Expanding the phenotype of *SLC12A6*-associated sensorimotor neuropathy

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SUMMARY

Hereditary motor and sensory neuropathy with agenesis of the corpus callosum (HMSN/ACC) is a rare autosomal recessive condition characterised by early-onset severe progressive neuropathy, variable degrees of ACC and cognitive impairment. Mutations in *SLC12A6* (solute carrier family 12, member 6) encoding the K+–Cl- transporter KCC3 have been identified as the genetic cause of HMSN/ACC. We describe fraternal twins with compound heterozygous mutations in *SLC12A6* and much milder phenotype than usually described. Neither of our patients requires assistance to walk. The female twin is still running and has a normal intellect. Charcot-Marie-Tooth Examination Score 2 was 8/28 in the brother and 5/28 in the sister. Neurophysiology demonstrated a length-dependent sensorimotor neuropathy. MRI brain showed normal corpus callosum. Genetic analysis revealed compound heterozygous mutations in *SLC12A6*, including a whole gene deletion. These cases expand the clinical and genetic phenotype of this rare condition and highlight the importance of careful clinical phenotyping.

BACKGROUND

Charcot-Marie-Tooth (CMT) disease or hereditary motor and sensory neuropathy (HMSN) is the most frequent inherited neuromuscular disorder.[1] *SLC12A6* (solute carrier family 12, member 6) encodes the K+–Cl-transporter KCC3 and pathogenic variants in this gene are associated with HMSN with variable degrees of agenesis of the corpus callosum in most cases (HMSN/ACC).[2] This is an autosomal recessive condition with onset in infancy, characterised by severe progressive sensorimotor neuropathy and cognitive impairment. The K+/Cl−cotransporter family are crucial in ion homeostasis, cell volume regulation and modulation of the cellular responses to GABA during neuronal development. HMSN/ACC was first described in Quebec[2] with fewer reports among populations outside French-Canada.

CASE PRESENTATION

27-year old fraternal twins had normal developmental milestones and walked by 15 months. There is no history of consanguinity.

The male twin, patient 1, started to trip at 5 years. He played sports in primary school but was always last in races. He has been unable to run since his teens and has worn orthotics since 21, having foot surgery at 26. He had difficulty holding a pen, writing, closing buttons and opening jars but no sensory symptoms. He required special educational needs assistance throughout school and a scribe for final exams due to his difficulty with handwriting; however, he remained in mainstream school and subsequently completed third-level education in an institute of technology. On examination, he had marked bilateral foot drop and walked with a broad base and waddle. He was unable to stand on his heels. He had a high arched palate and a convergent strabismus. He had scoliosis with winging of the left scapula and wasting from mid-forearms and knees distally with pes cavus and hammer toes bilaterally. He had length-dependent weakness predominantly affecting the intrinsic hand muscles, ankle dorsiflexion and eversion. He was areflexic throughout. Pinprick sensation was normal. Vibration was reduced to costal margin bilaterally. Joint position sense and temperature were normal. CMT examination score 2 (CMTES2) was 8/28 which is in the mild–moderate range.[3]

The female twin, patient 2, is less severely affected. She had difficulty in fitting shoes in the first decade and always had difficulty opening jars. She had foot surgery in the teens. She has worn orthotics since 20 years. She did not require any additional help in school and finished university. On examination, she had bilateral foot drop. She had a high arched palate and pes cavus bilaterally. She had mild weakness in the intrinsic hand muscles to MRC grade 4 and mild weakness of ankle dorsiflexion. She was areflexic throughout. Plantars were flexor. Pin and joint position sensation were normal. Vibration was reduced to costal margin bilaterally. CMTES2 score was 5/28 indicating mild severity.

INVESTIGATIONS

MRI brain showed normal corpus callosum without significant atrophy (figure 1). Neurophysiology demonstrated a length-dependent, sensorimotor axonal neuropathy with some evidence of dispersion and conduction block (table 1).

Analysis of 56 genes associated with inherited peripheral neuropathy using a next-generation sequencing (NGS) targeted panel assay revealed compound heterozygous variants in *SLC12A6* (NCBI RefSeq NM_133647.1): c.1592–2A>G, p.? in intron 11, not recorded in dbSNP, Exome Variant Server, or the Genome Aggregation Database and a heterozygous deletion of the entire coding region. Microarray comparative

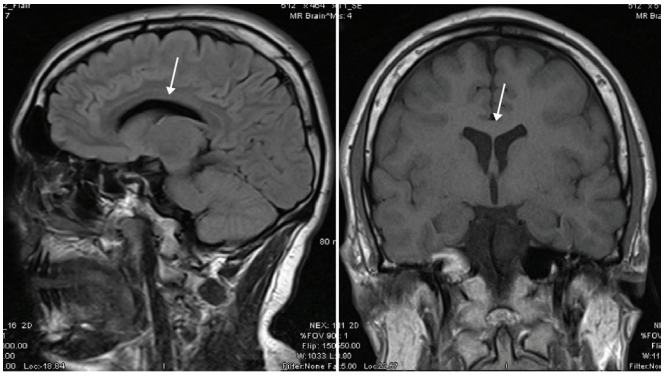

Figure 1 MRI brain of patient 1. MRI brain with intact corpus callosum.

genomic hybridisation using the 8×60K ISCA v2.0 array (Oxford Gene Technology) with reference GRCh37/hg19 showed a 1.8 Mb deletion of the long arm of chromosome 15, at bands q13.3-q14. The karyotype is arr 15q13.3q14(32,899,499-34,695,199)x1. 16 protein-coding genes including *SLC12A6* mapped to the minimum deleted segment; *ARHGAP11A, AVEN, CHRM5, EMC4, EMC7, FMN1, SMN1, GOLGA8A, GREM1, KATNBL1, LPCAT4, NOP10, NUTM1, RYR3, SCG5* and *SLC12A6*. Parental segregation was confirmed; their mother is heterozygous for the deletion and their father is heterozygous for the c.1592–2G>A variant.

OUTCOME AND FOLLOW-UP

Overall, both twins reported no change in their weakness and at 12-month follow-up assessment, both had very similar neurological examination. The female twin can still run and her brother remains independent.

DISCUSSION

These twins with compound heterozygous variants in *SLC12A6* have a considerably milder phenotype than previously described with recessive disease. Typically, patients have significant difficulties ambulating, either never walking or becoming wheelchair users in childhood[4] and marked mental retardation. Most die by their early 30 s.[5] Recessive *SLC12A6*-associated peripheral neuropathy has been reported in 113 individuals to date, the vast majority from Quebec (table 2). More recently, heterozygous de novo SLC12A6 variants were documented as causing early-onset progressive CMT with or without spasticity in four individuals. Neither of our patients requires assistance to walk or has dysmorphic facies. Both achieved third level education and remain fully ambulant. Both have a high arched palate and a sensorimotor neuropathy in keeping with *SLC12A6*-associated phenotype but neither has ACC.

In contrast to our twins with a much milder phenotype than usually described in recessive *SLC12A6*-associated peripheral neuropathy, normal cognitive development with normal MRI brain has been reported only in four unrelated individuals with heterozygous *de novo* dominant variants in the *SCL12A6* gene.[6 7]

The *SLC12A6* NM_133647.1:c.1592–2A>G, p.? has not been described previously and is classified as likely pathogenic as per the American College of Medical Genetics and Genomics (ACMG) guidelines. The guanine nucleotide is highly conserved and this substitution is predicted to affect splicing; potentially causing exon skipping and resulting in premature truncation of the mRNA product leading to nonsense-mediated decay. In addition, no protein would be produced from an allele carrying the whole gene deletion. It is therefore possible that c.1592–2A>G along with deletion of the entire coding region in trans could result in absence of SLC12A6 protein.

Table 1 Nerve conduction studies of fraternal twins with hereditary motor and sensory neuropathy due to compound heterozygous mutations in *SLC12A6*

	Patient 1			Patient 2		
Motor	**Lat (ms)**	**Amp (mV)**	**CV (m/s)**	**Lat (ms)**	**Amp (mV)**	**CV (m/s)**
Median-right						
Wrist-abductor pollicis brevis	5.55 (≤4.0)	1.93 (≥5.0)		2.96	4.6	
Elbow-wrist	11.3	1.64	43 (≥50)	7.59	4.0	47.5
Ulnar-right						
Wrist-abductor digiti minimi	2.32 (≤3.5)	6.0 (≥7.0)		2.83	5.6	
Below elbow-wrist	7.12	4.9	51 (≥50)	6.97	3.9	50.7
Peroneal-left						
Ankle-extensor digitorum brevis	19.9 (≤6.0)	(≥2.5)	(≥40)	4.46	1.84	
Below knee-ankle				12.7	0.53	37.5
Tibial-left						
Ankle-abductor halluces	4.29 (≤6.0)	3.7 (≥4.0)		3.57	6.4	
Knee-ankle	15.9	1.14	34.7 (≥40)	12.4	2.3	43.9
Sensory	**Lat (ms)**	**Amp (μV)**	**CV (m/s)**	**Lat (ms)**	**Amp (μV)**	**CV (m/s)**
Median-right (orthodromic)						
Digit III-wrist	2.75 (≤3.5)	12.6 (≥7)	51.3 (≥50)	2.18	16	54.1
Radial-right (antidromic)						
Radial forearm-snuffbox	Not performed			1.19 (≤2.9)	43.2 (≥16)	64.7 (≥50)
Sural-left (antidromic)						
Midcalf-ankle	2.29 (≤4.4)	1.99 (≥6.0)	42.8 (≥40)	2.12	3.3	46.2

Amp, amplitude; CV, conduction velocity; Lat, latency; ms, milliseconds; m/s, metres per second; mV, millivolt; μV, microvolt.

Table 2 Summary of cases described with *SLC12A6*-associated phenotype

Reference	n=Individuals (n=Families)	SLC12A6 variants	LD	NP	Dysmorphic Features	ACC	Other features
2	85 (unknown)	Hom c.2436delG; c.2436delG / Hom c.3031C>T; Hom c.2023C>T; c.1584_1585delCTinsG	NA	NA	NA	NA	?macrocephaly, stenosis Aq Sylvius?
8	2 (1)	c.1616G>A/ c.1118+1G>A		+		+in 1	
9	1	Hom for whole region of 15q13-q15	+	+	+	+	Seizure
10	3 (3)	c.(1478_1485)/(2032dup) Hom c.901del; Hom c.619C>T	+	+	+	+	White matter lesions on MRI
11	1	Hom c.571_572dup	+	+	+	+	Facial diplegia, eye movement abnormalities, cerebellar ataxia
12	1	Hom. c.3031C>T	+	+	+	+	Seizures
13	10 (6)	Hom c.3031C>T; Hom c.2994_3003del	+	+		+	Seizures, gaze palsy
14	3 (2)	Hom c.3402C>T; Hom c.619C>T	+	+		+	Seizures
15	3 (1)	Hom c.1073+1G>A	+	+	+	+	
16	2 (1)	c.2097dup /(?)	+	+		+	
17	1	Hom c.2604delT	+	+	+	+	
18	1	Hom c.1943+1G>T	+	+	—	—	
6	1	Het c.2971A>G	—	+	—	—	
7	3 (3)	Het c.620G>A Het c.2036A>G	—	+	—	—	Spasticity in 1

ACC, agenesis of the corpus callosum; het, heterozygous; hom, homozygous; LD, learning difficulty; NA, not available; NP, neuropathy.

These cases expand the clinical and genetic spectrum of *SLC12A6*-associated phenotype and highlight the importance of careful clinical phenotyping. With widespread availability of NGS, it is increasingly common to find potentially causative variants in unexpected genes. *SLC12A6* would not have been considered as a candidate gene for testing in these twins given their atypical presentation; however, NGS technology overcomes the limitations of pre-existing genotype–phenotype associations. The case also highlights the benefit of a co-ordinated NGS and microarray approach in order to provide an accurate diagnosis. With the advancement of genetic technology, further expansion of clinical phenotypes is anticipated.

Learning points

- *SLC12A6*-associated phenotype ranges from severe progressive sensorimotor neuropathy with agenesis of the corpus callosum and cognitive impairment to pure neuropathy.
- It is increasingly common to find potentially causative variants in unexpected genes with widespread availability of next-generation sequencing.
- These cases highlight the advantage of co-ordinated next-generation sequencing and microarray approach to provide an accurate diagnosis.
- Careful clinical phenotyping is crucial.

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