

Homozygous *HOXA1* mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development

Max A Tischfield^{1,2}, Thomas M Bosley^{3,4}, Mustafa A M Salih⁵, Ibrahim A Alorainy⁶, Emin C Sener⁷, Michael J Nester⁴, Darren T Oystreck³, Wai-Man Chan¹, Caroline Andrews¹, Robert P Erickson⁸ & Elizabeth C Engle^{1,2,9}

We identified homozygous truncating mutations in *HOXA1* in three genetically isolated human populations. The resulting phenotype includes horizontal gaze abnormalities, deafness, facial weakness, hypoventilation, vascular malformations of the internal carotid arteries and cardiac outflow tract, mental retardation and autism spectrum disorder. This is the first report to our knowledge of viable homozygous truncating mutations in any human *HOX* gene and of a mendelian disorder resulting from mutations in a human *HOX* gene critical for development of the central nervous system.

We observed an autosomal recessive syndrome in five consanguineous families, four Saudi Arabian and one Turkish (**Supplementary Fig. 1** online), which we called Bosley-Salih-Alorainy syndrome (BSAS; **Supplementary Table 1** online). All nine affected individuals had bilateral Duane syndrome, a congenital horizontal eye movement disorder (**Fig. 1a**). Eight affected individuals had profound sensorineural deafness, and three had external ear defects. Seven affected individuals had delayed motor milestones. Two individuals with BSAS from different Saudi Arabian families were cognitively and behaviorally impaired and met DSM-IV criteria for autism spectrum disorder. Their parents did not have features of BSAS.

Eight individuals with BSAS underwent brain magnetic resonance or computed tomography imaging (**Supplementary Methods** online). In thin magnetic resonance sections through the caudal pons from one affected individual, we could not identify exiting abducens cranial nerves, although we could identify them in control images. Otherwise, the cerebrum, cerebellum and brainstem appeared normal (**Fig. 1b**).

We imaged the inner ear in seven of eight individuals with deafness and found bilateral absence of the cochlea, semicircular canals and vestibule (common cavity deformity) in five of them (**Fig. 1c,d**) and

cochlea aplasia in two. The ninth individual had normal hearing and inner ear anatomy.

Three individuals with BSAS had computed tomography imaging of the skull base. One had bilateral absence and two had left-sided absence of the carotid canal (**Fig. 1e**), the foramen through which the internal carotid artery (ICA) normally enters the skull. Four individuals underwent magnetic resonance angiography (MRA) of both the head and neck, and three individuals underwent MRA of the head only. All had variable ICA malformations, ranging from unilateral hypoplasia to bilateral agenesis (**Fig. 1f-h** and **Supplementary Table 1** online).

We carried out SNP-based linkage analysis of the largest family with BSAS (**Supplementary Fig. 1** online) and identified a single, fully informative 8.5-Mb region flanked by rs763543 and rs177962 on chromosome 7p15.3–p14.3 in which only the affected children were homozygous. Further analysis with additional microsatellite markers (**Supplementary Table 2** online) confirmed coinheritance of the haplotype with disease status in all five pedigrees with BSAS and also identified a homozygous ~300-kb subregion on 7p15.2 that was haploidentical in affected Saudi Arabian individuals, suggestive of a founder mutation in the Saudi Arabian population (**Supplementary Fig. 1** online). The maximum combined two-point lod score was 7.7 (**Supplementary Table 3** online).

The *HOXA* cluster falls in the haploidentical region, and we analyzed *HOXA1* for mutations because there are similarities between the BSAS phenotype and the pathology of the *Hoxa1*^{-/-} mouse¹⁻³. Sequence analysis showed that Saudi Arabian individuals with BSAS carried a homozygous guanine insertion, 175-176insG, putatively resulting in a reading frame shift and the introduction of a premature stop codon (**Fig. 2a,b** and **Supplementary Fig. 1** online). The Turkish individual with BSAS had a homozygous 84C→G mutation, resulting in the substitution of a stop codon for a tyrosine residue (Y28X; **Fig. 2c** and **Supplementary Fig. 1** online). Both mutations were heterozygous in parents of affected individuals, cosegregated appropriately in each family and were not present on 354 chromosomes of mixed ethnicity (including 128 Saudi Arabian and 26 Turkish). The mutations are predicted to affect the synthesis of all three human *HOXA1* transcripts⁴ (**Fig. 2**) and to result in loss of *HOXA1* function.

Heterozygous mutations have been documented in four human *HOX* genes⁵⁻⁷ located near the 5' ends of the A or D clusters. These mutations lead primarily to malformed distal extremities. Human mutations have not been described in 3' *HOX* genes essential for head and central nervous system patterning. *Hoxa1*, the most 3' gene in cluster A, is the first of the *Hox* genes expressed in mammals and is

¹Department of Medicine, Program in Genomics, Children's Hospital Boston, Boston, Massachusetts, USA. ²Program in Neuroscience, Division of Medical Sciences, Harvard Medical School, Boston, Massachusetts 02115, USA. ³Neuro-ophthalmology Division, King Khaled Eye Specialist Hospital and ⁴Neuroscience Department, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. ⁵Division of Pediatric Neurology, Department of Pediatrics, College of Medicine, King Khalid University Hospital, Riyadh, Saudi Arabia. ⁶Department of Radiology and Diagnostic Imaging, College of Medicine, King Saud University, Riyadh, Saudi Arabia. ⁷Department of Ophthalmology, Hacettepe University Hospital, Ankara, Turkey. ⁸Department of Pediatrics, Molecular and Cellular Biology, University of Arizona College of Medicine, Tucson, Arizona, USA. ⁹Department of Neurology, Children's Hospital Boston, Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts 02115, USA. Correspondence should be addressed to E.C.E. (engle@enders.tch.harvard.edu).

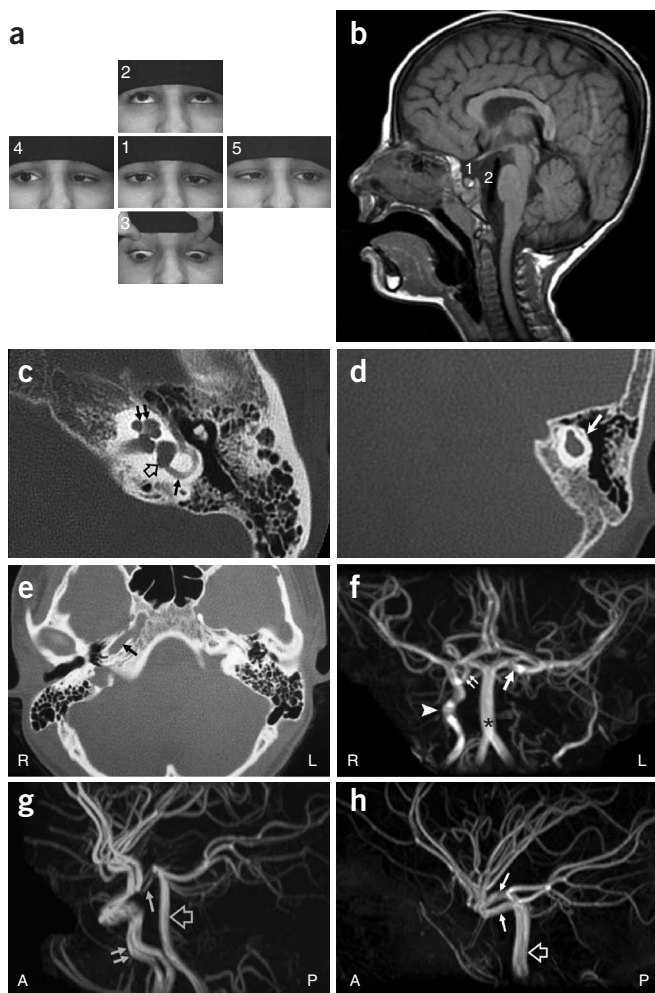


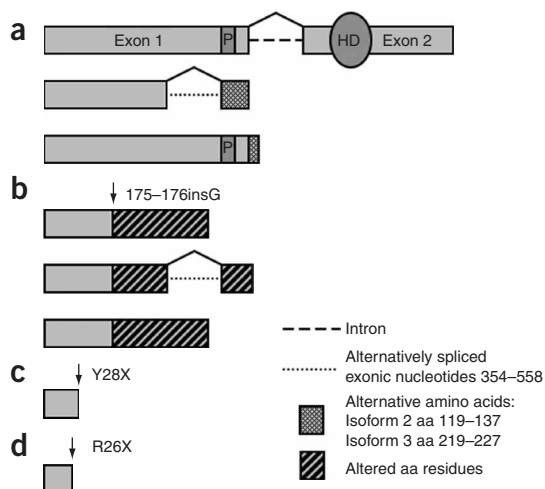
Figure 1 Clinical and radiological features of BSAS. **(a)** Photographs of an affected individual with bilateral Duane syndrome. Primary gaze (1), upgaze (2) and downgaze (3) are preserved, but with attempted horizontal gaze to the right (4) and left (5), there is limited abduction with retraction of the globe and narrowing of the palpebral fissure of the adducting eye. **(b)** Midsagittal brain magnetic resonance image showing normal appearance of the cerebrum and cerebellum. Note enlarged suprasellar (1) and prepontine (2) cisterns. **(c)** Control computed tomography scan of the temporal bone and left inner ear showing a normal cochlea (double arrows), vestibule (open arrow) and lateral semicircular canal (single arrow). **(d)** Computed tomography scan of the temporal bone in an individual with BSAS showing underdevelopment of the left inner ear with the presence of a common cavity (arrow). **(e, f)** Computed tomography scan of the skull base **(e)** and brain MRA **(f)** of an individual with BSAS with an absent left carotid canal **(e)** and left ICA **(f)**; the right carotid canal **(e; black arrow)** and the right ICA are present **(f; arrowhead)**. **(f)** An enlarged vertebrobasilar system (black asterisk) and left posterior communicating (Pcom) artery (large single arrow) provide compensatory blood flow to the middle and anterior cerebral arteries; the right Pcom (double arrows) is normal in size. **(e, f)** L, left; R, right. **(g)** Control brain MRA (lateral view) showing normal size basilar artery (open arrow), Pcom arteries (single arrow) and overlapped left and right ICAs (double arrows). **(h)** Brain MRA of an individual with BSAS showing absent ICAs bilaterally. Note the enlargement of the basilar (open arrow) and both Pcom (arrows) arteries that supply the anterior circulation on both sides. **(g, h)** A, anterior; P, posterior.

Two *Hoxa1*^{-/-} mouse models show variable abnormalities of hindbrain rhombomere segmentation and neural crest migration¹⁻³. They have reduction of neuronal cell bodies and exiting cranial nerves of the abducens and facial nuclei; absence of the superior olive (auditory relay); and variable defects in cranial autonomic and sensory ganglia, the skull and the external and inner ears.

We noted that the BSAS and *Hoxa1*^{-/-} mouse phenotypes overlap with that of an autosomal recessive disorder called Athabaskan brainstem dysgenesis syndrome (ABDS; OMIM 601536) reported in ten probands from two Native American tribes⁹. Similar to individuals with BSAS, these ten children with ABDS had horizontal gaze restriction, sensorineural deafness and delayed motor development⁹. Retrospective review of MRA available from three children with ABDS showed left ICA agenesis, right ICA hypoplasia and anterior cerebral artery aplasia, each in a different child. In contrast to the nine individuals with BSAS, however, all ten children with ABDS also had central hypoventilation and mental retardation, and some of them

among the first genes expressed in hindbrain neuroectoderm⁸. Although the spatial and temporal expression pattern of *HOXA1* has not been defined in humans, we found *HOXA1* transcripts in 7- to 8-week-old whole human embryos but not in 22-week-old human fetal tissue (including central nervous system) by RT-PCR (data not shown).

Figure 2 Schematic representation of three reported human *HOXA1* isoforms and corresponding predicted mutant proteins. **(a)** Wild-type isoforms. Isoform 1 (top) contains the PBX binding domain in exon 1 (P, amino acids 204–209) and homeodomain in exon 2 (HD, amino acids 229–288), which is critical for transcriptional activity. Alternatively spliced isoform 2 (middle) lacks the PBX binding domain and homeodomain and contains 19 alternative C-terminal amino acids. Isoform 3 (bottom) results from absent splicing and contains exon 1 followed by nine alternative C-terminal amino acids. Isoforms 2 and 3 have no known function. **(b)** The 175–176insG frameshift mutation found in Saudi Arabian individuals with BSAS is predicted to result in a mutant protein composed of the normal first 58 N-terminal amino acid residues followed by 118 altered residues in isoforms 1 (top) and 3 (bottom) and 81 altered residues in isoform 2 (middle). The 84C→G nonsense mutation found in Turkish individuals with BSAS **(c)** and the 76C→T nonsense mutation found in individuals with ABDS **(d)** are predicted to truncate all three isoforms at amino acid residues 28 (Y28X) and 26 (R26X), respectively. All mutant proteins lack known functional domains. aa, amino acids.



had facial weakness, vocal cord paralysis and conotruncal heart defects, including Tetralogy of Fallot and double aortic arch⁹.

We analyzed genomic DNA from five of the reported individuals with ABDS and four of their phenotypically normal parents. All five affected individuals were homozygous across the *HOXA1* locus, were haploidentical and carried a homozygous 76C→T *HOXA1* mutation resulting in the substitution of a stop codon for an arginine residue (R26X; **Fig. 2d** and **Supplementary Fig. 1** online). The mutation was heterozygous in the four parents and absent from 344 control chromosomes.

All individuals with *HOXA1*-related syndromes have horizontal gaze restriction, and all but one have sensorineural deafness. As in *Hoxa1*^{-/-} mice¹⁰, however, the phenotype is otherwise more variably expressed. There are also specific phenotypic differences between BSAS and ABDS. Central hypoventilation was observed in all individuals with ABDS but no individuals with BSAS; it can be fatal without medical intervention⁹. Similarly, *Hoxa1*^{-/-} mice have respiratory failure with perinatal death¹¹. Facial weakness was observed in six individuals with ABDS⁹ but no individuals with BSAS; this probably reflects loss of facial motoneurons as documented in *Hoxa1*^{-/-} mice³. Thus, the ABDS phenotype converges more closely with the phenotypic spectrum reported in *Hoxa1*^{-/-} mice¹⁻³ and suggests that hindbrain maldevelopment rostral to rhombomere 5 may be more severe in individuals with ABDS than in those with BSAS (**Supplementary Note** online). Phenotypic differences between BSAS and ABDS may result in part from genetic modifiers in these isolated human populations.

The ICA anomalies associated with *HOXA1*-related syndromes and the conotruncal heart defects associated with ABDS indicate that *HOXA1* has a previously unrecognized developmental function. Vascular defects have not been documented in *Hoxa1*^{-/-} mice beyond the occasional absence of the stapedial artery³, and *Hoxa1* expression has not been reported in the pharyngeal arches in mice⁸. More detailed studies are necessary to determine whether *HOXA1* regulates aspects of vasculogenesis or, alternatively, angiogenesis through its influence on migrating cranial neural crest cells, which are required for the remodeling of the primordial vasculature system¹².

Although the two cases of autism spectrum disorder among the individuals with BSAS may have occurred by chance, all individuals with ABDS have mental retardation. This is notable because forebrain and cerebellar defects have not been reported in *Hoxa1*^{-/-} mice¹⁻³, and the canonical rostral central nervous system expression boundary

of *Hoxa1* is at the border of rhombomeres 3 and 4, with a late phase of expression at the midbrain-forebrain boundary¹³. In addition, previous studies have proposed an association between *HOXA1* dysfunction and cognitive and behavioral impairment. Similarities of the hindbrain pathology of an autistic individual to that of the *Hoxa1*^{-/-} mouse¹⁴ prompted an association study that found linkage disequilibrium between the autism phenotype and an 218A→G SNP of *HOXA1* (ref. 15). Further studies in additional populations failed to replicate this finding. The phenotypes of individuals with BSAS and ABDS now show that loss of *HOXA1* function segregates with cognitive impairment and suggest that brainstem dysgenesis may lead to higher cortical dysfunction (**Supplementary Note** online).

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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