

Duane Radial Ray Syndrome (Okihiro Syndrome) Maps to 20q13 and Results from Mutations in *SALL4*, a New Member of the SAL Family

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Duane syndrome is a congenital eye movement disorder characterized most typically by absence of abduction, restricted adduction, and retraction of the globe on attempted adduction. Duane syndrome can be coinherited with radial ray anomalies as an autosomal dominant trait, referred to as “Okihiro syndrome” or “Duane radial ray syndrome” (DRRS). We ascertained three pedigrees with DRRS and mapped their disease gene to a 21.6-cM region of chromosome 20 flanked by markers D20S888 and D20S102. A new member of the SAL family of proposed C₂H₂ zinc finger transcription factors, *SALL4*, falls within the region. Mutation analysis of *SALL4* in the three pedigrees revealed one nonsense and two frameshift heterozygous mutations. *SALL4* represents the first identified Duane syndrome gene and the second malformation syndrome resulting from mutations in SAL genes and likely plays a critical role in abducens motoneuron development.

Several forms of congenital ophthalmoplegia are hypothesized to result from errors in the development of the ocular cranial nuclei (Nakano et al. 2001). The most common of these, Duane syndrome (MIM 126800 and MIM 604356), is characterized by restricted or absent abduction and variably restricted adduction with retraction of the globe on attempted adduction, and results from absence of the abducens (nVI) motoneurons and nerve, with sparing of the internuclear neurons (Hotchkiss et al. 1980; Miller et al. 1982). Two Duane syndrome loci (*DURS1* and *DURS2*) have been mapped, but neither gene has been identified (Vincent et al. 1994; Appukuttan et al. 1999). Duane syndrome can be coinherited with radial anomalies in an autosomal dominant fashion (fig. 1A). In affected individuals, radial dysplasia ranges from hypoplasia of the thenar eminence to absence of the radial bone or forearm. Variable expression of sensorineural hearing loss and cardiac, renal, vertebral, and lower extremity malformations can occur. De-

scribed in the 1970s and referred to as “Okihiro syndrome” or “Duane radial ray syndrome” (DRRS) (included in MIM 126800) (Okihiro et al. 1977; Temtamy and McKusick 1978; Appukuttan et al. 1999), this distinct disorder partially overlaps clinically with Townes-Brocks and Holt-Oram syndromes, as well as acroreno-ocular, oculo-otoradial, cervico-oculoacoustic, and oculoauriculovertebral syndromes.

To investigate the role that the DRRS gene plays in abducens development, we analyzed two white pedigrees and one Japanese pedigree (V, FN, and DA, respectively) segregating DRRS as an autosomal dominant trait with apparent full penetrance (fig. 1B–1D). The study was approved by Children's Hospital Boston Review Board, and appropriate informed consent was obtained from all participants. The clinical features of pedigrees DA and FN have been described elsewhere (Okihiro et al. 1977; Chun et al. 2001). Of the 13 affected participants in the three families, 12 have Duane syndrome. Among them, the Duane syndrome phenotype can be unilateral or bilateral and includes individuals with marked or complete limitation of abduction with minimal or no limitation of adduction (Duane syndrome type 1) and individuals with marked or complete limitation of both abduction and adduction (Duane syndrome type 3). None of the participants had marked or complete limitation of adduction with minimal or no limitation of abduction (Duane syndrome type 2). The presence of more than one Duane syndrome type within a single pedigree was also described in a family whose disease gene maps to the *DURS2* locus (Chung et al. 2000) and

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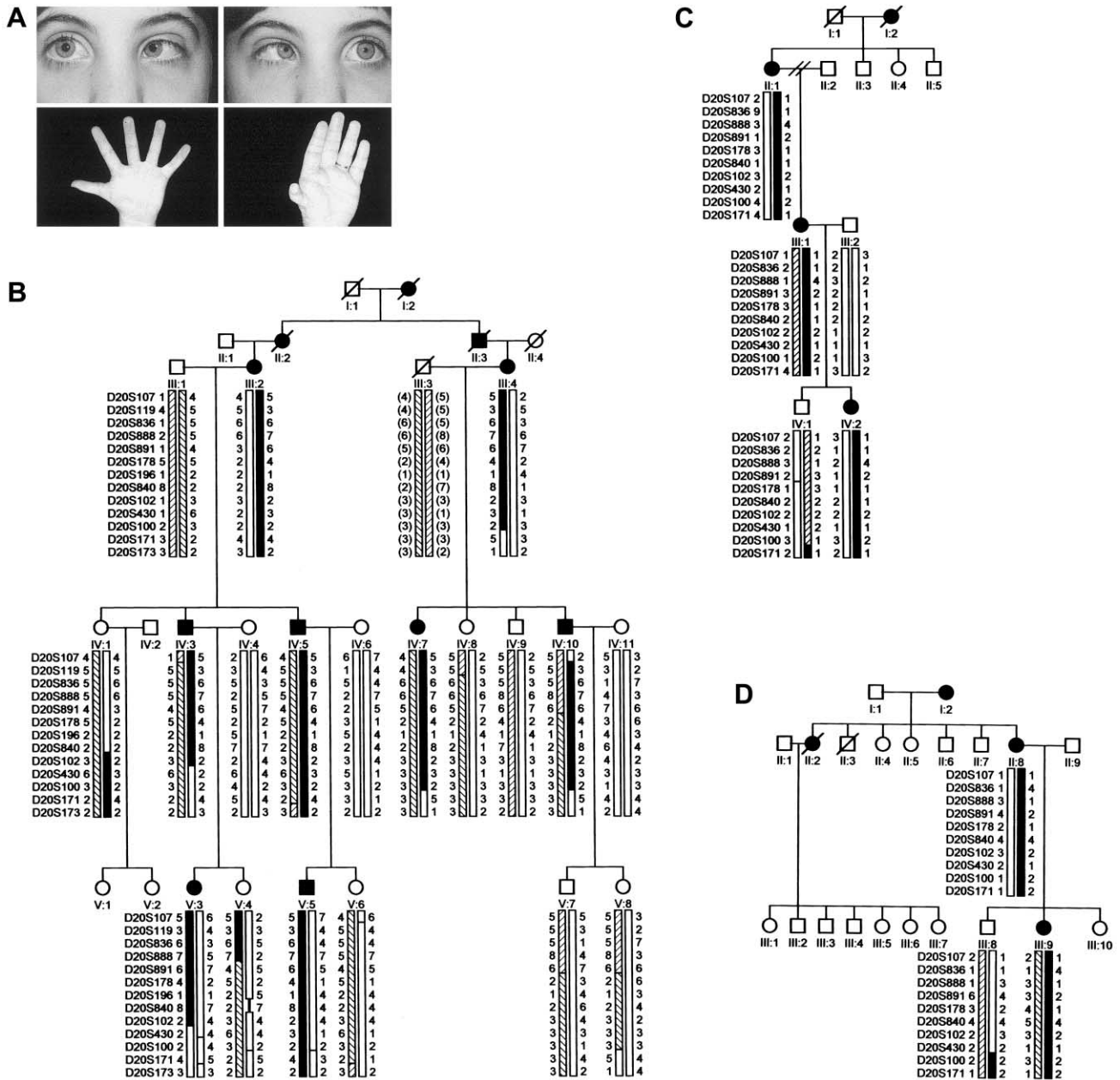


Figure 1 DRRS pedigrees. *A, Top*, attempted right and left gaze in an affected member of pedigree V (V:3) with Duane syndrome; *bottom*, thenar hypoplasia and thumb anomaly in affected members of pedigree V (V:3, left; IV:3, right). *B*, pedigree V. *C*, pedigree FN. *D*, pedigree DA. Affected individuals are indicated by filled symbols. Below the symbol for each participant are genotypes for chromosome 20q13.12-q13.31 markers. The disease-associated haplotype in each family is in black.

may result from variable expression of the primary gene mutations or from different patterns of secondary aberrant innervation. Twelve of the affected participants have radial dysplasia. The radial dysplasia can also be unilateral or bilateral and ranges from hypoplasia of the thenar eminence to foreshortening of the forearm with absent radial bone, radial pulse, and thumb. Only one participant has unilateral Duane syndrome and unilat-

eral radial anomalies on opposite sides. Two affected participants have sensorineural hearing loss. Facial weakness, abnormal pinna, fused cervical vertebrae, pectoralis muscle aplasia, and Hirschsprung disease were each detected once. No participants have known renal or cardiac anomalies. The clinical findings are summarized in table 1.

We conducted an ~10-cM genomewide linkage screen

Table 1
Clinical Synopsis of DRRS in Participating Family Members

PEDIGREE AND INDIVIDUAL	FINDING FOR CHARACTERISTIC									
	Duane Type	Thenar Hypoplasia	Thumb Defect	Absent Radial Pulse	Club Wrist	Short Forearm	Shoulder Dislocation	Short Neck	Fused Cervical Vertebrae	Sensorineural Hearing Loss
Pedigree V:										
III:2	L (1)	L	L	+		B	L	+	+	
IV:3	B (3)	B	B	+		B	+			
IV:5	R (1)	L	L		L	L	L			
V:3	B (1)	B	B	+			+			+
V:5	B (1)	B	B		B					
III:4	R (1)	R								
IV:7	B (3)	B	B	B	B	L	L			
IV:10	L (1)	—	—							
Pedigree FN:										
II:1	—		B							
III:1	R (1)		B							R
IV:2	R (1)		B							
Pedigree DA:										
II:8	B	R								
III:9	B (3)	B	B							

NOTE.—L = left, R = right, B = bilateral, + = present, — = absent, (1) = Duane type 1, (3) = Duane type 3.

of pedigree V's phenotype, using the ABI Prism Linkage Mapping Set Panels (Version 2; PE Applied Biosystems) and calculated two-point LOD scores as reported elsewhere (Engle et al. 1994). Adjacent screening markers D20S178, D20S196, and D20S100 revealed LOD scores of 2.6, 2.9, and 3.0 at $\theta = 0$. No other screening marker yielded a score >2 . Using radioactive methods, we analyzed 18 additional markers between D20S119 and D20S171 and refined the locus to an ~22-cM region of chromosome 20q13.12–q13.31 flanked by D20S888 and D20S102 (fig. 1B). We then analyzed FN and DA and found that their phenotypes were consistent with linkage (fig. 1C and 1D). Maximum LOD scores were 3.57, 0.6, and 0.3 at $\theta = 0$ in V, FN, and DA, respectively, and a maximum combined LOD score of 4.47 was obtained at the fully informative marker, D20S891 (table 2).

We reviewed positional candidate genes on the basis of the UCSC Human Genome Project Working Draft and chose to analyze a previously unreported gene, LOC57167, that has been annotated by GenBank as a new member of the SAL family (*SALL4*) and maps between D20S196 and D20S840. This gene was particularly interesting because heterozygous truncating mutations in *SALL1* cause Townes-Brocks (Kohlhase et al. 1998), a syndrome characterized by the distinct but overlapping complex of supernumerary/triphalangeal thumbs, hearing loss, malformed ears, and imperforate anus. We aligned the *SALL4* genomic and 3,162-bp cDNA sequences, identified four exons, amplified these exons and their flanking introns with 16 sets of primers (table A [online only]), and directly sequenced the PCR products. In the affected

members of pedigrees V and FN, we detected heterozygous exon 2 single-base-pair deletions 1904delT and 2425delG, respectively (fig. 2a and 2b). Both these mutations are predicted to result in frameshifts leading to a stop codon after 4 (pedigree V) and 46 (pedigree FN) altered amino acids. In pedigree DA, we detected a heterozygous 2593C→T change in exon 3, resulting in the transition of an arginine to a stop codon (R865X; fig. 2c). Five *SALL4* polymorphisms were also detected (table B [online only]). All three mutations cosegregate with the DRRS phenotype in each family, are not present on at least 174 chromosomes of mixed ethnicity (including 70 of Japanese ethnicity for the DA mutation), and, like *SALL1* mutations, are predicted to result in premature termination of the *SALL4* protein (fig. 2d).

SALL4 is predicted to encode a 1,053 aa protein with eight zinc finger motifs, three with SAL boxes, arranged in a characteristic fashion similar to *SALL1–3* (fig 2d). Thus, it is the fourth member of a group of human C₂H₂-type zinc finger proteins proposed to encode organ-specific transcription factors closely related to the *Drosophila melanogaster spalt (sal)* gene (Kohlhase et al. 1996, 1999). The *SALL4* protein shares 49%, 39%, 36%, and 29% identity Xsal-3, *SALL3*, *SALL1*, and *SALL2*, respectively. *SALL1* and mSall1 were recently demonstrated to be transcriptional repressors (Netzer et al. 2001; Kiefer et al. 2002) that localize to pericentromeric heterochromatin (Netzer et al. 2001). It is interesting that only the first 130 amino acids (including the amino-terminal H₂CH-ZF) of mSall1 are required for repression (Kiefer et al. 2002). This region is upstream of the known *SALL1* and *SALL4* mutations, sug-

Table 2
Pairwise LOD Scores of Chromosome 20 Markers with DRRS

LOCUS AND PEDIGREE	RECOMBINATION FRACTION (θ)					
	.00	.05	.10	.20	.30	.40
D20S119:						
V	−∞	2.36	2.31	1.86	1.24	.55
D20S836:						
V	−∞	1.83	1.84	1.54	1.09	.56
FN	.60	.56	.51	.41	.29	.16
DA	<u>.26</u>	<u>.22</u>	<u>.19</u>	<u>.11</u>	<u>.05</u>	<u>.01</u>
Total	−∞	2.61	2.54	2.06	1.43	.73
D20S888:						
V	−∞	2.36	2.31	1.86	1.24	.55
FN	.60	.56	.51	.41	.29	.16
DA	<u>−.78</u>	<u>−.49</u>	<u>−.33</u>	<u>−.15</u>	<u>−.06</u>	<u>−.01</u>
Total	−∞	2.43	2.49	2.12	1.47	.7
D20S891:						
V	3.57	3.25	2.91	2.21	1.47	.71
FN	.60	.56	.51	.41	.29	.16
DA	<u>.30</u>	<u>.26</u>	<u>.21</u>	<u>.13</u>	<u>.06</u>	<u>.02</u>
Total	4.47	4.07	3.63	2.75	1.82	.89
D20S178:						
V	2.63	2.55	2.36	1.83	1.20	.55
FN	.30	.26	.21	.13	.06	.02
DA	<u>.30</u>	<u>.26</u>	<u>.21</u>	<u>.13</u>	<u>.06</u>	<u>.02</u>
Total	3.23	3.07	2.78	2.09	1.32	.59
D20S196:						
V	2.90	2.58	2.25	1.57	.90	.33
D20S840:						
V	3.57	3.20	2.82	2.03	1.23	.51
FN	.60	.56	.51	.41	.29	.16
DA	<u>.00</u>	<u>.00</u>	<u>.00</u>	<u>.00</u>	<u>.00</u>	<u>.00</u>
Total	4.17	3.76	3.33	2.44	1.52	.67
D20S102:						
V	−∞	2.30	2.24	1.80	1.21	.57
FN	.00	.00	.00	.00	.00	.00
DA	<u>−.78</u>	<u>−.49</u>	<u>−.33</u>	<u>−.15</u>	<u>−.06</u>	<u>−.01</u>
Total	−∞	1.81	1.91	1.65	1.15	.56
D20S430:						
V	−∞	.56	.90	.96	.74	.40
FN	.30	.26	.21	.13	.06	.02
DA	<u>.30</u>	<u>.26</u>	<u>.21</u>	<u>.13</u>	<u>.06</u>	<u>.02</u>
Total	−∞	1.08	1.32	1.22	.86	.44
D20S100:						
V	3.04	2.76	2.47	1.88	1.26	.62
FN	.60	.56	.51	.41	.29	.16
DA	<u>−∞</u>	<u>−.72</u>	<u>−.44</u>	<u>−.19</u>	<u>−.08</u>	<u>−.02</u>
Total	−∞	2.60	2.54	2.10	1.47	.76
D20S171:						
V	−∞	.52	.88	.95	.72	.37
FN	−∞	−.72	−.44	−.19	−.08	−.02
DA	<u>−.78</u>	<u>−.49</u>	<u>−.33</u>	<u>−.15</u>	<u>−.06</u>	<u>−.01</u>
Total	−∞	−.69	.11	.61	.5	.34

gesting that the truncated proteins may have a dominant negative action as upregulated repressors (Kiefer et al. 2002). *SALL1–3* are reportedly expressed in restricted patterns in the embryonic nervous system (Kohlhase et al. 1996, 1999), with *SALL2* exclusively and *SALL3* selectively expressed in the fetal pons (potentially in the

developing abducens motoneurons). Thus, *SALL2* and *SALL3* are candidates for other forms of Duane syndrome. On the basis of our findings, *SALL4* is likely to have an important role in abducens motoneuron development and may serve as a unique marker for these developing neurons. This complements our previous report of *ARIX* mutations in CFEOM2, a much rarer form of congenital strabismus that selectively targets the developing oculomotor and trochlear motoneurons (Nak-

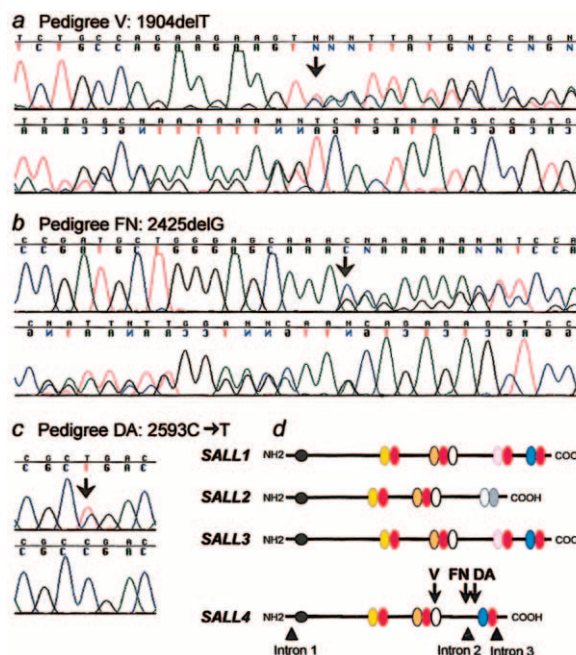


Figure 2 *SALL4* mutations and predicted SAL protein schematics. Forward (*top*) and reverse (*bottom*) strand direct sequence of an affected member of (*a*) pedigree V, showing the heterozygous 1904delT and of (*b*) pedigree FN, showing the heterozygous 2425delG. *c*, Direct sequence of an affected member of pedigree DA (*top*) and control (*bottom*) showing the heterozygous 2593C→T mutation. *d*, Predicted SALL1–4 protein structures and location of SALL4 DRRS mutations. SALL4 contains one NH₂-terminus C₂H₂-type zinc finger (ZF; black circle) and 7 C₂H₂-type ZF (oval) domains. The latter are arranged as double ZFs (DZF), with a third finger associated with the second doublet (white oval). The first finger of each pair contains a somewhat variable sequence (yellow, orange, and blue ovals), whereas the second finger of each pair contains the conserved SAL-box sequence, FT/STKGNLK (red ovals). The homologies to SALL1–3 are shown. SALL2 also contains three DZF motifs; the first two are highly similar to the other proteins, but the third is divergent (gray ovals). In contrast, SALL1 and SALL3 each contain three DZF motifs highly similar to SALL4 with an additional fourth DZF motif located between the second and third DZF in SALL4. The positions of the SALL4 splice junctions and the three DRRS mutations are superimposed on the schematic protein. The three mutations are predicted to result in unstable mRNAs or truncated proteins disrupting the fifth and/or lacking the sixth and seventh DZF motifs. Protein structures and similarities were predicted by alignment in MacVector 6.5.3 and by the prediction programs SWISS-PROT and TrEMBL on the ExPASy Molecular Biology Server.

ano et al. 2001). We hope that continued studies of these genes will lead to a definition of the cascade of factors that determine ocular motoneuron identity.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

ExPASy Molecular Biology Server, <http://us.expasy.org/> (for prediction programs SWISS-PROT and TrEMBL)
 GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for *SALL4* genomic, NT_011362; *SALL4* cDNA, NM_020436; *SALL4*, NP_065169; *SALL1*, NP_002959; *SALL2*, XP_033473; *SALL3*, AAK18311; *Xsal-3*, BAA85900)
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for *DURS1*, including *DRRS*, [MIM 126800] and *DURS2* [MIM 604356])
 UCSC Human Genome Project Working Draft, <http://genome.cse.ucsc.edu/> (for working drafts for the mouse genome and the human genome)

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