Two Novel CHN1 Mutations in 2 Families With Duane Retraction Syndrome

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Objective: To determine the genetic cause of Duane retraction syndrome (DRS) in 2 families segregating DRS as a dominant trait.

Methods: Members of 2 unrelated pedigrees were enrolled in a genetic study. Linkage analysis was performed on the CHN1 locus. Probands and family members were screened for CHN1 mutations.

Results: The 6 affected individuals in the 2 pedigrees have DRS. Both pedigrees are consistent with linkage to the locus. Sequence analysis revealed 2 novel heterozygous missense mutations, c.422C>T and c.754C>T, predicted to result in α2-chimaerin amino acid substitutions P141L and P252S, respectively.

Conclusions: Genetic analysis of 2 pedigrees revealed 2 novel DRS mutations, bringing the number of DRS pedigrees known to harbor CHN1 from 7 to 9. Both mutations alter residues that participate in intramolecular interactions that stabilize the inactive, closed conformation of α2-chimaerin and, thus, are predicted to result in its hyperactivation. Moreover, amino acid residue P252 was previously reported to be altered to a different residue in a previously reported DRS pedigree; thus, this is the first report of 2 CHN1 mutations altering the same residue, further supporting a gain-of-function etiology.

Clinical Relevance: Members of families segregating DRS as an autosomal dominant trait should be screened for mutations in the CHN1 gene, enhancing genetic counseling and permitting earlier diagnosis.


Methods: Two families that segregate DRS as a dominant trait were enrolled in an ongoing genetic study of congenital cranial dysinnervation disorders. The Children’s Hospital Boston institutional review board approved this study, and informed consent was obtained from participants or their guardians. The probands, their parents, and the half-sibling of the ACL proband underwent ophthalmologic examinations with full ocular motility testing. The affection status of the remaining participants was determined by review of ophthalmologic records, reported family history, or both. Each par-
RESULTS

Pedigree ABK is white of northern European ancestry and segregates DRS as an autosomal dominant trait with reduced penetrance in 4 generations, as described previously by Zhu-Tam and Gurwood.8 (Figure 1A). The proband has congenital bilateral limitation of abduction and globe retraction and narrowing of the palpebral fissure on abduction. Her mother was examined and has no defect in ocular motility but reported the family history of unilateral left-sided DRS as depicted in Figure 1A. Five family members (2 affected and 3 clinically unaffected) and 1 spouse participated in the genetic study.

Pedigree ACL is a previously unpublished family of African American ancestry that segregates DRS as an autosomal dominant trait in the proband (III-2) and his father (Figure 1B). The proband has bilateral DRS with esotropia and absent abduction. Globe retraction is present on attempted adduction bilaterally; adduction is full in the right eye and moderately limited with mild upshoot in the left eye. He also demonstrates a chin down posture when attempting fine focusing, with fusion on upgaze. The proband’s father also has bilateral DRS and had undergone surgery as a child. Both affected and 3 unaffected family members participated in the study.

Analysis of the 4 genetic markers flanking CHN1 in each pedigree revealed cosegregation of the DRS phenotype with the CHN1 locus. Pedigree ACL demonstrated complete penetrance. In pedigree ABK, III-2 and III-6 carry the disease-associated haplotype but do not or are not reported to manifest DRS, respectively.

A heterozygous missense mutation (c.422C>T) in CHN1 exon 6 was identified in the ABK proband, IV-1 (Figure 2A). This mutation was also present in the affected grandfather and in the unaffected mother and maternal aunt, who harbor the affected haplotype, and absent in the remaining participants from the family. This missense mutation is predicted to result in a conservative amino acid substitution of a nonpolar proline to a

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Figure 1. Schematic of pedigrees and haplotype analysis of families ABK (A) and ACL (B) segregating autosomal dominant Duane retraction syndrome at the CHN1 locus. Pedigree members are denoted by circles (females) and squares (males) and by generation and position. Solid circles and squares indicate clinically affected individuals; arrows, probands; solid bars, affected haplotypes; and open bar, unaffected haplotypes. Results are shown schematically for markers surrounding the CHN1 gene. Genotyping data and schematic segregating haplotype bars for 4 chromosome 2 markers and CHN1 mutation status are shown below the symbol for each individual who participated in the study. For CHN1+, indicates presence of the mutation; –, absence of the mutation. For pedigree ABK, the affected haplotype and CHN1 mutation are also inherited by 2 unaffected family members, III-2 and III-6.
nonpolar leucine at amino acid residue 141 (p.P141L), located in the SH2-C1 linker region of α-chimaerin (Figure 2B).

A heterozygous missense mutation (c.754C>T) in CHN1 exon 9 was identified in the ACL proband, III-2 (Figure 2A). The affected father, but none of the unaffected members of the pedigree, also harbored this mutation. This mutation is predicted to result in a nonconservative amino acid substitution of a nonpolar proline to a polar serine at amino residue 252 (P252S).

These 2 missense changes have not been previously reported and are not in single nucleotide polymorphism databases from the University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu) or the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP). Using PolyPhen (http://genetics.bwh.harvard.edu/pph),12 P141L is predicted to have a probably damaging impact and P252S is predicted to have a benign impact on the structure and function of α2-chimaerin. Neither change was present on 400 chromosomes of European-derived white ethnicity and 388 chromosomes of African American ethnicity. α2-Chimaerin p.P141L and p.P252S are evolutionarily conserved in multiple species and in α2-chimaerin’s close human paralog, β2-chimaerin (Figure 2C).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Nucleotide sequence, amino acid position, and conservation of the CHN1 mutations. A, Heterozygous CHN1 mutations in the probands of pedigrees ABK and ACL. Sequence chromatographs of the control individuals are normal (top row), whereas those of the affected individuals with Duane retraction syndrome each reveals a heterozygous CHN1 mutation (bottom row). The normal sequence and corresponding amino acid residues are indicated under each control sequence chromatograph (black), and the mutation and resulting amino acid substitution are denoted under each affected sequence (red). B, Predicted α2-chimaerin protein structure. The amino acid residues altered by the 2 novel heterozygous mutations are depicted in red, with red arrows above the protein pointing to their predicted positions. The 7 previously reported CHN1 mutations are indicated in black, and their locations are indicated by black arrows above the protein. C, Portions of the human CHN1 amino acid sequence that surround each substitution are aligned with the homologous sequence in 7 different species, followed by alignment with the paralogous β2-chimaerin sequence at the bottom. Identical amino acid residues are highlighted in light gray. The residues altered by the 2 new mutations are boxed in red.

We identified 2 novel heterozygous missense CHN1 mutations in 2 dominant DRS pedigrees. Clinical examination reveals that the probands from both families have isolated bilateral DRS with limited or no abduction and with retraction of the globe and narrowing of the palpebral fissure on attempted adduction. Although the affected father of the proband in pedigree ACL, II-2, also has bilateral DRS, all 3 affected relatives of the ABK proband, IV-1, have unilateral DRS. In addition, none of the affected family members in either pedigree were noted to have significant errors in vertical motility. Thus, although these DRS phenotypes fall within the spectrum of clinical findings from previously described DRS-positive families carrying CHN1 mutations, they are less atypical than most.6 Similar to ABK II-2 and III-6, we previously reported mutation-positive individuals in whom the DRS phenotype is not penetrant.6 Such clinically asymptomatic patients have not yet undergone detailed magnetic resonance imaging to determine whether they might harbor an endophenotype similar to that reported for CFEOM3.13

CHN1 encodes the Rac guanosine triphosphatase–activating (RacGAP) signaling molecule α2-chimaerin (Figure 3). When inactive, α2-chimaerin is found in the cytoplasm in a closed conformation. In response to diacylglycerol signaling, it unfolds and translocates to the membrane, exposing its RacGAP domain and inactivating Rac. Crystallization of its close relative, β2-chimaerin, and studies6,13,16 of mutant α2- and β2-chimaerin revealed that the inactive closed conformation is maintained by intramolecular interactions that impede access to the Rac and diacylglycerol binding sites (Figure 3). The protein modeling and functional studies of the 7 CHN1 mutations previously reported in DRS pedigrees revealed that each hyperactivates α2-chimaerin and lowers Rac guanosine triphosphate (Rac-GTP)–activating protein levels in the cell, and a subset do so by destabilizing the inactive closed conformation of α2-chimaerin, thus, increasing its translocation to the cell membrane and its signaling activity.6

Based on the positions of residues altered by mutations in pedigrees ABK and ACL, we predict that these mutations will behave in a similar manner as those reported previously.4 The mutation that segregates in pedigree ACL alters amino acid residue P252, which was also altered by 1 of the 7 original DURS2 mutations (Figure 3).4 The previous pedigree harbors CHN1 T755C>A (P252Q), and ACL harbors 754C>T (P252S), both mutations alter the polar uncharged proline in a conserved manner.
We previously established that P252Q enhances the translocation of α2-chimaerin to the membrane and lowers Rac-GTP levels in vitro. Thus, this is the first report of 2 DRS mutations altering the same amino acid residue. Because the ACL mutation alters P252 in a similar manner as the previous mutation, we predict that it will behave in a similar manner, despite its benign prediction by PolyPhen. Of note, this program also predicts the presence of amino acids predicted to be involved in intramolecular interactions that stabilize the closed conformation of α2-chimaerin based on homology with β2-chimaerin. The 7 previously reported mutations alter amino acid residues that are represented by green or blue circles or squares; those filled with green were previously demonstrated to enhance translocation of α2-chimaerin to the membrane when mutated, and those filled with blue did not. The red circle and the red and green-striped square represent the residues altered by the new novel mutations: P252S alters the same residue as P252Q (thus, the residue is striped), and P141L alters a residue predicted to interact with Y221. Thus, both residues are anticipated to destabilize the closed conformation of α2-chimaerin and result in its pathologic hyperactivation. Adapted with permission from Miyake et al.

Figure 3. Schematic of the α2-chimaerin structure in its closed conformation showing the predicted α2-chimaerin intramolecular interactions. The 3 domains of α2-chimaerin are depicted as follows: the N-terminal Src homology-2 (SH2) domain is depicted in blue, the C1 domain that binds to the second message-signaling lipid diacylglycerol is depicted in yellow, and the Rac guanosine triphosphatase (RacGAP)–activating protein domain that interacts with Rac and downregulates its activity is depicted in pink. Linker regions are depicted as black lines. Specific amino acid residues in a domain that interacts with Rac and downregulates its activity is depicted in pink. Linker regions are depicted as black lines. Specific amino acid residues altered by the new novel mutations: P252S alters the same residue as P252Q (thus, the residue is striped), and P141L alters a residue predicted to interact with Y221. Thus, both residues are anticipated to destabilize the closed conformation of α2-chimaerin and result in its pathologic hyperactivation. Adapted with permission from Miyake et al.

In conclusion, we identified 2 novel heterozygous missense CHN1 mutations that cause autosomal dominant bilateral DRS, bringing the total number of known CHN1 mutations to 9. These 2 new mutations alter residues previously shown to stabilize, or to be implicated in the stabilization of, the closed conformation of α2-chimaerin and provide further support that the DRS phenotype results from specific CHN1 mutations that hyperactivate the α2-chimaerin signaling molecule. Although we previously demonstrated that overexpression of mutant or wild-type α2-chimaerin in the embryonic chick ocular motor nerve results in axon stalling with aberrant branching or defasciculation, 6 the molecular pathway by which hyperactivation of α2-chimaerin in developing abducens and ocular motor axons results in the DRS phenotype has yet to be elucidated.

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