

# Two Pedigrees Segregating Duane's Retraction Syndrome as a Dominant Trait Map to the DURS2 Genetic Locus

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**PURPOSE.** The genetic bases of Duane's retraction syndrome (DRS) were investigated to determine its molecular etiologies. In prior studies, the transcription factors *SALL4* and *HOXA1* were identified as the genes mutated in DRS with radial anomalies, and in DRS with deafness, vascular anomalies, and cognitive deficits, respectively. Less is known, however, about the genetic etiology of DRS when it occurs in isolation, and only one genetic locus for isolated DRS, the DURS2 locus on chromosome 2, has been mapped to date. Toward the goal of identifying the DURS2 gene, two pedigrees have been ascertained that segregate DRS as a dominant trait.

**METHODS.** Members of two large dominant DRS pedigrees were enrolled in an ongoing study of the genetic basis of the congenital cranial dysinnervation disorders, and linkage analysis was conducted to determine whether their DRS phenotype maps to the DURS2 locus.

**RESULTS.** By haplotype analysis, the DRS phenotype in each family cosegregates with markers spanning the DURS2 region. Linkage analysis reveals maximum lod scores  $>2$ , establishing that the DRS phenotype in these two pedigrees maps to the DURS2 locus.

**CONCLUSIONS.** These two pedigrees double the published pedigrees known to map to the DURS2 locus and can thus contribute toward the search for the DURS2 gene. The affected members represent a genetically defined population of DURS2-linked DRS individuals, and hence studies of their clinical and structural features can enhance understanding of the DURS2 phenotype, as described in the companion paper. (*Invest Ophthalmol Vis Sci.* 2007;48:189-193) DOI:10.1167/iovs.06-0631

**N**amed for Alexander Duane,<sup>1</sup> Duane's retraction syndrome (DRS) is the most common of the congenital cranial dysinnervation disorders (CCDDs) and accounts for 1% to 5% of all cases of strabismus.<sup>2,3</sup> Affected eyes have limited horizontal

gaze and retraction of the globe into the orbit on attempted adduction, resulting in secondary narrowing of the palpebral fissure in adduction. DRS can be clinically categorized into three types.<sup>4</sup> Type I (DRS-I) is characterized by poor abduction with little or no limitation of adduction; type II (DRS-II) is characterized by poor adduction with little or no limitation of abduction; and type III (DRS-III) is characterized by both poor abduction and poor adduction.

Early studies of DRS reported fibrosis, abnormal insertions, and adhesions of the lateral (LR) or medial (MR) rectus muscles and suggested a primary myopathic etiology.<sup>1,5,6</sup> Subsequently, two postmortem examinations in cases of DRS revealed absence of the abducens nucleus and cranial nerve VI (CN6) on the affected side(s), and partial innervation of the LR muscle(s) by branches of the oculomotor nerve (CN3).<sup>7,8</sup> Electromyographic (EMG) studies have revealed that simultaneous activation of the MR and LR muscles is associated with cocontraction and globe retraction.<sup>9,10</sup> Magnetic resonance imaging (MRI) has verified the absence of CN6 at the pons<sup>11</sup> and has documented cocontraction of the MR and LR on attempted adduction<sup>12-14</sup> in sporadic DRS. These studies suggest that at least a subset of DRS results from aberrant development of CN6, with varying amounts of primary or secondary anomalous innervation of the LR by CN3.

Although DRS is most commonly a sporadic trait, it can be inherited. Identification of the genes mutated in inherited DRS can provide insight both into the cause of the disorder and the molecular pathways essential to ocular motoneuron and axon development. Using this approach, we have identified several gene defects that result in syndromic DRS. Mutations in the transcription factor *SALL4* cause DRS in association with variably penetrant radial ray deformities and deafness.<sup>15,16</sup> Homozygous loss-of-function mutations in the homeodomain transcription factor *HOXA1* result in DRS in association with variable penetrance of deafness, hypoventilation, internal carotid and cardiac outflow vascular defects, and cognitive deficits.<sup>17</sup> Recessive mutations in the axon guidance molecule *ROBO3* result in absent horizontal eye movements and progressive scoliosis.<sup>18,19</sup>

In most individuals, DRS occurs in isolation without additional congenital defects, and among individuals with isolated DRS, a positive family history is reported in only 2% to 20% of cases.<sup>3,6,20-27</sup> Individuals with isolated DRS have not been found to harbor mutations in *HOXA1*<sup>28</sup> or *SALL4*,<sup>29</sup> supporting the hypothesis that isolated familial and sporadic DRS is genetically different from syndromic DRS.

It is rare to find large multigenerational families with isolated DRS that are amenable to linkage analysis and, hence, to the identification of isolated DRS genes. In 1999, however, Appukuttan et al.<sup>30</sup> successfully ascertained a four-generation family from Mexico with fully penetrant isolated DRS and mapped their phenotype to a 17.8-cM region of 2q31 flanked by *D2S2330* and *D2S364*, now referred to as the DURS2 locus (OMIM 604356; Online Mendelian Inheritance in Man; <http://www.ncbi.nlm.nih.gov/Omim/> provided in the public domain by the National Center for Biotechnology Information, Be-

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thesda, MD). A maximum lod score of 11.73 was obtained at  $\theta = 0$ . A detailed clinical description of the pedigree<sup>31</sup> revealed that, of the 25 affected participants, 24 (96%) had bilateral DRS, with DRS-I noted in 20 (80%) and DRS-III in 5 (20%). Nineteen (76%) had strabismus in primary gaze (10 esotropic, 1 exotropic, 8 manifest hypertropia, and 4 dissociated vertical deviation). In addition, 48% had amblyopia, 12% had trochlear nerve palsy, and a majority had vertical as well as horizontal movement abnormalities. Two affected individuals (8%) did not have retraction, as was true of 5% in Duane's original study.<sup>1</sup>

In 2000, Evans et al.<sup>32</sup> analyzed a four-generation British pedigree with fully penetrant isolated DRS and confirmed linkage to the DURS2 locus with a maximum lod score of 3.3 at  $\theta = 0$ . A recombination event in one affected individual reduced the DURS2 critical region to 8.8 cM flanked by *D2S326* and *D2S364*. All nine affected members of this family had bilateral disease. Five had DRS-I, 2 had DRS-III, and 2 had DRS-I on the right and DRS-III on the left. The *HOXD* gene cluster falls within the DURS2 region but no mutations of *HOXD1*, *HOXD3*, and *HOXD4* were identified in affected members of either family.

We have now ascertained two large, previously unreported pedigrees that cosegregate Duane's syndrome as an autosomal dominant trait. In this report, we describe their genetic mapping to the DURS2 locus. In the companion paper, we describe their clinical examinations and brain stem and orbital MRI.<sup>33</sup>

## METHODS

### Clinical Examinations

Pedigrees segregating isolated dominant DRS were enrolled in an ongoing genetic study of the CCDDs. After informed consent was obtained, the ophthalmic and general medical histories of participating family members were obtained, and a peripheral blood sample was drawn for genomic DNA isolation. Verbal histories, medical records, and photographs of participants were reviewed and, whenever possible, participants were examined. The diagnosis of DRS was made based on the presence of limitation of abduction and/or adduction in one or both eyes, with globe retraction and lid fissure narrowing on adduction of affected eyes. The study was approved by relevant institutional review boards; informed consent was obtained in conformity with the Declaration of Helsinki.

### Linkage Analysis

High-molecular-weight genomic DNA was extracted from each blood sample using the Puregene kit (Gentra, Minneapolis, MN). Linkage studies were conducted using six fluorescently labeled microsatellite markers spanning the *DURS2* locus (*D2S2330*, *D2S335*, *D2S326*, *D2S2314*, *D2S364*, and *D2S117*), five spanning the *SALL4* locus (*D20S119*, *D20S178*, *D20S196*, *D20S100*, and *D20S171*), and five spanning the *HOXA1* locus (*D7S493*, *D7S1821*, *MT26723*, *MT27012*, and *D7S516*). Fluorescently labeled primers were purchased from Invitrogen (Carlsbad, CA), and amplicons were generated by 30 cycles of PCR amplification containing 10 to 30 ng of genomic DNA in 5- $\mu$ L reaction volumes of *Taq* PCR master mix (Qiagen, Valencia, CA) containing 2 picomoles of each fluorescent primer pair, 1 nanomole each of dATP, dTTP, dGTP, and dCTP, and 0.15 U *Taq* polymerase. The products were analyzed in a DNA analyzer (model 3730; Applied Biosystems [ABI], Foster City, CA).

For linkage analysis, an individual was scored as affected based on clinical examination and/or clinical examination records. Lod scores were calculated with the MLINK (v5.1 with 2-point autosomal data) part of the LINKAGE package,<sup>34</sup> assuming autosomal dominant inheritance with 95% penetrance and a disease incidence of 1 in 1000,000 births. Because of the absence of specific allele frequencies for the two

ethnic groups represented in the study, we assumed 10 marker alleles of equal frequency.

### *SALL4* Mutation Analysis

The four coding exons and flanking introns of *SALL4* were amplified, and the PCR products were directly sequenced as previously reported.<sup>15</sup>

## RESULTS

### Pedigrees

Two pedigrees segregating isolated DRS as a dominant trait were enrolled in the study (Figure 1). FY is a Hispanic family originally from Aguascalientes, Mexico, and pedigree JH is a white pedigree from Texas.

Twenty-three members of family FY participated in the study, including five of the six living affected members. Of the five affected participants, three had bilateral DRS-III (V:3, V:12, V:14), one had right unilateral DRS-III (V:6), and one had left unilateral DRS-I (IV:2). It appears that the DRS phenotype may be partially penetrant in this pedigree, given the report that deceased relatives I:1, I:2, and II:3 did not have DRS, and examination of photographs of II:3 did not reveal strabismus.

Fourteen members of family JH were studied, including all six affected by DRS. One affected member had bilateral DRS-III (IV:3), whereas three had right-side DRS-III and left-side DRS-I (II:1, III:3, and III:6), and one had right DRS-I and left DRS-III (V:1). Individual III:1 had unilateral left DRS-I. In addition, III:6 had Klippel-Feil syndrome.

Four affected (V:3, V:6, V:12, and V:14) and three unaffected (V:5, VI:4, and VI:6) participants from pedigree FY, and four affected (III:3, III:6, IV:3, and V:1) participants from pedigree JH also participated in our CCDD MRI study. These individuals underwent complete ophthalmic examination by one of the authors (JLD), and most also underwent high-resolution MRI of the orbits and cranial nerves at the brain stem, as detailed in the companion paper.<sup>33</sup>

### Linkage and Haplotype Analysis

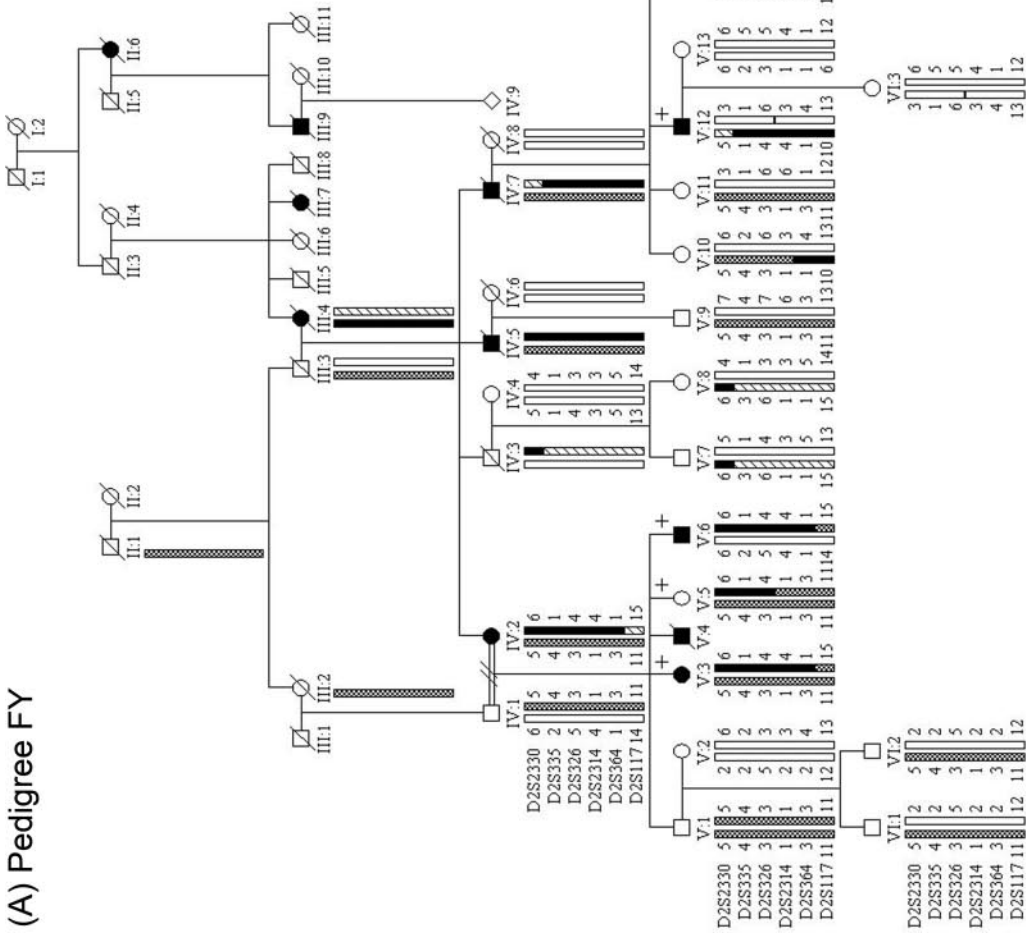
Analysis of the six genetic markers across the 8.8 cM DURS2 critical region, including the flanking markers *D2S326* and *D2S364* and one internal marker *D2S2314*, revealed cosegregation of the DRS phenotype in both pedigrees to the DURS2 locus. Maximum lod scores of 2.1 and 2.3 were obtained at *D2S2314* by pedigrees FY and JH, respectively (Table 1). These are the maximum lod scores obtainable, given the pedigree structure and family participants, and lod scores of  $>2$  are considered significant for confirmation of a previously established disease locus.<sup>35</sup> Pedigree JH demonstrated complete cosegregation of the affected haplotype with the DRS phenotype, consistent with full penetrance of the DURS2 mutation. Consistent with the apparent incomplete penetrance of the DURS2 mutation in FY II:3, however, FY VI:4 carries the entire disease-associated haplotype and FY V:5 carries a portion. Both FY VI:4 and V:5 had normal ophthalmic examinations (refer to companion paper<sup>33</sup>).

Linkage analysis at the previously reported DRS loci, *HOXA1* and *SALL4*, revealed that neither pedigree mapped to the *HOXA1* locus, regardless of whether the data were interpreted as cosegregation of a dominant or recessive trait. FY was not linked to the *SALL4* locus, whereas JH was consistent with linkage but with a penetrance of only 66%. No mutations were detected in *SALL4* in affected members of pedigree JH.

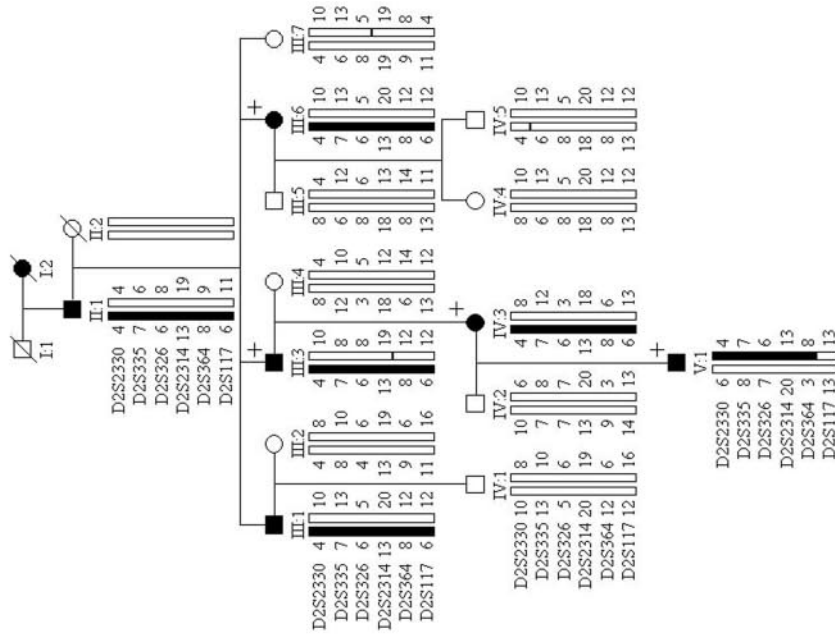
## DISCUSSION

Our data establish that the DRS phenotypes in pedigrees FY and JH segregate with and are linked to the previously defined

(A) Pedigree FY



(B) Pedigree JH



**FIGURE 1.** Haplotype analysis of pedigrees FY and JH at the DURS2 locus. *Black symbols:* those individuals who are clinically affected with DRB. Genotyping data and schematic segregating haplotype bars for 2q13 markers are shown below the symbol for each study participant, and allele sizes are equivalent between families for a given marker. *Black bars:* the potential disease-associated region; *hatched or white bars:* the inheritance of the non-disease-associated

haplotypes. Assumed haplotype bars without corresponding allele data are provided for some nonparticipating pedigree members, to assist in tracing inheritance patterns. References to specific individuals within the text refer to the generation number (Roman numeral) and position within generation (Arabic numeral). A superscript (+) sign to the upper right of an individual denotes participation in the MRI study, as described in the companion paper.<sup>35</sup>

TABLE 1. Lod Scores of 2q31 Markers with DRS

Locus	Pedigree	Recombination fraction ( $\theta$ )					
		0.00	0.05	0.10	0.20	0.30	0.40
D2S2330	FY	-7.2	-0.1	0.1	0.3	0.3	0.1
	JH	1.4	1.3	1.2	1.0	0.7	0.4
D2S335	FY	1.0	1.3	1.4	1.3	0.9	0.5
	JH	2.3	2.1	1.9	1.5	1.0	0.5
D2S326	FY	1.2	1.5	1.5	1.3	1.0	0.4
	JH	2.0	1.8	1.6	1.2	0.8	0.4
D2S2314	FY	2.1	2.1	2.0	1.6	1.0	0.4
	JH	2.3	2.1	1.9	1.5	1.0	0.5
D2S364	FY	1.3	1.2	1.1	0.9	0.6	0.3
	JH	2.0	1.9	1.7	1.3	0.9	0.4
D2S117	FY	-9.4	0.1	0.5	0.7	0.6	0.3
	JH	-3.7	0.8	1.0	0.9	0.6	0.3

DURS2 locus<sup>30,32</sup> with lod scores  $>2.0$ , thus confirming this genetic locus and doubling the pedigrees reported to map to it. Unlike the two previously reported DURS2 pedigrees, however, pedigree FY included one unaffected child who carried the disease-associated haplotype and was clinically unaffected, establishing that DURS2 gene mutations can be clinically non-penetrant. Unfortunately, this child was too young to undergo MRI and, hence, we could not determine whether he harbored a clinically undetected endophenotype.

Similar to the previously reported DURS2-linked DRS pedigrees, affected members of these two families have DRS-I or DRS-III, and most but not all family members are bilaterally affected. No affected members of DURS2-linked DRS pedigrees,<sup>31,32</sup> *SALL4*-linked DRS pedigrees,<sup>15</sup> or *HOXA1*-linked pedigrees<sup>17</sup> have had a diagnosis of DRS-II, suggesting that DRS-II is a genetically distinct disorder.

The current 8.8 cM DURS2 region corresponds to 9.9 Mb and contains approximately 45 candidate genes. The only recombination event within this critical region in pedigrees FY and JH occurred in participant FY V:5, whose clinical examination results were normal. Because DRS appeared to be partially penetrant in this pedigree and it was not known whether V:5 harbored the mutation, this recombination event cannot be used to reduce the DURS2 critical region.

Pedigrees FY and JH were of different ethnicities and did not share disease-associated alleles at the markers examined, suggesting their DURS2 mutations arose independently. However, the initial DURS2 pedigree reported by Appukuttan et al.<sup>30</sup> is from Oaxaca, Mexico, approximately 600 miles south of Aguascalientes. It is possible that FY shares a common founder mutation with this original pedigree and, if so, defining the genetic distance over which they share a disease-associated haplotype could reduce the DURS2 region. Thus, pedigrees FY and JH should assist in the identification of the DURS2 gene, given that the pedigrees are likely to provide two new DURS2 founder mutations or, alternatively, to provide one new founder mutation and the potential to reduce the critical region through a second shared founder mutation.

Establishing that the DRS phenotype in pedigrees FY and JH map to the DURS2 locus has provided an opportunity to define further the DURS2-linked DRS phenotype. By defining these pedigrees genetically, we can now compare clinical and MRI findings within and among DURS2-linked DRS families, leading to a more precise description of the DURS2 clinical and endophenotype. Results of such a study should aid in clinical diagnosis, permit the comparison of the DURS2 phenotype to that found in syndromic and sporadic DRS, and provide guidance for future examinations of the role of the DURS2 gene in ocular motor development. Toward these goals, the clinical

and MRI studies of members of pedigrees FY and JH are presented in a companion paper,<sup>33</sup> and provide evidence that DURS2-linked DRS is a diffuse congenital cranial dysinnervation disorder not limited to the abducens nucleus and cranial nerve 6.

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