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Two pedigrees segregating Duane's retraction syndrome as a dominant trait map to the DURS2 genetic locus

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Abstract

PURPOSE—To determine the molecular etiologies of Duane's retraction syndrome (DRS), we are investigating its genetic bases. We have previously identified the transcription factors *SALL4* and *HOXA1* as the genes mutated in DRS with radial anomalies, and in DRS with deafness, vascular anomalies, and cognitive deficits, respectively. We know less, 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, we have ascertained and studied two pedigrees that segregate DRS as a dominant trait.

METHODS—We enrolled members of two large dominant DRS pedigrees into our ongoing study of the genetic basis of the congenital cranial dysinnervation disorders, and conducted linkage analysis to determine if their DRS phenotype maps to the DURS2 locus.

RESULTS—By haplotype analysis, the DRS phenotype in each family co-segregates with markers spanning the DURS2 region, and linkage analysis reveals maximum lod scores of >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 our understanding of the DURS2 phenotype, as described in the companion paper.

Keywords

Duane's syndrome; linkage analysis; DUR2

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Named for Alexander Duane¹, Duane's retraction syndrome (DRS) is the most common of the congenital cranial dysinnervation disorders (CCDDs), and accounts for 1–5% of all strabismus cases^{2,3}. 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 types⁴. 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 rectus (MR) muscles, and suggested a primary myopathic etiology^{1,5,6}. Subsequently, two postmortem examinations of cases of DRS revealed absence of the abducens nucleus and cranial nerve (CN6) on the affected side(s), and partial innervation of the LR muscle(s) by branches of the oculomotor nerve (CN3)^{7,8}. Electromyographic (EMG) studies have revealed that simultaneous activation of the MR and LR muscles is associated with co-contraction and globe retraction^{9,10}. Magnetic resonance imaging (MRI) has verified the absence of (CN6) at the pons¹¹ and has documented co-contraction of the MR and LR on attempted adduction^{12–14} 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^{15,16}. 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 deficits¹⁷. Recessive mutations in the axon guidance molecule *ROBO3* result in absent horizontal eye movements and progressive scoliosis^{18,19}.

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 – 20% of cases^{3,6,20–27}. Individuals with isolated DRS have not been found to harbor mutations in *HOXA1*²⁸ or *SALL4*²⁹, supporting the hypothesis that isolated familial and sporadic DRS is genetically unique from syndromic DRS.

It is rare to find large multi-generational 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*³⁰ successfully ascertained a 4-generation family from Mexico with fully penetrant isolated DRS and mapped their phenotype to a 17.8-cM region of chromosome 2q31 flanked by *D2S2330* and *D2S364*, now referred to as the DURS2 locus (OMIM 604356). A maximum LOD score of 11.73 was obtained at $\theta = 0$. A detailed clinical description of the pedigree³¹ 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, 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, similar to 5% in Duane's original study¹.

In 2000, Evans *et al*³² analyzed a 4-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*. Of the nine affected members of this family, all were bilaterally affected. Five had DRS-1, 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 co-segregate Duane's syndrome as an autosomal dominant trait. In this manuscript, we describe their genetic mapping to the DURS2 locus. In the companion paper, we describe their clinical examinations and brainstem and orbital MRI³³.

METHODS

Clinical examinations

Pedigrees segregating isolated dominant DRS were enrolled in an ongoing genetic study of the CCDDs. After informed consent was obtained, the ophthalmologic 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 to the Declaration of Helsinki.

Linkage analysis

High-molecular weight genomic DNA was extracted from each blood sample using the Puregene kit (Gentra). 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*, *D20S171*), and five spanning the *HOXA1* locus (*D7S493*, *D7S1821*, *MT26723*, *MT27012*, *D7S516*). Fluorescently labeled primers were purchased from Invitrogen, and amplicons were generated by 30 cycles of PCR amplification containing 10–30 ng of genomic DNA in 5- μ l reaction volumes of Qiagen's Taq PCR Master Mix containing 2 pmol of each fluorescent primer pair, 1 nmol each of dATP, dTTP, dGTP, and dCTP, and 0.15 U Taq polymerase. The products were analyzed in an Applied Biosystems 3730 DNA Analyzer.

For linkage analysis, an individual was scored as affected based on clinical examination and/or clinical examination records. Lod scores were calculated using the MLINK (v5.1 with 2-point autosomal data) part of the LINKAGE package³⁴ assuming autosomal dominant inheritance with 95% penetrance and a disease incidence of 1 in 1,000,000 births. Because of the absence of specific allele frequencies for the two ethnic groups represented in the study, we assumed ten marker alleles of equal frequency.

SALL4 mutation analysis

The 4 coding exons and flanking introns of *SALL4* were amplified and the PCR products were directly sequenced as previously reported¹⁵.

RESULTS

Pedigrees

Two pedigrees segregating isolated DRS as a dominant trait were enrolled in the study (Figure). FY is a Hispanic family originally from Aguascalientes, Mexico while pedigree JH is a Caucasian 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 have bilateral DRS-III (V:3, V:12, V:14), one has right unilateral DRS-III (V:6), and one has 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 photos of II:3 does not reveal strabismus.

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

Four affected (V:3, V:6, V:12, V:14) and three unaffected (V:5, VI:4, VI:6) participants from pedigree FY, and four affected (III:3, III:6, IV:3, V:1) participants from pedigree JH also participated in our CCDD MRI study. These individuals underwent complete ophthalmologic examination by one of the authors (J.L.D.), and most also underwent high-resolution MRI of the orbits and cranial nerves at the brainstem as detailed in the companion paper³³.

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 co-segregation 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). 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 locus³⁵. Pedigree JH demonstrated complete co-segregation 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 and FY V:5 carries a portion of the disease-associated haplotype. Both FY VI:4 and V:5 had normal ophthalmologic examinations, and FY V:5 had normal MR imaging of the orbits and brainstem (refer to companion paper³³).

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 was interpreted as co-segregation of a dominant or recessive trait. FY was not linked to the *SALL4* locus, while 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 DURS2 locus^{30,32} 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 includes one unaffected child who carries the disease-associated haplotype and is clinically unaffected, establishing that DURS2 gene

mutations can be clinically nonpenetrant. Unfortunately, this child was too young to undergo MRI and, hence, we cannot determine if he harbors 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^{31,32}, SALL4-linked DRRS pedigrees¹⁵, or HOXA1-linked pedigrees¹⁷ have been diagnosed with 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 occurs in participant FY V:5, whose clinical examination is normal. Because DRS appears to be partially penetrant in this pedigree and it is not known whether V:5 harbors the mutation, this recombination event cannot be used to reduce the DURS2 critical region.

Pedigrees FY and JH are of different ethnicities and did not share disease-associated alleles at the markers examined, suggesting their DURS2 mutations arose independently. Interestingly, however, the initial DURS2 pedigree reported by Appukuttan *et al*³⁰ 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, 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 further define 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 paper³³, 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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Figure.

Haplotype analysis of pedigrees FY and JH at the DURS2 locus. Black symbols denote those individuals who are clinically affected with DRS. Genotyping data and schematic segregating haplotype bars for chromosome 2q13 markers are shown below the symbol for each study participant, and allele sizes are equivalent between families for a given marker. Black bars denote the potential disease-associated region. Hatched or white bars highlight the inheritance of the non-disease-associated haplotypes. Assumed haplotype bars without corresponding allele data are provided for some non-participating 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 magnetic resonance imaging study, as described in the companion paper³³

Table

Lod scores of chromosome 2q31 markers with DRS										
Locus	Pedigree	Recombination fraction (θ)								
		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			