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CHN1 Mutations are not a Common Cause of Sporadic Duane's Retraction Syndrome

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To the Editor:

Duane retraction syndrome (DRS) is a congenital disorder characterized by restricted horizontal eye movement with globe retraction and palpebral fissure narrowing on attempted adduction. DRS is observed in ~0.1% of the general population, accounts for 1-5% of all strabismus [DeRespinis et al., 1993; Kirkham 1970], and if untreated in childhood can result in loss of binocular vision and amblyopia. Post-mortem examinations of individuals with sporadic DRS have revealed absence of the abducens motor neurons and abducens cranial nerve on the affected side(s), and aberrant innervation of the lateral rectus by axons of the oculomotor nerve that normally innervate the medial rectus muscle [Hotchkiss et al., 1980; Miller et al., 1982].

Most individuals with DRS are affected unilaterally and have no family history of the disorder [Kirkham, 1970]. Approximately 10% of individuals have a positive family history, however, and most of these individuals are bilaterally affected and segregate DRS as an autosomal dominant trait. In addition, a quarter to half of affected individuals have syndromic DRS, with additional congenital defects most typically involving ocular, skeletal, auricular, or neuronal structures [Pfaffenbach et al., 1972]. These additional findings can co-segregate within DRS families, and led to the identification of *SALL4* mutations in patients

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with DRS and radial ray anomalies (DRRS; OMIM 607323) [Al-Baradie et al., 2002; Kohlhasse et al., 2002], and *HOXA1* mutations in patients with DRS, facial weakness, deafness, hypoventilation, vascular and cardiac outflow anomalies, and developmental delay/autism (OMIM 601536) [Tischfield et al., 2005]. Neither of these genes was found to be mutated in isolated, sporadic DRS [Tischfield et al., 2006; Wabbels et al., 2004].

We recently identified *CHN1* as the disease gene at the autosomal dominant DURS2 locus on chromosome 2 [Miyake et al., 2008]. This is the only locus for nonsyndromic DRS mapped by linkage analysis, and four pedigrees had been reported to map to this location [Appukuttan et al., 1999; Engle et al., 2007; Evans et al., 2000]. We analyzed these four pedigrees as well as additional families segregating DRS as an autosomal dominant trait and identified seven different causative heterozygous missense mutations in seven pedigrees [Miyake et al., 2008]. Notably, in comparison to individuals with sporadic DRS, the affected members of these families had a higher incidence of bilateral DRS and of vertical movement abnormalities. In addition, magnetic resonance imaging revealed hypoplasia of the oculomotor as well as the abducens nerve [Demer, 2007 #3090]. *CHN1* encodes alpha2-chimaerin, a Rac guanosine triphosphatase-activating protein (RacGAP). We demonstrated that the seven DRS *CHN1* missense mutations increase the activity of alpha2-chimaerin, lower Rac-GTP levels in the cell, and result in failure of oculomotor axons to innervate their target extraocular muscles in the developing chick embryo (Miyake et al., 2008).

To determine whether *CHN1* mutations are a common cause of sporadic DRS, we have now reviewed all probands enrolled in our genetic study of complex strabismus and identified the 140 DRS probands with a negative family history for DRS. This study was prospectively reviewed and approved by the Children's Hospital Boston institutional review board and informed consent was obtained from all participants and/or their guardians. Ophthalmological and general examinations were either performed at Children's Hospital Boston or were obtained from the proband's medical record. Each participant provided a salivary or blood sample for DNA extraction.

The 140 probands were of diverse ethnicity and from diverse geographic locations, including North, Central, and South America, Europe, Middle East, Asia, India, and Australia. Of these, 36 had additional congenital anomalies and 6 had known chromosomal abnormalities not overlapping with the DURS2 locus. Detailed ophthalmological data was available from 90 probands and, of these, 72% had DRS type 1, 20% had DRS type 3, and 8% had DRS type 2 as per the Huber classification [Huber, 1974]. In addition, 86% had unilateral DRS and, of these, 73% were left-sided. Of the 18 individuals with bilateral DRS, five had associated non-ocular findings. Overall, the clinical features of the cohort correspond to other cohorts in the literature [DeRespinis et al., 1993].

We screened DNA extracted from blood or saliva from the 140 probands for sequence variants in the 13 coding exons and exon-intron boundaries of the *CHN1* gene (primer sequences available on request). Amplicons were analyzed through a combination of denaturing high performance liquid chromatography (dHPLC) (WAVE; Transgenomic, Inc., Omaha, NE) and direct sequencing as previously reported [Miyake et al., 2008]. Each variant detected by dHPLC was sequenced.

No *CHN1* mutations were detected in any of the 140 DRS probands. We did detect 7 heterozygous single nucleotide substitutions (Table I), of which three are known polymorphisms (SNPs) and a fourth, 261-24A>T in intron 5, we found in 1 of 187 controls of mixed ethnicity. Although we did not detect the remaining 3 variants (1-35G>T in the 5'UTR, 588C>T in exon 7, and 964+54A>G in intron 10) on 420 control alleles of mixed ancestry screened by dHPLC, only 588C>T is in coding sequence and it results in a

synonymous amino acid substitution (E196E), and none are predicted to be promoter/enhancer regions or to alter splicing by either ESE finder 3.0 (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi>), or splice site prediction by neural network from Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/splice.html). Thus, these are most likely rare polymorphisms and not disease-causing.

The absence of *CHN1* coding mutations in 140 individuals with sporadic DRS is in contrast to the 35% detection rate of *CHN1* mutation in familial DRS (7/20 pedigrees) [Miyake et al., 2008]. There are several possible explanations for this discrepancy. The individuals harboring *CHN1* mutations identified to date were from families that segregate autosomal dominant isolated DRS with a high incidence of bilateral involvement and additional abnormalities of vertical gaze. Only 13 of the 90 well-defined probands with sporadic DRS screened in this study had bilateral DRS in the absence of additional anomalies and only 1 of the 13 had additional vertical gaze abnormalities noted. Thus, it is possible that the *de novo* *CHN1* mutation rate is low and our cohort contained too few individuals with sporadic, isolated, nonsyndromic DRS to detect new mutations. We have not, however, eliminated the possibility that individuals with sporadic DRS harbor somatic *CHN1* mutations that were not present in the buccal epithelial cells and/or lymphocytes from which their DNA was obtained. It is also possible that these individuals have an extra copy of *CHN1* or harbor mutations in undefined *CHN1* regulatory regions that we did not sequence, either of which could result in gain-of-function of the alpha2-chimaerin protein and, hence, the DURS2 phenotype.

If *CHN1* gain-of-function mutations simply cause a rare variant form of DRS and we are not missing somatic or germ-line *CHN1* mutations, what causes the common sporadic form of this disorder? Although we are not aware of families segregating DRS that are large enough for informative linkage analysis and do not harbor *CHN1* mutations, only 35% of the dominant pedigrees we reported harbor *CHN1* mutations, supporting the presence of additional genetic causes of DRS in small families and potentially sporadic cases. Some sporadic cases may represent autosomal recessive transmission and, consistent with this possibility, 4 probands in the current cohort are offspring of closely related parents. It is also interesting that there are several reports of individuals with sporadic DRS and chromosomal anomalies [Cullen et al., 1993; Pizzuti et al., 2002], including four probands enrolled in this study, suggesting that *de novo* rearrangements or copy number variations may underlie a subset of DRS. Finally, some cases of sporadic DRS likely result from epigenetic and/or environmental factors. Indeed, prenatal exposure to thalidomide has been shown to cause DRS [Miller, 1991].

We conclude that *CHN1* mutations are not a major cause of DRS among individuals with sporadic disease. Based on our findings, we recommend *CHN1* mutation screening in individuals with isolated DRS that segregates as an autosomal dominant trait. Among these families, we would hypothesize that those whose affected members have bilateral DRS and additional vertical movement abnormalities are most likely to be mutation positive.

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Table 1
***CHN1* single nucleotide variants observed among DRS probands**

# probands with variant	Location of change ^a	Amino acid substitution	dbSNP reference ID	# controls with variant
1	1-35G>T, 5'UTR	none	none	0/213
1	261-24A>T, intron 5	none	none	1/187
1	588C>T, exon7	E196E	none	0/264
1	886+15A>G, intron 9	none	rs12613075	
2	964+54A>G, intron 10	none	none	0/285
3	964+123A>T, intron 10	none	rs56694150	
6	965-100G>A, intron 10	none	rs57776382	

^a Nucleotide numbering refers to the cDNA sequence specifying the 1,380 amino acid residues of *CHN1* alpha2-chimaerin splice form, commencing at the +1 position of the initiation codon (NM_001822).