Apparent digenic trialleleic inheritance in early-onset ataxia through whole exome sequencing

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Abstract

We have identified alterations in both alleles of the *CACNA1A* gene and in one allele of the *TGM6* gene in a 4 year old girl with early-onset ataxia. As identified through the use of whole exome sequencing, she has a homozygous c.3043G>A missense variant in *CACNA1A*, inherited from carrier parents, and a heterozygous c.115A>T missense variant in *TGM6*, inherited from her father. The paternal grandfather also carries one allele of each alteration. The 4 year old has mild global developmental delays, moderate gait ataxia, mild intention tremor, and moderately dysarthric speech. Neither parent nor the paternal grandfather has ataxia by history or by physical examination. Heterozygous mutations in the *CACNA1A* gene, which encodes the pore-forming a1 subunit of the P/Q type calcium channel, are associated with a range of autosomal dominant neurologic phenotypes including: episodic ataxia type 2 (EA2), usually associated with truncating mutations; familial hemiplegic migraine, usually associated with missense mutations; and spinocerebellar ataxia type 6 (SCA6), associated with polyglutamine expansions. To our knowledge, this is the first report of an individual with biallelic *CACNA1A* gene alterations. *CACNA1A* mutations may also modify other genetic disorders, such as in SCN1A-associated Dravet syndrome where it increases severity. Mutations in the *TGM6* gene, which encodes a transglutaminase, are associated with spinocerebellar ataxia type 35 (SCA35), a dominant adult-onset ataxia associated with missense mutations. Based on our findings in this family, we propose that the combined effects of the three altered alleles in the *CACNA1A* and *TGM6* genes are causative of this child's neurologic signs and symptoms. Both proteins function in the central nervous system suggesting different means, both direct and indirect, by which their mutations may synergistically cause ataxia and other neurologic findings.

Introduction:

Genetic ataxias are a heterogeneous group of diseases that exhibit both locus and allelic heterogeneity. The etiology remains unknown for approximately 40% of patients with genetic cerebellar ataxia, likely due to rare and as yet unidentified disease genes (Sailer and Houlden, 2012).

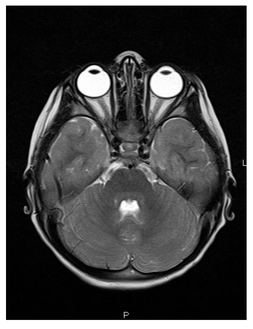
There are over 40 autosomal dominant forms of hereditary ataxia known. Many of these result from trinucleotide repeat expansions with the size of the expansion correlating with the severity of disease manifestations. The spectrum of disease severity is vast and even within a single disease entity there may be significant differences in age of onset and severity of symptoms, sometimes even within a single kindred.

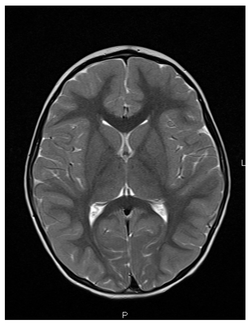
There are over 23 genes associated with autosomal recessive ataxia which also display wide phenotypic variability, age of onset, and degree of severity and are due to virtually all types of mutation. Among these genes, it has been observed generally that patients with "conventional" mutations (missense, nonsense, small insertions/deletions) show an earlier age of onset but a less severe phenotype which progresses less rapidly than patients with repeat expansions or complex rearrangements.

Case History

This four-year old girl manifested developmental delays by her second year of life and began developmental therapies. Her speech remains dysarthric and difficult to comprehend. She ambulates independently but unsteadily and also has a mild baseline tremor. She has a limited attention-span, though she is not currently considered to have ADHD. Vision, hearing, and general medical health are all normal. Investigation has included normal head MRI (at age 3, see below) and EEG. There is no history of seizure activity. Prior genetic and metabolic testing was normal, including oligonucleotide chromosomal microarray, Fragile X DNA testing, urine organic acids, and very long chain fatty acids.

Family history is notable for ADHD in both parents. Neither parent has ataxia, dysarthria, or seizures. The paternal grandparents also lack these findings, though the paternal grandmother has migraines and poor balance.





Results

NUMBER OF GENES & ALTERATIONS IDENTIFIED BASED ON BIOINFORMATICS & INTERPRETATION*

	Post-Inheritance Model Filtering	Post-Medical Review**		Notable Candidate Genes
		Post-alteration review	Post-clinical association review	
Autosomal Dominant Genes (Alterations)	44 (46)	43 (45)	1 (1)	**1 (1)**
Autosomal Recessive Genes (Alterations)	5 (9)	5 (9)	1 (1)	**1 (1)**
X-linked Recessive Genes (Alterations)	0 (0)	0 (0)	0 (0)	**0 (0)**
X-linked Dominant Genes (Alterations)	1 (1)	1 (1)	0 (0)	**0 (0)**
Y-linked Genes (Alterations)				
TOTAL GENES (Alterations)	**50 (56)**	**49 (55)**	**2 (2)**	**2 (2)**

*First-Tier Exome analysis: Reported are genes that are known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databases or the medical literature (the alterations within these genes are located in parentheses). "Clinically novel" genes are not analyzed or reported. Please inquire with the laboratory for more information about post-medical review variant filtered genes and alterations.

**Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign.

GENE-RELATED INFORMATION

Gene/ Locus	Location	Protein	Genomic coordinates (GRCh37)	Genomic Size (bp)	Exons
CACNA1A	19p13	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit (2261 aa)	chr19:13317256-13617274	300019	47
TGM6	20p13	Transglutaminase 6 (706 aa)	chr20:2361554-2413399	51846	13

FAMILIAL CO-SEGREGATION RESULTS:

GENE(S)	INHERIT. MODEL	ALTERATION	PATIENT	FATHER	MOTHER	PAT. GR-MOTHER	PAT. GR-FATHER
CACNA1A	AD (AR?)	c.3043G>A (p.E1015K)	+/+	+/-	+/-	+/-	-/-
TGM6	AD	c.115A>T (p.S39C)	+/-	+/-	-/-	+/-	-/-

Discussion

The affected child has homozygous c.3043G>A mutations in the *CACNA1A* gene. The A-allele has a frequency of 0.43% (34/7976) total alleles studied and is not observed in the homozygous state (NHLBI Exome Sequencing Project). This missense mutation replaces glutamic acid with lysine at an amino acid position that is moderately conserved in vertebrate species. The alteration is predicted to be benign by PolyPhen and tolerated by SIFT *in silico* analyses. Although there are no prior reports of homozygous or compound heterozygous *CACNA1A* mutations, mouse models with Cacna1a gene alterations are most commonly recessive, and have milder phenotypes with missense versus truncating mutations (Fletcher et al, 1996; Noebels and Sidman, 1979).

Heterozygous *CACNA1A* alterations are associated with a wide range of autosomal dominant neurological syndromes including episodic ataxia type 2 (EA2), familial hemiplegic migraine (FHM), progressive spinocerebellar ataxia (SCA6), and alternating hemiplegia of childhood (AHC). Missense mutations, although associated most commonly with FHM, have been associated with EA2, FHM, SCA6, AHC, progressive ataxia, and non-fluctuating cerebellar ataxia. The mechanism underlying the variable expressivity and reduced penetrance associated with *CACNA1A* alterations is unclear, but may be explained by genetic modifiers, recessive disease inheritance, and/or oligogenic inheritance.

The child also has a heterozygous 115A>T mutation the *TGM6* gene. The T-allele has an overall frequency of 0.05% (5/10758) total alleles studied (NHLBI Exome Sequencing Project). This missense mutation replaces serine with cysteine, an amino acid with dissimilar properties. While this amino acid position is poorly conserved in vertebrates, it is located within the pro-ß sandwich which forms a functional unit with the core domain. It is predicted to be possibly damaging by PolyPhen and tolerated by SIFT *in silico* analyses.

TGM6 encodes a transglutaminase and is expressed abundantly in the central nervous system, most prominently in the cell layers containing neuronal cell bodies of the cerebral cortex, the olfactory lobe, and the Purkinje cells of the cerebellum. Missense alterations in the *TGM6* gene have been reported in three Chinese families with spinocerebellar ataxia.

Given the clinical and molecular findings in the affected child, and the lack of apparent clinical findings in her parents and paternal grandparents through whom these mutations are segregating, in addition to the wide phenotypic variability commonly associated with genetic ataxias, we propose that the abnormal protein products of these three missense alleles act together (whether directly or indirectly) to cause her ataxia, dysarthria, and developmental delay. Such oligogenic etiology is an ever increasingly appreciated theme in neurogenetic disorders.

Methods

Genomic DNA was isolated from whole blood from the patient, her parents, and her paternal grandparents. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).

Exome data undergoes alignment, base calling, and variant calling. Passing base calls have at least 10x coverage and quality scores of Q30 or higher, which translates to a base call error rate probability of 1:1000, or a base call read accuracy of 99.9%. Exons plus at least 2 bases into the 5' and 3' ends of all the introns are analyzed and reported. Variants were filtered further based on family history and possible inheritance models. Data is annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, predicted functional impact, and clinical disease associations (Human Gene Mutation Database (HGMD; Stenson, 2009; OMIM; and several other databases).

A molecular geneticist performed interpretive filtering based on the deleterious nature of the candidate alterations literature search and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype.

Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation analysis.

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