A *Grammastola spatulata* mechanotoxin-4 (GsMTx4)-sensitive cation channel mediates increased cation permeability in human hereditary spherocytosis of multiple genetic etiologies

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Supplemental Table 1 Legend.

Hematologic indices were measured by automated analysis on the ADVIA 2120 (Siemens). Bilirubin and LDH were measured by automated analysis on the COBAS (Roche).
For candidate gene sequencing, $\leq$ 1 mg total RNA prepared from whole blood of each patient (Rneasy Kit, Qiagen) was used for first strand cDNA synthesis (Retroscript, Ambion). Genomic DNA (gDNA) was also isolated from whole blood of each patient (Dneasy Blood and Tissue Kit, Qiagen). RT-PCR products (36-38 cycles) and/or genomic PCR products of the *SLC4A1*, *SPTB* and *ANK1* genes (36 cycles) were analyzed in 1% agarose gel. Fragments of expected size were excised, purifed (QIAquick Gel Extraction Kit, Qiagen) and subjected to Sanger sequencing. Pathogenic variants were discovered in samples of patients HS3-HS6, HS8, HS9, and HS11 (Table 1).
For whole exome sequencing, gDNA (1 μg) from whole blood of patient HS1 was fragmented by mechanical shearing (Covaris) to obtain fragments of length ~250 nt. DNA fragments were adenylated, adapter-ligated, and hybrid-captured, then processed for library preparation and paired-end sequencing using Novaseq 6000 at 100X coverage. Sequencing data was processed using a workflow including raw reads quality assessment by FastQC, adapter- and quality-trimming by Trimmomatic, alignment using BWA-MEM with hg19, post-alignment quality and removal of PCR duplicates by SAMtools and Picard-Tools (http://picard.sourceforge.net). Variants and indels were detected by GATK and annotated by ANNOVAR.

Exonic DNA fragments from 100 ng sheared gDNA from HS10 and HS12 were captured using the Illumina WES Nextera Kit. Exonic DNA fragments from HS2, HS7 and HS13 were captured using the Nimblegen SeqCap EZ Exome Capture Kit. Next-generation sequencing was conducted with an Illumina HiSeq4000 instrument using a paired-ends 2x100 base-pair protocol. Best practices pipeline recommendations for quality control and variant calling of reads were followed using the Genome Analysis Toolkit (GATK version 3.4-46) [1]. Sequenced reads were aligned to hg19 and analyzed. Varian calling was by GATK Haplotype Caller, and variant annotation was by Variant Effect Predictor (VEP)[2]. Mean target coverage was >91%, and >85% of bases were read at 10x coverage. Of 415,187 detected variants, 109,188 remained after selecting nonsynonymous variants with consequences matching the following terms: splice_acceptor, splice_donor_variant, stop_gained, frameshit_variant, stop_lost, start_lost, protein_altering_variant, missense_variant, coding_sequence_variant. Each pathogenic variant discovered by whole exome sequencing from patients HS1, HS2, HS7, HS10, HS12 and HS13 (Table 1) was subsequently validated by Sanger sequencing.

Supplemental Table 1. Hemolytic indices of patients.

Subject #	Genetic Dx	RBC (x 10^6)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HDW (g/dL)	RDW (%)	Retic (%)	Retic (x10^6/mL)	Bili (T/D) (mg/dL)	LDH (U/L)
HS1	SLC4A1 E68X SLC4A1 R180H	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
HS2	SLC4A1 R150X/E40K Band 3 Lyon in tandem w Band 3 Montefiore	3.75	12.5	34.7	92.7	33.5	36.1	3.38	15.2	5.0	0.189	1.1/0.2	294
HS3, HS4 (sibs)	SLC4A1 R490C sib1	3.88	11.8	32.3	83.2	30.4	36.5	3.83	20.1	7.5	0.365	2.1/0.3	287
	Band 3 Bicetre sib2	3.00	10.0	27.3	81.4	29.8	36.6	3.83	19.6	9.7	0.326	1.5/0.2	398
HS5	SLC4A1 M663del Band 3 Osnabruck	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
HS6	SLC4A1 R870W/K56E Band 3 Prague 3	4.35	12.0	34.9	80.2	27.5	34.3	2.79	13.8	1.5	0.067	<0.1/<0.1	305
HS7	ANK1 E924X SPTA1 R1074H	4.47	12.9	34.3	76.7	28.8	37.5	4.37	18.5	2.3	0.105	0.8/0.2	208
HS8	ANK1 A1110-G1111del	5.03	12.8	34.8	69.3	25.5	36.8	4.44	18.3	3.0	0.151	0.6/0.1	240
HS9	ANK1 K1140Gfs.X87	3.4	8.8	24.6	72.4	25.9	35.8	4.0	17.7	5.6	0.190	0.8/0.2	294
	SLC4A1 P854L/K56E												
HS10	ANK1 E1289Gfs86X	3.69	11.6	30.2	81.9	31.4	38.4	4.68	19.5	10.0	0.369	2.4/0.2	370
HS11	SPTB R1255G ANK1 R619H	3.42	11.1	31.0	90.4	32.4	35.9	3.48	13.9	4.8	0.140	1.2/0.2	197
HS12	SPTB G1450Rfs41X	4.20	12.5	33.1	78.9	29.7	37.7	4.41	18.6	10.8	0.454	2.2/0.2	384
HS13	SPTB E1815AfsX	4.29	12.1	32.5	75.8	28.3	37.3	4.19	17.5	10.0	0.429	2.0/0.3	361
Normal range		3.92-4.72	11.0-12.8	31.5-36.8	76.8-83.3	26.8-29.4	34.2-35.7	2.75-3.21	13.2-14.5	0.8-2.0	0.029-0.080	0.3-0.0 - 1.2-0.4	110-295