HHS Public Access

Author manuscript

J Med Genet. Author manuscript; available in PMC 2024 May 07.

Published in final edited form as:

J Med Genet. 2020 April ; 57(4): 283–288. doi:10.1136/jmedgenet-2019-106273.

De novo variants in *SLC12A6* cause sporadic early-onset progressive sensorimotor neuropathy

Joohyun Park[1,2], **Bianca R. Flores**[3], **Katalin Scherer**[4], **Hanna Kuepper**[5], **Mari Rossi**[6], **Katrin Rupprich**[7], **Maren Rautenberg**[1], **Natalie Deininger**[1], **Annette Weichselbaum**[5], **Alexander Grimm**[2], **Marc Sturm**[1], **Ute Grasshoff**[1], **Eric Delpire**[3], **Tobias B. Haack**[1,8]

[1]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany

[2]Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany

[3]Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[4]Neuromuscular Clinic, Children's Clinic for Rehabilitative Services, Tucson, Arizona, USA

[5]Department of Neuropediatrics, University of Tübingen, Tübingen, Germany

[6]Department of Clinical Diagnostics, Ambry Genetics, Aliso Viejo, California, USA

[7]Department of Neuropediatrics, Essen University Hospital, Essen, Germany

[8]Centre for Rare Diseases, University of Tübingen, Tübingen, Germany

Abstract

Background—Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. Biallelic variants in *SLC12A6* have been associated with autosomal-recessive hereditary motor and sensory neuropathy with agenesis of the corpus callosum (HMSN/ACC). We identified heterozygous *de novo* variants in *SLC12A6* in three unrelated patients with intermediate CMT.

Methods—We evaluated the clinical reports and electrophysiological data of three patients carrying *de novo* variants in *SLC12A6* identified by diagnostic trio exome sequencing. For functional characterization of the identified variants, potassium influx of mutated KCC3 cotransporters was measured in Xenopus oocytes.

Correspondence to Dr Tobias B Haack, Institute of Medical Genetics and Applied Genomics, University of Tübingen, 72076 Tübingen, Baden-Württemberg, Germany, tobias.haack@med.uni-tuebingen.de.

Contributors

JP collected and analyzed clinical and genetic data and was responsible for drafting the manuscript. BRF conducted functional experiments, collected and analyzed the functional data and contributed to manuscript. KS, HK, KR, AW, AG and UG contributed to phenotyping, acquisition, and analysis of clinical and electrophysiological data. MR, MR, ND and MS contributed to analysis and interpretation of genetic data. TBH and ED were responsible for the conception, design and supervision of the study and writing of the manuscript. All authors revised the manuscript for intellectual content.

Competing interests: None declared.

Patient consent for publication: Obtained.

Results—We identified two different *de novo* missense changes (p.Arg207His and p.Tyr679Cys) in *SLC12A6* in three unrelated individuals with early-onset progressive CMT. All presented with axonal/demyelinating sensorimotor neuropathy accompanied by spasticity in one patient. Cognition and brain MRI were normal. Modelling of the mutant KCC3 cotransporter in *Xenopus* oocytes showed a significant reduction in potassium influx for both changes.

Conclusion—Our findings expand the genotypic and phenotypic spectrum associated with *SLC12A6* variants from autosomal-recessive HMSN/ACC to dominant-acting de novo variants causing a milder clinical presentation with early-onset neuropathy.

INTRODUCTION

Charcot-Marie-Tooth disease (CMT), also known as hereditary sensory and motor neuropathy (HSMN), is the most common inherited disorder of the peripheral nervous system with a variety of inheritance pattern that can be autosomal-dominant, autosomal-recessive or X linked.[1] Demyelinating CMT with median motor nerve conduction velocity (MNCV) below 38 m/s is classified as CMT1 and axonal CMT with MNCV above 45 m/s as CMT2. For CMT with axonal and demyelinating features with MNCV between 25 and 45 m/s, the term intermediate CMT has been introduced.[1–3] To our knowledge, six genes (DNM2, YARS, MPZ, INF2, GNB4 and NEFL) have been associated with autosomal-dominant intermediate CMT (DI-CMT) and assigned with phenotype Mendelian Inheritance in Man numbers.[2] Due to advances in sequencing technologies, the number of CMT-associated genes almost doubled in the past 10 years.[4] The contribution of de novo sequence variation in the pathogenesis of a number of sporadic early-onset neurological phenotypes is increasingly recognised.[5,6] In particular, the application of trio exome sequencing led to the identification of numerous disease-causal de novo mutations, thereby expanding the phenotypic spectrum associated with many disease loci and linking dominant phenotypes to genes that have originally been reported in the context of recessive disorders.[4,7] Potassium chloride cotransporters (KCC1, KCC2, KCC3 and KCC4) are encoded by the SLC12A gene family (*SLC12A4*, *SLC12A5*, *SLC12A6* and *SLC12A7*) and are involved in regulation of the intracellular ionic milieu by mediating electroneutral potassium and chloride efflux in response to osmotic changes.[8] By modulating intracellular chloride concentrations, KCC plays an important role in the maintenance of cell volume, neural excitability and epithelial transport.[9] KCC3, which is encoded by SLC12A6, is predominantly expressed in the brain and spinal cord (neurons and glial cells) of the central nervous system and in the dorsal root ganglion of the peripheral nervous system.[10, 11] Dysregulation of chloride concentrations in neurons may influence the neuronal activity and their response to GABA.8 However, the specific pathophysiology of peripheral neuropathy caused by KCC3 defects is yet unclear.

Biallelic loss-of-function variants in *SLC12A6* are associated with hereditary motor and sensory neuropathy with agenesis of the corpus callosum (HMSN/ACC), also known as Andermann syndrome (OMIM #218000), which is characterized by severe progressive motor and sensory neuropathy, developmental delay, intellectual disability and variable degrees of agenesis of the corpus callosum.[12–14] A single heterozygous de novo missense variant (p.Thr991Ala) has been postulated to act as a gain-of-function variant causing severe motor neuropathy without agenesis of the corpus callosum in a 10-year-old patient.[15] Using

diagnostic trio exome sequencing, we identified two new de novo variants in *SLC12A6*, affecting evolutionarily highly conserved amino acid residues, in three unrelated patients with early-onset, severe and progressive CMT without intellectual disability. Our results further support the hypothesis of dominant-acting heterozygous de novo SLC12A6 variants as the underlying disease mechanism in a subset of CMT patients in addition to the well-established recessive-type *SLC12A6* variants causing a more severe clinical presentation.

MATERIALS AND METHODS

Patients

Patients were examined by neuropaediatricians and referred for diagnostic genetic testing to different centres. The investigated patients or their guardians provided informed written consent for diagnostic exome sequencing and data analysis in the context of the respective disease phenotypes in a routine diagnostic setting. In a second step, clinical and genetic data were retrospectively evaluated in more detail after receiving additional informed consents from the patients' families including also the permission for data publication.

Diagnostic exome sequencing

Diagnostic exome sequencing was performed on DNA isolated from patients' and parental EDTA-blood as described previously.[16]

Functional analysis

KCC3-mediated K^+ transport was measured using unidirectional ^{86}Rb uptakes in groups of 20–25 *Xenopus laevis* oocytes injected with 5 ng c-myc-tagged wild-type or mutant KCC3 cRNA.[17, 18] Oocytes were incubated with 1 mL isosmotic or hyposmotic flux solution containing 5 μCi ^{86}Rb for 1 hour then washed four times with ice-cold solution. ^{86}Rb uptake in single oocyte was measured by β-scintillation counting, and K^+ influx was expressed in pmoles K^+ per oocyte per hour. For Western blot analysis, groups of 8 oocytes were homogenized in CHAPS-containing lysis buffer, subjected to 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membrane, incubated with mouse monoclonal anti-cmyc antibody (clone 9E10, Thermo Fisher Scientific) followed by horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibody.

RESULTS

Clinical presentation

Detailed clinical and electrophysiological data are summarized in table 1.

Patient 1 (Arg207His)—This 14-year-old boy is the second child of healthy German parents (Figure 1A). He learnt to walk freely at 18 months and showed bilateral muscle weakness in the lower limbs with frequent falls. He did not show any learning difficulties and he now has normal speech and cognition. On examination, he presented with an intermediate type sensorimotor predominantly demyelinating neuropathy and diminished patellar and deep tendon reflexes. He had distal more than proximal muscle atrophy

and weakness, but clinically intact proprioception and nociception. Mean MNCV was determined between 23 and 33 m/s, distal motor latencies were prolonged and sensory nerve conduction velocity was reduced. Nerve hypertrophy was not observed in the nerve sonography of the roots, the large upper and lower limb nerves. He required a right ankle foot orthosis due to contractures at the age of 9 years. Mild haemolytic anaemia was observed since the age of 4 years, which could not be explained so far. MRI scans of the brain were normal. Epileptic seizures were not reported.

Patient 2 (Arg207His)—This 11-year-old boy was born by vaginal delivery; he is the fourth of five siblings (Figure 1A). His early motor milestones were normal. Walking was delayed and his gait remained impaired. He never achieved running. MRI of the brain and lumbar spine were normal. He developed spells of shaking which did not have an EEG correlate at age 9 years and were diagnosed as anxiety. One EEG showed a 2 s burst of generalized spike and wave activity in sleep. He never had a clinical seizure, and his cognition was normal. EMG at age 5 years showed a severe motor and sensory peripheral neuropathy. Repeat EMG at age 11 years showed progression of a severe mixed axonal/demyelinating (predominantly demyelinating) chronic neuropathy. His gait and fine motor skills progressively worsened. On examination, he has length-dependent sensory loss, and distal more than proximal muscle weakness, unobtainable tendon reflexes and muscle atrophy (Figure 1B). He ambulates with braces and falls frequently. He was recently diagnosed with obstructive sleep apnea.

Patient 3 (Tyr679Cys)—Patient 3 (figure 1A) is a 15-year-old girl who demonstrated mild motor developmental delay. She learnt to walk independently at 24 months. At 13 years, she was small for her age with 38 kg (10th centile) and 141 cm (3rd centile). Head circumference has always been within normal range (52.2 cm, 10th centile). She showed marked distal lower limb weakness and hand weakness. Apart from ankle contractures and pes planovalgus, other deformities such as scoliosis or dysmorphic features were not noted. Neurological examination revealed spastic gait with brisk patellar and deep tendon reflexes. Vibration sense and pinprick sensation was normal in the limbs but reduced in the periumbilical region. Electrophysiological examinations over the course of 3 years revealed a progressive axonal/demyelinating neuropathy with MNCV between 33 and 45 m/s. MRI of the brain and spine were normal. She suffered from celiac disease and occasional migraine attacks. There were neither organic malformations, complicated hospitalizations nor any evidence of seizures.

Genetic evaluation

Prior to diagnostic trio exome, mutations in CMT-related genes, including copy number variants in PMP22 gene were excluded in patients 1 and 2. Diagnostic trio whole exome sequencing revealed de novo missense variants in *SLC12A6* (NM_133647.1) in patients 1, 2 and 3. Patients 1 and 2 carry the same missense variant c.620G>A, p.Arg207His and patient 3 carries the missense variant c.2036A>G, p.Tyr679Cys (figure 1A, table 1). Both variants are absent from public databases (1000 Genomes project, Genome Aggregation Database (gnomAD r2.0.2), Exome Aggregation Consortium (ExAC r0.3), 04/2019) as well as an in-house database.

Functional analyses

To assess the functional consequence of single amino acid substitutions in KCC3, we performed standard K^+ influx measurements in *Xenopus laevis* oocytes injected with wild-type or mutant mouse KCC3 RNA. The mouse sequence is highly conserved, and all mutated and neighboring residues are identical between the two species. In fact, these residues are also conserved with all members of the SLC12A family of transporters (Figure 1C) and within KCC3 are conserved from *C. elegans* to *H. sapiens* (Figure 1D). As seen in Figure 1E, the level of K^+ influx under isosmotic conditions (grey boxes) in KCC3-injected oocytes is not different from that of water-injected oocytes. This reflects the known absence of KCC3 function under isotonic conditions. When oocytes are subjected to a hypotonic shock (black boxes), K^+ influx is significantly higher in KCC3-injected oocytes than basal flux in water injected oocytes. This KCC3-mediated activity is absent in oocytes injected with either Arg270His or Arg270Cys mutant transporters ($p < 0.001$, one way analysis of variance (ANOVA)). We also tested function of the Tyr679Cys mutant and although while its function was not completely eliminated like in the Arg270 mutants, the level of flux was significantly reduced compared with wild-type KCC3 ($p < 0.05$, one way ANOVA).

DISCUSSION

Clinical features observed in the three patients reported in this manuscript are milder than the previously reported patients with Andermann syndrome with biallelic truncating or missense variants in *SLC12A6*, but similar to the patient reported by Kahle et al (2016)[15] with a heterozygous *de novo* variant, p.Thr991Ala.[15] All our patients presented with early motor developmental delay, muscle weakness and progressive intermediate, mixed mostly predominantly demyelinating neuropathy, particularly affecting the lower limbs. Nerve enlargement has not been found in patient 1. All three patients had normal cognition and brain MRI. No seizures, learning difficulties, hearing problems or dysmorphic features were observed, although patient 2 (p.Arg207His) had pathological EEG findings without any reported seizures. Patient 3 with p.Tyr679Cys exhibited spasticity of the lower limbs in addition to sensorimotor neuropathy. Foot and spine deformities such as scoliosis, pes cavus and planovalgus foot were also observed. In contrast, patients with Andermann syndrome present with a more severe and complex neurodevelopmental disorder with progressive sensorimotor neuropathy, dysmorphic features, developmental delay, intellectual disability, seizures and agenesis of the corpus callosum.[12,13] Similarly, $Slc12a6^{-/-}$ mice exhibit a severe phenotype with impaired motor function and significant axonal swelling accompanied by hypomyelination while $Slc12a6^{-/+}$ mice are asymptomatic.[19] Although p.Thr991Ala was found in a heterozygous state in one patient, the heterozygous KCC3 Thr991Ala mice do not display any abnormalities while homozygous KCC3 Thr991Ala mice demonstrate a severe phenotype comparable to the knockout mice.[15] However, not significant, a slightly decreased sciatic nerve amplitude could be observed in the heterozygous mice compared with that of the wild-type mice.[15] While the heterozygote mice do not display an overt phenotype, their nerve fibres showed intermediate shrinkage when compared with wild-type or homozygote mice.[20]

To date, mostly truncating variants (nonsense, splice and frameshift) have been reported to cause the recessive disorder. One missense variant, p.Gly539Asp, has been identified in a compound-heterozygous state with a truncating variant and a homozygous missense variant p.Arg207Cys was reported in a patient affected by the Andermann syndrome.[13, 14] The missense variant p.Arg207His, affecting the same codon, was found recurrent in a heterozygous state in two of our patients. Functional studies demonstrated a complete loss-of-function for the variants p.Arg207His and p.Arg207Cys, while p.Tyr679Cys only partially reduced the influx of potassium ions in Xenopus oocytes. In contrast, the previously published de novo variant p.Thr991Ala showed increased potassium influx indicating a gain-of-function. This was demonstrated in mouse fibroblasts natively expressing the mutant transporter,15 as well as in HEK293 cells overexpressing the mutated threonine residue.[8, 21] It is striking that p.Arg207His and p.Arg207Cys show different inheritance patterns, although they affect the same amino acid codon and show similar biophysical properties. Although the heterozygous de novo variants cause a homogeneous phenotype, our experimental data assessed in *Xenopus* oocytes displayed different functional features in all identified variants. Thus, our current data do not reveal a distinct correlation between the biophysical properties of mutant cotransporters and the difference in inheritance pattern, which have to be caused by other, so far unknown factors.

CONCLUSION

Diagnostic trio exome sequencing has proven an efficient tool to establish new disease genes and/or variants that are involved in early-onset neuropathies. We provide evidence that heterozygous *de novo* variants in *SLC12A6* cause early-onset progressive CMT with or without spasticity, which is milder than the previously reported recessive phenotype. Thus, autosomal-dominant inheritance of *SLC12A6* variants also needs to be considered in patients with early-onset neuropathies. Further functional studies are needed to determine the exact mechanism by which different variants in *SCL12A6* that might even affect the same amino acid residue, exert their detrimental effect either in an autosomal-recessive or autosomal-dominant fashion.

Acknowledgements

We thank all families and patients for their declaration of consent to the publication.

Funding

This study was supported by the German Bundesministerium für Bildung und Forschung (BMBF) through the Juniorverbund in der Systemmedizin 'mitOmics' (FKZ 01ZX1405C to TBH), the intramural fortüne program (#2435-0-0) and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer (418081722)

REFERENCES

1. Tazir M, Hamadouche T, Nouioua S, Mathis S, Vallat J-M. Hereditary motor and sensory neuropathies or Charcot–Marie–Tooth diseases: an update. J Neurol Sci 2014; 347: 14–22. [PubMed: 25454638]
2. Berciano J, García A, Gallardo E, Peeters K, Pelayo-Negro AL, Álvarez-Paradelo S, Gazulla J, Martínez-Tames M, Infante J, Jordanova A. Intermediate Charcot–Marie–Tooth disease: an

electrophysiological reappraisal and systematic review. J Neurol 2017; 264: 1655–1677. [PubMed: 28364294]

3. Nicholson G, Myers S. Intermediate forms of Charcot-Marie-Tooth neuropathy. Neuromolecular Med 2006; 8: 123–130. [PubMed: 16775371]
4. Rossor AM, Polke JM, Houlden H, Reilly MM. Clinical implications of genetic advances in Charcot–Marie–Tooth disease. Nat Rev Neurol 2013; 9: 562–571. [PubMed: 24018473]
5. Veltman JA, Brunner HG. De novo mutations in human genetic disease. Nat Rev Genet 2012; 13: 565–575. [PubMed: 22805709]
6. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WSW, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OT , Thorsteinsdottir U, Stefansson K. Rate of de novo mutations and the importance of father's age to disease risk. Nature 2012; 488: 471–475. [PubMed: 22914163]
7. Fassio A, Esposito A, Kato M, Saitsu H, Mei D, Marini C, Conti V, Nakashima M, Okamoto N, Olmez Turker A, Albuz B, Semerci Gündüz CN, Yanagihara K, Belmonte E, Maragliano L, Ramsey K, Balak C, Siniard A, Narayanan V, Ohba C, Shiina M, Ogata K, Matsumoto N, Benfenati F, Guerrini R. De novo mutations of the ATP6V1A gene cause developmental encephalopathy with epilepsy. Brain 2018; 141: 1703–1718. [PubMed: 29668857]
8. Rinehart J, Maksimova YD, Tanis JE, Stone KL, Hodson CA, Zhang J, Risinger M, Pan W, Wu D, Colangelo CM, Forbush B, Joiner CH, Gulcicek EE, Gallagher PG, Lifton RP. Sites of regulated phosphorylation that control K-Cl cotransporter activity. Cell 2009; 138: 525–536. [PubMed: 19665974]
9. Gagnon KB, Delpire E. Physiology of SLC12 transporters: lessons from inherited human genetic mutations and genetically engineered mouse knockouts. Am J Physiol Cell Physiol 2013; 304: C693–C714. [PubMed: 23325410]
10. Pearson MM, Lu J, Mount DB, Delpire E. Localization of the K+–Cl− cotransporter, KCC3, in the central and peripheral nervous systems: expression in the choroid plexus, large neurons and white matter tracts. Neuroscience 2001; 103: 481–491. [PubMed: 11246162]
11. Shekarabi M, Salin-Cantegrel A, Laganière J, Gaudet R, Dion P, Rouleau GA. Cellular expression of the K+–Cl− cotransporter KCC3 in the central nervous system of mouse. Brain Res 2011; 1374: 15–26. [PubMed: 21147077]
12. Howard HC, Mount DB, Rochefort D, Byun N, Dupré N, Lu J, Fan X, Song L, Rivière J-B, Prévost C, Horst J, Simonati A, Lemcke B, Welch R, England R, Zhan FQ, Mercado A, Siesser WB, George AL, Bouchard J-P, Bouchard J-P, Mathieu J, Delpire E, Rouleau GA. The K–Cl cotransporter KCC3 is mutant in a severe peripheral neuropathy associated with agenesis of the corpus callosum. Nat Genet 2002; 32: 384–392. [PubMed: 12368912]
13. Uyanik G, Elcioglu N, Penzien J, Gross C, Yilmaz Y, Olmez A, Demir E, Wahl D, Scheglmann K, Winner B, Bogdahn U, Topaloglu H, Hehr U, Winkler J. Novel truncating and missense mutations of the KCC3 gene associated with Andermann syndrome. Neurology 2006; 66: 1044–1048. [PubMed: 16606917]
14. Rudnik-Schöneborn S, Hehr U, Tvon Kalle, Bornemann A, Winkler J, Zerres K. Andermann Syndrome can be a Phenocopy of Hereditary Motor and Sensory Neuropathy – Report of a Discordant Sibship with a Compound Heterozygous Mutation of the KCC3 Gene. Neuropediatrics 2009; 40: 129–133. [PubMed: 20020398]
15. Kahle KT, Flores B, Bharucha-Goebel D, Zhang J, Donkervoort S, Hegde M, Begum G, Duran D, Liang B, Sun D, Bönnemann CG, Delpire E. Peripheral motor neuropathy is associated with defective kinase regulation of the KCC3 cotransporter. Sci Signal 2016; 9: ra77. [PubMed: 27485015]
16. Fritzen D, Kuechler A, Grimmel M, Becker J, Peters S, Sturm M, Hundertmark H, Schmidt A, Kreiß M, Strom TM, Wieczorek D, Haack TB, Beck-Wödl S, Cremer K, Engels H. De novo FBXO11 mutations are associated with intellectual disability and behavioural anomalies. Hum Genet 2018; 137: 401–411. [PubMed: 29796876]
17. Delpire E, Gagnon KB, Ledford JJ, Wallace JM. Housing and husbandry of Xenopus laevis affect the quality of oocytes for heterologous expression studies. J Am Assoc Lab Anim Sci 2011; 50: 46–53. [PubMed: 21333163]

18. Ding J, Ponce-Coria J, Delpire E. A Trafficking-Deficient mutant of KCC3 reveals dominant-negative effects on K–Cl cotransport function. PLoS One 2013; 8: e61112. [PubMed: 23593405]
19. Byun N, Delpire E. Axonal and periaxonal swelling precede peripheral neurodegeneration in KCC3 knockout mice. Neurobiol Dis 2007; 28: 39–51. [PubMed: 17659877]
20. Flores B, Schornak CC, Delpire E. A role for KCC3 in maintaining cell volume of peripheral nerve fibers. Neurochem Int 2019; 123: 114–124. [PubMed: 29366908]
21. Adragna NC, Ravilla NB, Lauf PK, Begum G, Khanna AR, Sun D, Kahle KT. Regulated phosphorylation of the K-Cl cotransporter KCC3 is a molecular switch of intracellular potassium content and cell volume homeostasis. Front Cell Neurosci 2015; 9: 255. [PubMed: 26217182]

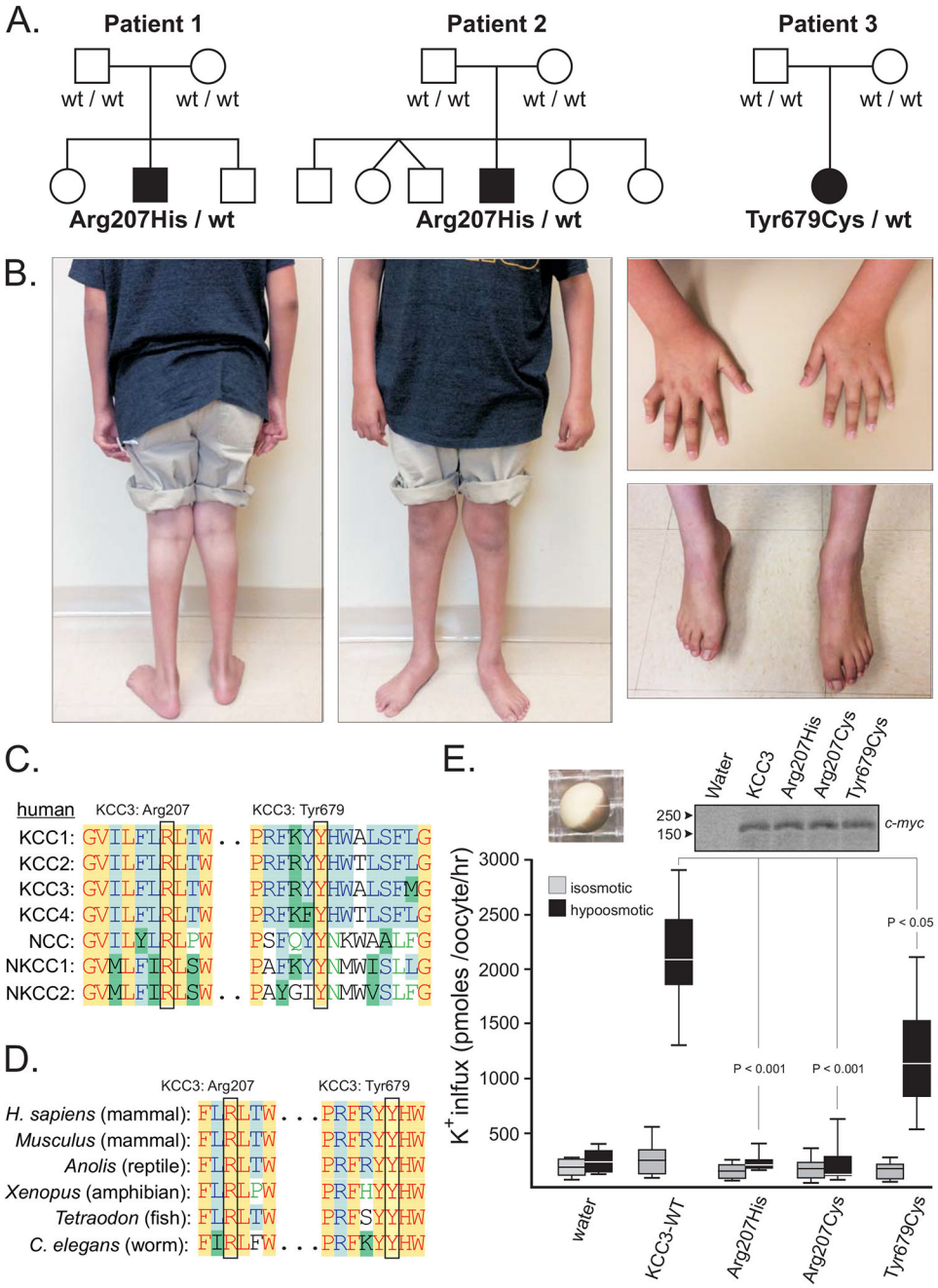

Figure 1.

(A) Pedigrees of the three unrelated affected patients (closed symbols) with de novo variants in *SLC12A6* and healthy family members (open symbols). Wildtype is depicted as wt. **(B)** Images of patient 2 at 14 years show muscle atrophy predominantly in distal lower limbs, contractures of fourth and fifth fingers and flat feet. **(C)** Conservation of KCC3 Arg207 and Tyr679 among the different members of the SLC12A (cation-chloride) cotransporters. The arginine and tyrosine residues are boxed. **(D)** Conservations of same residues within KCC3 transporters from different species. **(E)** K^+ influx measured in oocytes injected with

water, KCC3-WT, Arg270His, Arg270Cys and Tyr679Cys. Oocytes were incubated under isotonic (grey bars) or hypertonic (black bars) conditions. Statistical analysis was done using one way analysis of variance, groups of 20–25 oocytes. Top inset: Western blot showing expression of all transporters in oocytes and absence of expression in water-injected oocytes. Antibody was mouse monoclonal anti-cmyc.

Table 1

Clinical and genetic findings in patients with *SLC12A6 de novo* variants

	Patient 1	Patient 2	Patient 3	Kahle et al (2016)	Andermann Syndrome (13,14,19)
Age at onset (current age)	1-2 years (11 years)	1-2 years (11 years)	1-2 years (15 years)	1-2 years	Infancy
Sex	Male	Male	Female	Male	Male and female
Mutation	c.620G>A, p.Arg207His	c.620G>A, p.Arg207His	c.2036A>G, p.Tyr679Cys	c.2971A>G, p.Thr991Ala	missense, splice and truncating
Inheritance	*De novo* (dominant)	*De novo* (dominant)	*De novo* (dominant)	*De novo* (dominant)	Autosomal recessive
Main diagnosis	Polyneuropathy	Polyneuropathy	Spastic paraparesis, neuropathy	Polyneuropathy	Complex neurodevelopmental disorder, Intellectual disability, neuropathy
First sign	Frequent falls and tripping	Delayed walking	Spastic gait and delayed walking	Foot dragging at 9months, foot drops at 15 months	Developmental delay, generalized hypotonia, areflexia
Motor development	Delayed, walking at 18 months	Delayed	Delayed, first steps at 24 months	Delayed	Delayed, standing and walking at 4-6 years
Neuropathy					
Motor or sensory	Both, predominant motor	Both	Both	Both, predominant motor	Both
Axonal or demyelinating	Both, predominant demyelinating with intermediate type	Both, predominant demyelinating	Both, predominant axonal	Both, predominant axonal, Secondary demyelinating	Both
Sensory loss	No	Absent vibration sense and reduced perception of touch	Periumbilical hypaesthesia	No	Yes
Reflexes	Diminished BR, PR and AR	Absent BR, PR and AR	Normal BR, brisk and extended reflex zones in PR and AR	Absent AR	Absent
Spasticity	No	No	Yes	No	No
UL weakness prox. (MRC Scale)	Shoulder and elbow extension (5−/5), otherwise normal (5/5)	Yes (4/5)	No	No	Yes, severe
UL weakness dist. (MRC Scale)	Handwriting problems, otherwise stable	Yes (3/5) hand intrinsics	Hands (5−/5), otherwise normal	Wrist and finger extension (2/5)	Yes, severe
LL weakness prox. (MRC Scale)	Hip abduction (4+/5), hip flexion (5−/5)	Minimal (4+/5)	No	Yes	Yes, severe
LL weakness dist. (MRC Scale)	Foot dorsiflexion (4−/5), difficulties standing on heels	Yes (3/5)	Diminished (4/5)	Severe (1-2/5)	Yes, severe
UL muscle atrophy prox./dist.	No/no	No/yes	No/no	Yes/yes	Yes/yes

	Patient 1	Patient 2	Patient 3	Kahle et al (2016)	Andermann Syndrome (13,14,19)
LL muscle atrophy prox./dist.	No/yes	No/yes	No/yes	Yes/yes	Yes/yes
UL motor nerve					
DML	Prolonged median: 5.5 ms	Prolonged, median: 2.1 ms, ulnar: 3.8-5.9 ms	Normal	Prolonged, median: 4.8 −5.5ms, ulnar: 4.3-6.3 mV	Prolonged
NCV	Reduced, median: 33 m/s	Reduced, median: 32-35 m/s, ulnar 25-27 m/s	Normal	Reduced, median: 14-31 m/s, ulnar: NR-14 m/s	Variably reduced
CMAP	Reduced, median: 1.2mV	Reduced-normal, median: 3.7-7.3 mV, ulnar: 0.8-1.4 mV	Normal	Reduced, median: 0.2 mV, ulnar: 0.1 mV	Reduced
UL sensory nerve	Reduced, median; SNAP (3.5mV), NCV (21 m/s)	NR	Normal	Reduced, median: SNAP (9mV), NCV (44 m/s)	Reduced or NR
LL motor nerve					
DML	Prolonged, tibial: 5.2 ms	Prolonged, tibial: NR, peroneal: 9.2-44ms	Prolonged, tibial: 3.9-5.5 ms, peroneal: 4.6-5.4 ms	Prolonged, tibial: 6.1 ms, peroneal: NR	Prolonged
NCV	Reduced, tibial: 23 m/s	Tibial: NR, peroneal: normal 90 m/s	Reduced, tibial: 33-40 m/s, peroneal 37-44 m/s	NR, tibial and peroneal	Reduced
CMAP	Reduced, tibial: 3.5mV	Reduced, tibial: NR, peroneal: 0.2-2.3 mV	Reduced, tibial: 1-1.9 mV, peroneal: 0.5-0.8 mV	Reduced, tibial: 0.4 mV, peroneal: NR	Reduced
LL sensory nerve	NR	NR	NR	Normal SNAP, reduced sural NCV: 27 m/s	Reduced or NR
Brain and spinal MRI	Normal	Normal	Normal, non-specific minimal white matter lesions periventricular, stable over 2 years	Normal	Variable degrees of agenesis of the corpus callosum (complete, partial and also normal)
Seizures, EEG	No	No, bursts of generalized spine and polyspike and wave discharges in sleep EEG	No	No	Yes (>15%)
Intellectual disability	No	No	No	No	Yes
Dysmorphism	No	No	No	No	Yes
Deformities (foot, scoliosis,…)	Achilles tendon retraction	Scoliosis, hand and foot deformities	Achilles tendon retraction, plenovalgus foot	Unknown	Scoliosis (>80%) and achilles tendon retraction (>45%)
Other	Recurrent mild haemolytic anaemia, no hypertrophic Nerves in nervesonography	None	Celiac disease,short stature, migraine, bladder and bowel incontinence	Lost the ability to independently ambulate at 9 years	Wheelchair or bedridden by adolescence, average age of death 33 years
Disease progression	Yes	Yes	Yes	Yes	Yes

AR, achilles reflex;BR, biceps reflex; CMAP, compound muscle action potential; DML, distal motor latency; LL, lower limb; MRC Scale, Medical Research Council scale for muscle strength; NCV, nerve conductance velocity; NR, no response; PR, patellar reflex; SNAP, sensory nerve action potential; UL, upper limb; dist., distal; prox., proximal.