayed at 100, 105 and 113 bases, respectively, and black bars correspond to control probes. Control probes displayed at 208 and 238 bases correspond to X- and Y-chromosomal loci, respectively.

Table S1. Primer Sequences for Amplification and Sequencing of *SPINT2* in Genomic DNA

Name	Sequence	fragment length (bp)
SPINT2 1f	ACCTGATCGCGAGACCCC	285
SPINT2 1r	GAACGCCATCAAGTAGCCC	
SPINT2 2f	GATTGCCCTGCCAAGCTAAC	242
SPINT2 2rnn	AACAAAAGCTCCAACTACTGCC	
SPINT2 3f	CTGGCAGTCTCTCGAAAGC	288
SPINT2 3r	CCCATAAGGATGCTGGAGC	
SPINT2 4f	GCCCAGCCTCCCTAACAC	193
SPINT2 4rnn	AGGATGGTCTTGATATCCTGACC	
SPINT2 5f	TCAGGCACTTTCTGGCTTGC	366
SPINT2 5r	GGCTTAGAGGCCTTGCTGC	
SPINT2 6f	CCATGGAGGCCCTGGCTG	265
SPINT2 6rnn	TCACGCAGAAACATGACTTTCTG	
SPINT2 7f	ACTCTGGCTGCAACTCCCC	310
SPINT2 7r	ACTCAAATCCGAGTCAATCCC	
SPINT2 7R3	CACCATCACGAACAGCCCC	89

Table S2. Primer Sequences to Amplify SPINT2 cDNA in Two Overlapping Fragments

SPINT2 1f	ACCTGATCGCGAGACCCC
SPINT2 c5r	gtgactgcgttggcggtgc
SPINT2 c4f	tcctctgtcccaagtgctcc
SPINT2 7r	ACTCAAATCCGAGTCAATCCC

Table S3. Oligos for SPINT2 cDNA Cloning

TopoF	CACCATGGCGCAGCTGTGCGGGC
V5R	CAGGACATATGTGTTCTTCACCAGC
TopoM1F	CACCTTGGCGCAGCTGTGCGGGC

Table S4. Synthetic MLPA Probes to Target Six out of the Seven *SPINT2* Exons

Exon #	Name	5´ modification and 5´-3´ sequence including primer annealing sequence	Complete probe length (bases)
1	SPINT2 1_5	FAM-gggttccctaagggttggaGGCTGAGGCGGAGCCGGGCGTTTCTCG	96
	SPINT2 1_3	Pho-CCCTGCTGGGATCGCTGCTCCTCTCTGtctagattggatcttgctggcac	
2	SPINT2 2_5	FAM-gggttccctaagggttggaCAATGTCACTGACGGATCCTGCCA	105
	SPINT2 2_3	Pho-GCTGTTTGTGTATGGGGGCTGTGACGGAAACAGCAATAAtctagattggatcttgctggcac	
5	SPINT2 5_5	FAM-gggttccctaagggttggaACGCTGGTACTTTGACGTGGAGAGGAACT	100
	SPINT2 5_3	Pho-CCTGCAATAACTTCATCTATGGAGGCTGCtctagattggatcttgctggcac	
7	SPINT2 7_5	FAM-gggttccctaagggttggaAGGAACCAGGAGCGTGCCCTGCGCACCGTCTGGAG	113
	SPINT2 7_3	Pho-CTCCGGAGATGACAAGGAGCAGCTGGTGAAGAACACtctagattggatcttgctggcac	
4	SPINT2 4_5	FAM-gggttccctaagggttggaCAGCTCCCAGAAGGCAGGATTCTGAAGACCACTCCAGC	117
	SPINT2 4_3	Pho-GATATGTTCAACTATGAAGGTAAAACTCCAAAGAGGCtctagattggatcttgctggcac	
3	SPINT2 3_5	FAM-gggttccctaagggttggaCTCTTTCACTGTGCTGTTTCTTTGTCCCCTTGCAGAGAATGCCACGGG	122
	SPINT2 3_3	Pho-ACGGGTGACCTGGCCACCAGCAGGAATGCAGCtctagattggatcttgctggcac	

Synthetic probes were designed according to MRC-Holland (Amsterdam, The Netherlands), http://www.mrc-holland.com/pages/support_desing_synthetic_probespag.html. MLPA analysis was performed using standard conditions.