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 CHN1 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.


CONGENITAL CRANIAL DYSINNERRATION disorders are associated with abnormal cranial motor neuron and axon development, causing errors in ocular and facial muscle innervation.1 Duane retraction syndrome (DRS) is the most common of the congenital cranial dysinnervation disorders.2,3 Individuals with DRS typically manifest limitation or absence of globe abduction, variable limitation of adduction, and palpebral fissure narrowing on attempted adduction secondary to globe retraction. Postmortem studies4,5 of individuals with DRS have found absence of abducens motor neurons and nerve and aberrant innervation of the lateral rectus muscle by a branch of the oculomotor nerve.

We previously reported that 7 families segregating DRS as an autosomal dominant trait each harbor a unique heterozygous missense mutation in the CHN1 (GenBank NM_001822) gene.6 Affected family members had a higher incidence of bilateral DRS and vertical movement abnormalities than is typical of sporadic DRS,7,8 and magnetic resonance imaging of their orbits revealed small or absent abducens nerves and, in some cases, small oculomotor nerves.9 We also examined a large cohort of individuals with sporadic DRS and did not identify any CHN1 mutations among them.10 Herein we ascertained 2 additional pedigrees (ABK and ACL) that cosegregate DRS as autosomal dominant trait linkage and mutational analyses were performed to determine whether these families segregate CHN1 mutations.

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-
Puregene Extraction Kit (QIAGEN GmbH, Hilden, Germany). Weight DNA was extracted from blood samples using the Puregene Extraction Kit (QIAGEN GmbH, Hilden, Germany).

For CHN1, schematic segregating haplotype bars for 4 chromosome 2 markers and D2S2330, D2S2314, D2S364, D2S326 are shown in the pedigree. Haplotype analyses of pedigrees are shown schematically for markers surrounding the CHN1 locus. Pedigree ACL demonstrated cosegregation of the DRS phenotype with the CHN1 locus. 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.

The proband from each family was screened for mutations in the 13 coding exons and exon-intron boundaries of the CHN1 gene (primer sequences available from the author on request). The amplicons were analyzed using a combination of denaturing high-performance liquid chromatography (Transgenic Inc, Omaha, Nebraska) and direct sequencing as previously reported. Variants that were detected by denaturing high-performance liquid chromatography were confirmed by direct sequencing. When a sequence variant was identified in the proband, the participating family members and control samples were also examined for the presence or absence of the variant by either denaturing high-performance liquid chromatography or direct sequencing.

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. The proband has congenital bilateral limitation of abduction and globe retraction and narrowing of the palpebral fissure on adduction. 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.

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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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 386 chromosomes of mixed ethnicity. In addition, 754C>T was also absent from 200 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).

We identified 2 novel heterozygous missense CHN1 mutations in 2 dominant DRS pedigrees. Clinical examinations reveal 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 III-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 studies6,14,16 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 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.6 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).6 The previous pedigree harbors CHN1 T755C>A (P232Q), and ACL harbors T754C>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 previously reported I126M amino acid substitution to be benign, despite the finding that it lowers RacGAP levels and causes DRS.

The mutation that segregates in pedigree ABK alters residue P141. Based on the crystal structure of β2-chimaerin, we previously predicted that α2-chimaerin residues P141 and Y143, both in the SH2-C1 linker region, form intramolecular interactions with residue Y221 in the C1 domain, thus stabilizing the closed conformation of α2-chimaerin (Figure 3). Moreover, 1 of the original 7 DURS2 pedigrees harbored the heterozygous CHN1 mutation 427C>T, resulting in Y143H, and this mutation enhanced the translocation of α2-chimaerin to the membrane and lowered Rac-GTP levels in vitro. Thus, we predict that P141L will behave in a similar manner.

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 oculomotor nerve results in axon stalling with aberrant branching or defasciculation, the molecular pathway by which hyperactivation of α2-chimaerin in developing abducens and oculomotor axons results in the DRS phenotype has yet to be elucidated.

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REFERENCES