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Supplemental Data

Defective Presynaptic Choline Transport

Underlies Hereditary Motor Neuropathy

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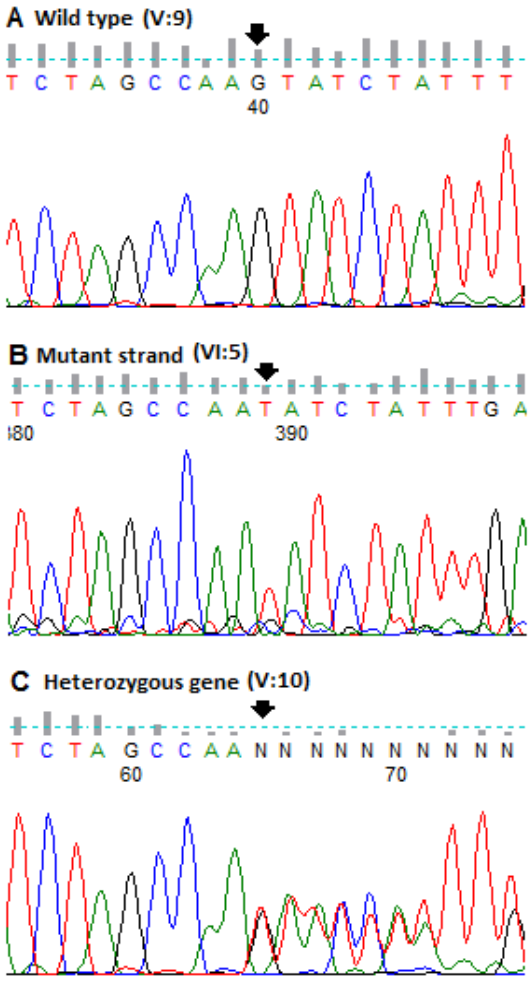

Figure S1. DNA Sequence Analysis of *SLC5A7*

Chromatogram showing the wild-type and c.1497delG variation in *SLC5A7* exon 9 resulting in the NP_068587.1: p.(Lys499Asnfs*13) frameshift mutation. Mutant and wild type sequences were derived by sub-cloning of genomic PCR products (B). Products were cloned using the TA Cloning kit pCR 2.1 vector (Invitrogen) according to the manufacturer's protocol, and the products were subsequently transformed into *E. coli.* BL21(DE3) Singles Competent Cells (Novagen) according to the TA Cloning kit pCR 2.1 vector protocol.

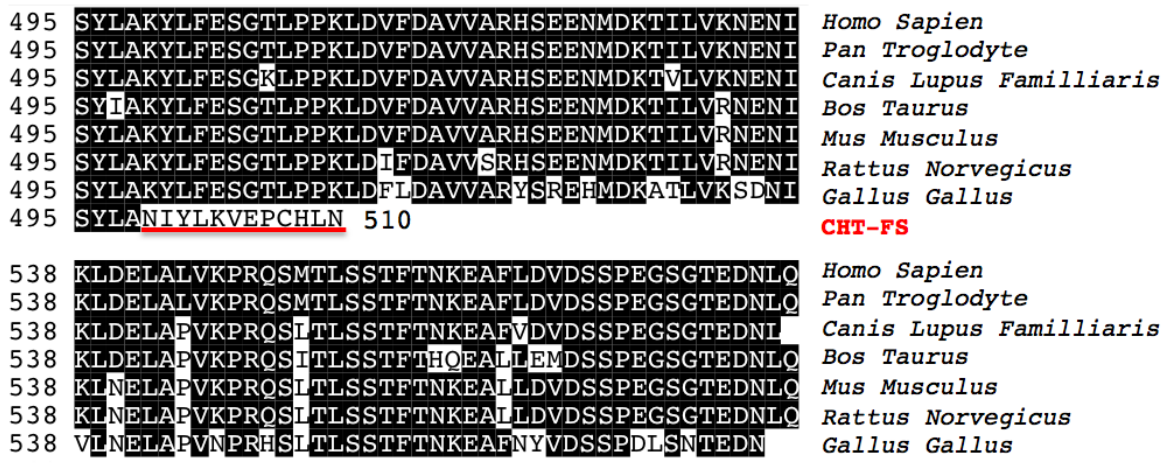

Figure S2. Protein Homology of SLC5A7 in Various Species and the Mutant Molecule

The mutation results in an amino acid substitution at Lys499, the premature truncation of the protein by 82 amino acids and the inclusion of 12 aberrant amino acids at the highly conserved 3' terminus (ClustalW2).

Table S1. Conversion Table of Pedigree Numbering Compared with Previously Published Pedigree

Barwick et al. (2012)	Dick et al. (2001)
V:1	V:1
V:2	V:2
V:3	V:3
V:4	V:4
VI:2	VI:2
V:9	V:10
V:10	V:9
VI:4	VI:6
VI:5	VI:5
V:11	V:11
V:12	V:12
VI:6	VI:7
VI:7	VI:8
VI:8	VI:9
V:16	V:14
V:17	V:13
VI:9	VI:10
VI:10	VI:11
IV:9	IV:9
IV:10	IV:10
V:18	V:22
V:19	V:21
VI:11	VI:12
V:20	V:18
V:21	V:13
V:12	VI:13

Table S2. Sequencing Matrices

Bases	9,877,091,850 (9.8 Gb)
% Q20 bases	96.87%
% Q30 bases	91.57%
Reads used	131,694,558
% Duplication	7.69%
Reads mapped	120,632,748 (91.6%)
Reads mapped (high mapping quality ≥ Q30)	103,839,839 (78.8%)
Mean depth on target regions	107.5
Array	SureSelect All Exon 50Mb (solution)
Mapped to	GRCh37_53 (Human)

Single nucleotide variants (SNVs) were called using two programs (GATK, SamTools) while whereas insertion-deletions (indels) were called using (GATK and Dindel). All variants were annotated using dbSNP (132) and the 1000 genomes pilot study. SNP Effect Predictor (VEP) was used for predicting the variant consequences on the protein structure based on Ensembl (version 61). Both SNVs and indels called by the three programs were then merged into a single file for downstream analysis.

GATK (version 1.0 rebuild 130910)
Reads were mapped to the reference genome, duplicate fragments marked (Picard) and base qualities were recalibrated (GATK). GATK was used to call indels using IndelGenotyper and variants were called using GATK UnifiedGenotyper. Poor quality sites were filtered out near indels and using the following hard filters:

QUAL < 30.0 || AB > 0.75 && DP > 15 || HRun > 5 || SB > -0.10 || DP < 4 || DP > 2000"

Dindel (version 1.01)
Reads were mapped to the reference genome, duplicate fragments marked (Picard) and base qualities were recalibrated (GATK). Dindel was used to generate a list of indel candidates in the union of the bait and design target regions +/- 25bp.

Samtools (version 0.1.7)
Reads were mapped to the reference genome, duplicate fragments marked (Picard) and base qualities were recalibrated (GATK). Samtools was used to call variants only from reads mapped in good pairs (correct insert size range) within the union of the bait and designed target regions. Variants were filtered out if the read depth < 4x or > 1200x, if the consensus quality < 20 or if the SNP quality < 25.

Table S3. Average Number of High-Quality Reads (≥Q30) per Exon in *SLC5A7*

***SLC5A7* Exons**	**Average Number of Read Depth**	**Log10 Average Number of Reads**
Chr2:108602978-108603219	8	0.903089987
Chr2:108604563-108604789	400	2.602059991
Chr2:108608561-108608675	172	2.235528447
Chr2:108609427-108609583	401	2.603144373
Chr2:108614293-108614442	804	2.905256049
Chr2:108618352-108618496	305	2.484299839
Chr2:108622504-108622658	324	2.51054501
Chr2:108624920-108625138	291	2.463892989
Chr2:108626687-108627317	875	2.942008053

SLC5A7 exons are well covered including the 9th exon that harbours the frameshift mutation under study. There are fewer reads covering the first exon due to high GC content**.**

Table S4. Fatiguibility and Decremental EMG Response

Subject	Age/Sex	Repetitive Stimulation	Single Fibre EMG
V:19	56y/M	Recording of right ADM at rest and up to 1 min post activation showed no significant decrement.	Right EDC: no spontaneous activity, an excess of complex polyphasic units on volition, quantitative jitter studies showed an excess of units with jitter and blocking.
V1:12	28y/M	Recording of right ADM at rest and up to 1 min post activation showed no significant decrement.	Right EDC: no spontaneous activity, an excess of stable polyphasic units noted on volition, quantitative jitter studies showed an excess of complex units with increased jitter and occasional blocking.

ADM=adductor digiti minimi, EDC=extensor digitorum communis, M=male.

At 2- 3 Hz stimulation or abnormal jitter and blocking on single fibre EMG are seen often in CMS. Specialist NMJ electrophysiology evaluation was carried out in 2 family members. Repetitive stimulation showed no significant decrement. EMG quantitative jitter studies showed an excess of jitter and blocking. This is suggestive of disturbance of the NMJ but it is not clear if this is all secondary to reinnervation or if there is an element of primary disturbance of NMJ as well. The changes were less marked in the younger patient with less progressed disease.