Published in final edited form as:
Am J Med Genet A. 2006 April 15; 140(8): 900–902. doi:10.1002/ajmg.a.31167.

HOXA1 Mutations are Not a Common Cause of Duane Anomaly

Max A Tischfield[1,3], **Wai-Man Chan**[1], **Jann-Frederik Grunert**[1], **Caroline Andrews**[1], and **Elizabeth C Engle**[1,2,3]

[1] Department of Medicine, Program in Genomics, Boston, Massachusetts, USA

[2] Department of Neurology, Children's Hospital Boston, Boston, Massachusetts, USA

[3] Program in Neuroscience, Division of Medical Sciences, Harvard Medical School, Boston, Massachusetts 02115, USA

To the Editor:

Most patients with congenital horizontal gaze abnormalities meet diagnostic criteria for Duane anomaly (DA, also referred to as Duane syndrome), a relatively common disorder that accounts for 1–5% of strabismus cases [Engle 2002]. Affected individuals with DA typically have unilateral or bilateral restriction in their ability to move their affected eye(s) outward (abduction), and when they attempt to move the affected eye(s) inward (adduction), it retracts into the orbit resulting in narrowing of the width of the palpebral fissure. Restricted adduction is often present as well and can occur along with abduction deficits in the same eye. Abnormal antagonistic co-contraction of the opposing medial and lateral recti are thought to account for globe retraction in DA. This is supported by post-mortem examinations of individuals with DA that demonstrate absence of the abducens motoneurons and cranial nerve on the affected side(s), and misinnervation of the lateral rectus by axons of the oculomotor nerve that normally innervate the medial rectus [Hotchkiss et al., 1980; Miller et al., 1982]. Some individuals have horizontal gaze defects without retraction, which we refer to as horizontal gaze palsy (HGP), and they presumably lack this aberrant innervation.

Duane anomaly is most commonly a sporadic trait that occurs in isolation [Kirkham 1970]. A large dominant pedigree with isolated DA led to the identification of the DURS2 locus on chromosome 2 [Appukuttan et al., 1999; Evans et al., 2000], although the DURS2 gene has not yet been identified. Both DA and HGP can also occur in association with other congenital anomalies including skeletal defects, limb and digit dysplasia, scoliosis, and facial weakness [Cross 1972; Kirkham 1970; Pfaffenbach et al., 1972; Verzijl et al., 2003]. In at least three circumstances, these additional anomalies are co-inherited with DA/HGP as familial traits and permit genetic analysis. Patients with Duane radial ray syndrome (DRRS) have DA and anomalies of the radial aspect of the hand and arm, with a lower penetrance of deafness. These individuals harbor heterozygous mutations in the transcription factor *SALL4* [Al-Baradie et al., 2002; Kohlhase et al., 2002]. Patients with horizontal gaze palsy and progressive scoliosis (HGPPS) harbor homozygous mutations in *ROBO3*, a member of the *roundabout* family of axon guidance receptors [Jen et al., 2004].

We recently described the genetic basis of a third cause of syndromic DA and HGP, the "HOXA1-related syndromes", identified in consanguineous Saudi Arabian, Turkish, and Native American families [Tischfield et al., 2005]. The HOXA1-related syndrome

Corresponding author: Elizabeth C. Engle, MD, Program in Genomics, Enders 560.2, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115, Tel 617-919-4030, fax 617-730-0253, engle@enders.tch.harvard.edu.

phenotype is variable and the only completely penetrant finding in all patients was a defect in horizontal gaze, diagnosed as Duane anomaly in the Saudi and Turkish patients and as horizontal gaze palsy in the Native American patients. Sensorineural deafness, motor delay, craniofacial malformations, and internal carotid artery defects were present with variability in both the Middle Eastern and Native American patients. Autism was found in ~ 20% of the Middle Eastern patients. Mental retardation and hypoventilation was found in all Native Americans, and a subset had facial weakness and conotruncal heart defects. Parents of affected children were phenotypically normal. We mapped this disorder to chromosome 7 and identified unique homozygous truncating *HOXA1* mutations in the affected members of each of the three genetically isolated populations [Tischfield et al., 2005].

One of the Saudi HOXA1-related syndrome patients presented with isolated Duane anomaly, and had normal hearing, normal inner ear morphology by MR imaging, and no other symptoms [Tischfield et al., 2005]. As part of our larger study, it was determined that he had a hypoplastic left internal carotid artery, but this was clinically silent and, thus, could be present in patients believed to have isolated DA. Therefore, we wished to determine whether homozygous or compound heterozygous *HOXA1* mutations might be an etiology of DA in the general population.

We reviewed all probands enrolled in our genetic study of complex strabismus approved by the Children's Hospital Boston institutional review board, and identified 131 probands with Duane anomaly who are not known to harbor a mutation in *SALL4* or *ROBO3*, and are not a member of a pedigree whose phenotype maps to the *DURS2* locus. Informed consent had been obtained from all participants and/or their guardians, and each participant had undergone ophthalmologic and general examinations and had donated a blood or salivary samples for DNA extraction. This panel of 131 DA probands includes 25 familial and 106 sporadic cases of diverse ethnicity, including individuals of Turkish, Saudi Arabian, Hispanic, Asian, Indian, and US, European, and Australian ancestry. Ten probands are offspring of consanguineous marriages. Four probands had chromosomal anomalies not involving chromosome 7. Overall, DA type 1 was more common that type 3, and there were only a few cases of DA type 2. Unilateral cases were more common than bilateral cases. Of the 131 probands, 101 had isolated DA and 30 had one or more additional physical finding. These findings included developmental delay, mental retardation, autism, micro- or macrocephaly, hearing loss, craniofacial anomalies, vertebral anomalies, scoliosis, radial ray and other distal limb anomalies, cardiovascular anomalies, and/or malformed kidneys.

Six primer sets were designed to PCR amplify the coding exons and exon-intron boundaries of *HOXA1*. Genomic DNA from patients and control individuals was amplified and analyzed for *HOXA1* coding sequence mutations by Denaturing High Performance Liquid Chromatography (dHPLC) (WAVE; Transgenomic, Inc., Omaha, NE) using appropriate denaturing temperatures and acetonitrile gradients. Variant *HOXA1* amplicons found by WAVE analysis were directly sequenced as previously described [Tischfield et al., 2005], and all variants were numbered according to Genbank #BC032547 and base changes identified using the conversion proposed by den Dunnen et al. [den Dunnen and Antonarakis 2000] in which the A of the ATG of the initiator Met codon is denoted as nucleotide +1.

No mutations were detected in *HOXA1* in any of the 131 probands. We did find two of the three previously published polymorphisms within the *HOXA1* coding sequence, all of which are located within a series of 10 histidine residues in amino acid positions 65-74 and encoded by exon 1. Sixteen DA participants were heterozygous for SNP A218G, a commonly reported non-synonymous coding sequence polymorphism (H73R) that has been implicated in autism [Ingram et al., 2000]. None of these 16 DA patients had autism or mental retardation. Ten DA participants were heterozygous for a deletion of one histidine

codon, 220_222delCAC. This deletion was also detected in the heterozygous state in 9 of 96 individuals from a mixed ethnicity control panel (rare allele frequency 0.047), and similar single and multiple codon deletions and insertions have been previously reported [Ingram et al., 2000; Paraguison et al., 2005]. We also identified an unpublished polymorphism located within a second series of five histidine residues in amino acid positions 142-146 and encoded by exon 1. This SNP, 436C>A, results in the substitution of an asparagine residue for a histidine at amino acid position 146 (H146N) and was found in the heterozygous state in two patients with sporadic DA and six of 86 individuals from a mixed ethnicity control panel (rare allele frequency 0.035). Parental DNA from one of these two patients was available, and one clinically unaffected parent was also heterozygous for this SNP. Only one sporadic patient harbored two heterozygous polymorphisms, the A218G and the 436C>A. Parental DNA is not available to determine if these SNPs are allelic in this patient.

Although these changes are polymorphisms, it is interesting to note that both of the histidine repeat regions are highly conserved among vertebrates [Ingram et al., 2000], and it has been proposed that the longer histidine repeat region may be involved in protein-protein interactions [Hong et al., 1995]. Although it is still unknown what effect, if any, these polymorphisms may have on *HOXA1* function and human development, a recent study demonstrated that histidine expansions within the longer repeat region result in the aggregation of HOXA1 in nuclei of cultured cells with subsequent cell death, perhaps due to improper protein folding [Paraguison et al., 2005].

We conclude that *HOXA1* mutations are a rare cause of isolated DA and mutational analysis is not recommended for sporadic DA cases born to unrelated parents. Pending the results of a larger study, however, it may still be indicated to screen patients with bilateral DA who are offspring of consanguineous marriages or are deaf, given that the DA/HGP has been bilateral in all *HOXA1*-related syndrome patients examined to date, and deafness was found to be ~95% penetrant among these patients. We also recommend screening for clinically silent vascular anomalies in these patients.

Acknowledgments

We thank the many clinicians who referred patients for this study, and the patients and their families for their participation. Supported by NIH-R01-EY015298 and NIH-P30-HD-18655 (E.C.E),

References

Al-Baradie R, Yamada K, St Hilaire C, Chan WM, Andrews C, McIntosh N, Nakano M, Martonyi EJ, Raymond WR, Okumura S, Okihiro MM, Engle EC. Duane Radial Ray Syndrome (Okihiro Syndrome) Maps to 20q13 and Results from Mutations in SALL4, a New Member of the SAL Family. Am J Hum Genet. 2002; 71:1195–1199. [PubMed: 12395297]

Appukuttan B, Gillanders E, Juo SH, Freas-Lutz D, Ott S, Sood R, Van Auken A, Bailey-Wilson J, Wang X, Patel RJ, Robbins CM, Chung M, Annett G, Weinberg K, Borchert MS, Trent JM, Brownstein MJ, Stout JT. Localization of a gene for Duane retraction syndrome to chromosome 2q31. Am J Hum Genet. 1999; 65:1639–1646. [PubMed: 10577917]

Cross HE, Pfaffenbach David D. Duane's retraction syndrome and associated congenital malformations. American Journal of Ophthalmology. 1972; 73:442–450. [PubMed: 4622381]

den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat. 2000; 15:7–12. [PubMed: 10612815]

Engle EC. Applications of molecular genetics to the understanding of congenital ocular motility disorders. Ann N Y Acad Sci. 2002; 956:55–63. [PubMed: 11960793]

Evans JC, Frayling TM, Ellard S, Gutowski NJ. Confirmation of linkage of Duane's syndrome and refinement of the disease locus to an 8.8-cM interval on chromosome 2q31. Hum Genet. 2000; 106(6):636–8. [PubMed: 10942112]

Hong YS, Kim SY, Bhattacharya A, Pratt DR, Hong WK, Tainsky MA. Structure and function of the HOX A1 human homeobox gene cDNA. Gene. 1995; 159:209–214. [PubMed: 7622051]

Hotchkiss MG, Miller NR, Clark AW, Green WG. Bilateral Duane's retraction syndrome: A clinical-pathological case report. Arch Ophthalmol. 1980; 98:870–874. [PubMed: 7378011]

Ingram JL, Stodgell CJ, Hyman SL, Figlewicz DA, Weitkamp LR, Rodier PM. Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. Teratology. 2000; 62(6):393–405. [PubMed: 11091361]

Jen JC, Chan WM, Bosley TM, Wan J, Carr JR, Rub U, Shattuck D, Salamon G, Kudo LC, Ou J, Lin DDM, Salih MAM, Kansu T, Dhalaan H, Al Zayed Z, MacDonald DB, Stigsby B, Plaitakis A, Dretakis EK, Gottlob I, Pieh C, Traboulsi EI, Wang Q, Wang L, Andrews C, Yamada K, Demer JL, Karim S, Alger J, Geschwind DH, Deller T, Sicotte NL, Nelson SF, Baloh RW, Engle EC. Mutations in a human ROBO gene disrupt hindbrain axon pathway crossing and morphogenesis. Science. 2004; 304(5676):1509–1513. [PubMed: 15105459]

Kirkham T. Inheritance of Duane's syndrome. British Journal of Ophthalmology. 1970; 54:323–329. [PubMed: 5428664]

Kohlhase J, Heinrich M, Schubert L, Liebers M, Kispert A, Laccone F, Turnpenny P, Winter RM, Reardon W. Okihiro syndrome is caused by SALL4 mutations. Hum Mol Genet. 2002; 11:2979–2987. [PubMed: 12393809]

Miller NR, Kiel SM, Green WR, Clark AW. Unilateral Duane's retraction syndrome (type 1). Archives of Ophthalmology. 1982; 100:1468–1472. [PubMed: 7115176]

Paraguison RC, Higaki K, Sakamoto Y, Hashimoto O, Miyake N, Matsumoto H, Yamamoto K, Sasaki T, Kato N, Nanba E. Polyhistidine tract expansions in HOXA1 result in intranuclear aggregation and increased cell death. Biochem Biophys Res Commun. 2005

Pfaffenbach D, Cross H, Kearns T. Congenital anomalies in Duane's retraction syndrome. Archives of Ophthalmology. 1972; 88:635. [PubMed: 4628563]

Tischfield MA, Bosley TM, Salih MA, Alorainy IA, Sener EC, Nester MJ, Oystreck DT, Chan WM, Andrews C, Erickson RP, Engle EC. Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development. Nat Genet. 2005; 37(10):1035–1037. [PubMed: 16155570]

Verzijl HT, van der Zwaag B, Cruysberg JR, Padberg GW. Mobius syndrome redefined: a syndrome of rhombencephalic maldevelopment. Neurology. 2003; 61:327–333. [PubMed: 12913192]