Heterozygous *De Novo* Alteration in *SLC12A6* in a Patient with Progressive Sensorimotor Polyneuropathy and Abnormal EEG.

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BACKGROUND

- Different mutations in the same gene can cause similar phenotypes through **different disease mechanism**, complicating diagnosis.
- The *SLC12A6* (OMIM:604878) on chromosome 15q14 encodes the potassium-chloride cotransporter 3, isoform A (KCC3), a transmembrane protein that regulates cell volume and controls neuronal activity by transporting K+ and Cl- ions across the plasma membrane.[1]
  - **Biallelic loss of function** (LoF) mutations in *SLC12A6* cause autosomal recessive agenesis of the corpus callosum with peripheral neuropathy (ACCPN, OMIM:218000) associated with hydrocephalus, developmental delay, intellectual disability and seizures.[2,3,4]
  - **Heterozygous gain of function** (GoF) alteration in *SLC12A6* cause progressive, early-onset, predominantly motor neuropathy and axonal degeneration with normal brain structure and function.[2]
- Herein, we report the second patient with a *de novo* **heterozygous missense** alteration (c.620G>A, p.R207H) in the *SLC12A6*, and severe, progressive **polyneuropathy and abnormal EEG**.
- Finely tuned KCC3 activity is required for structure and function of the peripheral nervous system, and disruptions lead to disease through impaired cell volume regulation and subsequent neurodegeneration.[2]

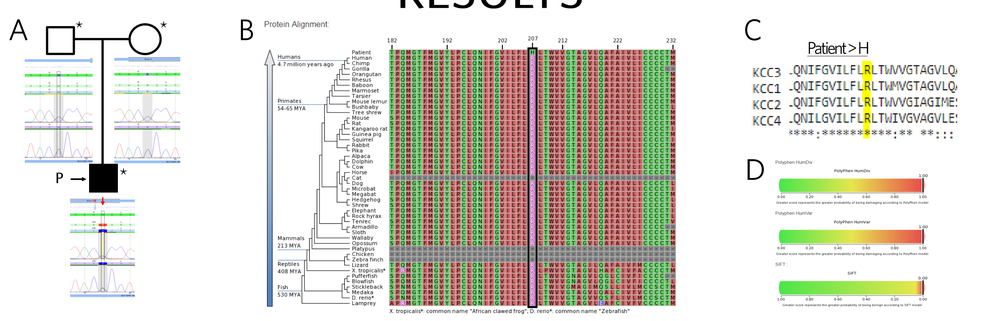
CLINICAL HISTORY

- 10-year-old male of Native American ancestry
- Severe progressive sensorimotor polyneuropathy
  - Mixed axonal and demyelinating features
  - distal and proximal weakness
  - Neck flexion weakness, occasional dysphagia
  - Wide-based gait, toe walking, bilateral heel cord tightness
  - Vibratory sense absent on toes and ankles, reduced at the knees and fingers
- Abnormal EEG (bursts of spike and polyspike wave discharges mainly in sleep)
- Nerve conduction studies: decreased nerve conduction velocities and compound motor action potentials, active denervation/reinnervation
- Unresponsive to IVIG
- Negative findings
  - MRI: brain and lumbar spine MRI normal
  - No intellectual disability or seizures

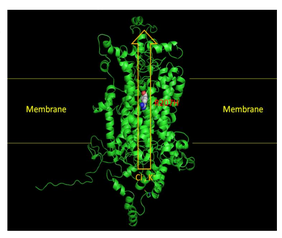
METHODS

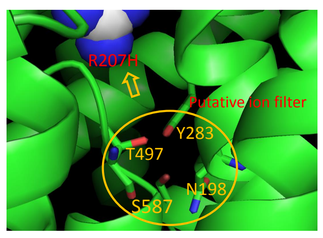
- Diagnostic exome sequencing (DES) was performed on genomic deoxyribonucleic acid (gDNA) isolated from whole blood from the proband and both parents. Targeted Sanger sequencing was performed on gDNA isolated from whole blood. Informed consent was obtained from all family members involved in the testing process.
- Exome library preparation, sequencing, bioinformatics, data analysis, results categories, and analysis and interpretation of novel gene findings are as previously described.[6,7]
- The structure of SLC12A6 was modelled by Rosetta[8] templating the several crystal structures of homologues (PDB id: 2xut,3gi8, 3ru1,4yms, and 5j1d). The energy differences between the wildtype and adjacent variants ware calculated in Rosetta score[9]. Model graphics were generated using Pymol.[10] Motifs were identified using the web server Pfam.[11]

RESULTS



Conservation, *in silico,* and co-segregation data for c.620G>A p.R207H.
A) DES identified a *de novo* heterozygous missense alteration c.620G>A in the *SLC12A6* gene and results confirmed with Sanger sequencing. **B)** Amino acid is completely conserved in available vertebrate species and **C)** across other human KCC family members. **D)** Predicted probably damaging by Polyphen and deleterious by SIFT *in silico* analysis. The c.620G>A p.R207H is absent in all population databases.





C **Energy Score (Rosetta)**

Assessment	Variant	Allele Frequency	ΔΔG
Wildtype	R207R		-1828.83
	R207H		-1854.54
Pathogenic	R207C	1.65e-05	-1838.78
Benign	M282L	4.96e-05	-1823.62
Benign	S495C	3.25e-05	-1829.96
Benign	F492L	8.23e-06	-1807.70

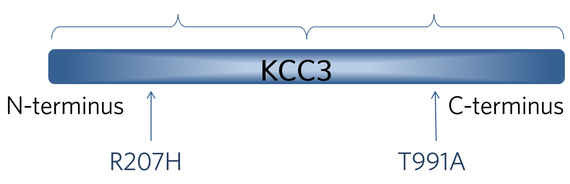
Figure 3. Homology based 3D structural modeling for the p.R207H.
A) R207H is located on the first transmembrane domain of SLC12A6 and on the ion pore, and directly involved in ion transportation. **B)** R207H is located close to the putative ion filter domain. **C)** Energetically, R207H overstabilizes the local structure more than R207C or benign variants around. The Arg to His at 207 changes the charge and thus likely the environment of ion pathway.

CLINICAL FEATURES ACROSS DIFFERENT *SLC12A6* MUTATION TYPES

	Current case	Kahle *et al.* 2016	Uyanik *et al.* 2006 (pt3)	Typical ACCPN[2,3,4,12]
Mutation	c.620G>A p.R207H	c.2971A>G p.T991A	c.619C>T p.R207C	Multiple
State	Heterozygous	Heterozygous	Homozygous	Biallelic
Type	Missense	Missense	Missense	Splice, truncating
Mutational mechanism	Unknown	Gain of function	Loss of function	Loss of function
Age at onset	Infancy	~9 months	~4 months	< 1 years
Central Nervous System				
Intellectual disability	-	-	+	>90%
Agenesis of the corpus callosum	-	-	+***	~70%
Seizures	- *	-	-	>15%
Developmental delay	-	-	+	+
Peripheral Nervous System				
Peripheral motor neuropathy	+	+	+	+
Peripheral sensory neuropathy	+	+**	+	+
Areflexia	+	-	+	+
Absent deep tendon reflexes	+	+	reduced	+
Axonal neuropathy	+	+	+	+
Demyelinating neuropathy	+	+	+	+
Onion bulbs	-	-	+	+
Hypotonia	-	?	+	+
Muscle, soft tissue				
Progressive distal and proximal symmetric limb weakness	+	+	+	+
Denervation/reinnervation	+	+	+	+
Skeletal				
Scoliosis/Kyphosis	-	-	+	>80%
Syndactyly	-	-	-	~10%
Behavioral Psychiatric Manifestations				
Psychotic episodes	-	-	-	~40%
Typical facial features	-	-	+	+

*Abnormal EEG
**Mild sensory neuropathy
***Additional disseminated white matter hyperintensities observed in MRI

Severity of the phenotype



Human disease mutations in *SLC12A6* disrupt KCC3 function by two mechanisms.
N-terminal mutation decrease KCC3 plasma membrane localization and result in KCC3 retention in the endoplasmic reticulum (ER).[5] C-terminal truncations lead to absence of regulatory sites; C-terminal KCC3 domain directly interacts with brain-specific creatine kinase (CK-B)[13], and contains two phosphorylation sites.[14]

DISCUSSION

- Biallelic LoF mutations in *SLC12A6* cause a severe form of ACCPN while heterozygous missense mutations cause milder form of the disease.
- Similarly, *Slc12a6*$^{-/-}$ mice exhibit more severe phenotype over *Slc12a6*$^{-/+}$ mice with impaired motor function and significant axonal swelling accompanied by hypomyelination[14].
- We report the second patient with a heterozygous missense alteration in *SLC12A6* and a milder form of ACCPN compared to typical ACCPN.
- A homozygous missense alteration affecting the same codon (p.R207C) was reported in a patient with less severe disease compared to truncating KCC3 mutations. The alteration leads to decreased KCC3 transport activity and plasma membrane localization, and results in KCC3 retention in the ER.[4] Heterozygous carrier parents are asymptomatic.
- Based on structural modeling, R207H is more disrupted over R207C, with the assumption that over stabilized energy disrupts the transportation function.
- This patient was also submitted to RNA-sequencing studies to confirm the state of the second allele.

TAKE-HOME POINTS

- Both biallelic and monoallelic alterations in the *SLC12A6* cause ACCPN through different disease mechanisms.
- Biallelic *SLC12A6* mutations cause more severe disease compared to heterozygous mutations.
- DES is a tool for dissecting the mutational and phenotypic spectrum of diseases.

REFERENCES

1. Dupré N, *et al.* (2003) *Ann Neurol* **54**(1):9-18.
2. Howard HC, *et al.* (2002) *Nat Genet* **32**(3):384-392
3. Salin-Cantegrel J, *et al.* (2011) *Biol Chem***286**(32):28456-28465.
4. Uyanik G, *et al.* (2006) *Neurology***66**(7):1044-1048.
5. Kahle KT, *et al.* (2016) *Sci Signal* **2**(439):ra77.
6. Farwell KD, *et al.* (2015) *Genet Med* **17**(7): 578-586.
7. Farwell Hagman KD, *et al.* (2016) *Genet Med* **19**(2):224-235.
8. The Rosetta Commons Modeling Software, Version 3.6 (https://www.rosettacommons.org/).
9. O'Meara MJ *et al.* (2015) *J Chem Theory Comput.**11**(2)*:609-622
10. The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.
11. The Pfam database (http://pfam.xfam.org/).
12. Dupré N, *et al.* (2006) (Updated Jun 12 2014). Hereditary Motor and Sensory Neuropathy with Agenesis of the Corpus Callosum In: GeneReviews® [Internet]. Copyright, University of Washington, Seattle. 1993-2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1372/
13. Salin-Cantegrel A, *et al.* (2008) *Hum Mol Genet* **17**(17):2703–2711.
14. Rinehart J, *et al.* (2009) *Cell* **138**(3):525–536.
15. Howard HC, *et al.* (2002) *Nat Genet.***32**(3):384–392.

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