The identification and analysis of pedigrees with rare congenital oculomotility syndromes has led to the definition of the congenital cranial dysinnervation disorders. These disorders appear to result from mutations in genes that are essential to the normal development and/or connectivity of cranial motoneurons. This review highlights the clinical features and genetic etiology of 3 congenital cranial dysinnervation disorders: the human homeobox A1 (HOXA1) syndromes, in which early motoneuron development is disrupted; horizontal gaze palsy with progressive scoliosis, in which there is aberrant axonal targeting onto abducens motoneurons; and congenital fibrosis of the extraocular muscles type 1, in which there is aberrant axonal targeting onto the extraocular muscles.

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This review describes several rare oculomotility disorders and presents some of the accumulating evidence that these disorders result from disruptions in motor neuron development. My interest in the etiology of congenital oculomotility disorders began when a toddler was admitted to the neurology service at Children's Hospital Boston while I was a senior resident in 1992. He was born with congenital bilateral ptosis and with his eyes fixed in a downward position. He underwent evaluation for myasthenia, mitochondrial disorders, and congenital myopathies. We also asked for our ophthalmology consultants' opinion and were somewhat surprised when they diagnosed him as having congenital fibrosis of the extraocular muscles (CFEOM), a disorder with which none of us were familiar. We learned that CFEOM had been described in the ophthalmologic literature since the 1800s and was classified as one of the ocular fibrosis syndromes, the most common form being Duane syndrome. In 1992, the diagnoses of the ocular fibrosis syndromes were clinical, and the name arose from the common belief that they resulted from primary fibrosis of the extraocular muscles.¹ ²

The toddler was a member of a large family in which CFEOM was transmitted as an autosomal dominant trait. In the early 1990s, the Human Genome Project was developing maps of polymorphic short tandem repeat markers across the genome, revolutionizing our ability to perform linkage analysis.³ Therefore, after completion of my residency training and with an interest in neurogenetics, I entered the laboratories of Louis Kunkel, PhD, and Alan Beggs, PhD, to begin research of CFEOM. I had 2 primary goals. The first was to use linkage analysis to map and eventually identify the gene causing CFEOM in the toddler's family. The second was to determine whether CFEOM might result not from primary fibrosis of the extraocular muscles but rather from errors in the development of brainstem motor neurons and axonal targeting of these muscles. In the intervening years, my laboratory has extended these studies beyond CFEOM to include other forms of ocular fibrosis syndromes and, based on the results from our laboratory and others, we have renamed these syndromes the congenital cranial dysinnervation disorders (CCDDs).⁴

Collaborating with clinicians worldwide, we have ascertained study participants with mendelian syndromes that include variable forms of congenital...
ophthalmoplegia and enrolled these families into our ongoing study. We sort these syndromes by phenotype, considering whether we thought the primary abnormality fell in the distribution of oculomotor, trochlear, or abducens innervated extraocular muscles (Table). We use linkage analysis to map the phenotypes and positional cloning techniques to identify the mutated gene. Once the gene and its spectrum of mutations is defined, we study the role of these normal and abnormal gene products in neurodevelopment. In this review, I highlight the genetic, neuroanatomic, and neurodevelopmental bases of 3 of these disorders: horizontal gaze palsy with progressive scoliosis (HGPPS) and the homeobox A1 (HOXA1) syndrome. They are unable to abduct their eyes and, when they attempt to look inward, there is narrowing of the palpebral fissure secondary to retraction of the globe into the orbit. In the early 1980s, 2 autopsies of individuals with Duane syndrome were conducted at The Johns Hopkins University,6,7 Baltimore, Md, which revealed absence of the abducens nerve, absence of the motor neurons in the abducens nucleus, and aberrant innervation of the lateral rectus muscle by branches of the oculomotor nerve. These autopsies were some of the earliest evidence that the fibrosis syndromes may indeed be neurogenic. Consistent with the Duane syndrome autopsy findings, when high-resolution magnetic resonance (MR) imaging sections through the pons were obtained in a patient with BSAS, no exiting abducens nerve was identified. Patients with BSAS have additional congenital anomalies, including severe bilateral sensory-neural hearing loss secondary to the absence of the cochlea, vestibule, and semicircular canals. This rudimentary inner ear defect is referred to as a common cavity deformity and is often accompanied by absence of the eighth cranial nerve.5 Skull-based computed tomography revealed that most patients had unilateral or bilateral hypoplastic or absent carotid canals, and MR angiograms revealed a variety of internal carotid artery malformations, including bilateral absence of the internal carotid artery defect is referred to as a common cavity deformity and is often accompanied by absence of the eighth cranial nerve.5 Skull-based computed tomography revealed that most patients had unilateral or bilateral hypoplastic or absent carotid canals, and MR angiograms revealed a variety of internal carotid artery malformations, including bilateral absence of the internal carotid artery.

**THE HUMAN HOXA1 SYNDROMES**

The human HOXA1 story began in our laboratory when collaborators identified an autosomal recessive syndrome that included abnormal ocular motility in 4 Saudi Arabian pedigrees.7 We subsequently named this BSAS in recognition of the collaborators who defined the phenotype. Bosley-Salih-Alorainy syndrome is recessive, requiring an individual to harbor 2 mutated copies of the gene to express the phenotype, and all 4 of the Saudi pedigrees were consanguineous, with affected individuals being the offspring of first- or second-cousin marriages. Once this phenotype was defined, we realized we had previously enrolled a Turkish patient with similar findings who was the only child of his first-cousin parents. Individuals with BSAS are born with bilateral Duane syndrome. They are unable to abduct their eyes and, when they attempt to look inward, there is narrowing of the palpebral fissure secondary to retraction of the globe into the orbit. In the early 1980s, 2 autopsies of individuals with Duane syndrome were conducted at The Johns Hopkins University,6,7 Baltimore, Md, which revealed absence of the abducens nerve, absence of the motor neurons in the abducens nucleus, and aberrant innervation of the lateral rectus muscle by branches of the oculomotor nerve. These autopsies were some of the earliest evidence that the fibrosis syndromes may indeed be neurogenic. Consistent with the Duane syndrome autopsy findings, when high-resolution magnetic resonance (MR) imaging sections through the pons were obtained in a patient with BSAS, no exiting abducens nerve was identified. Patients with BSAS have additional congenital anomalies, including severe bilateral sensory-neural hearing loss secondary to the absence of the cochlea, vestibule, and semicircular canals. This rudimentary inner ear defect is referred to as a common cavity deformity and is often accompanied by absence of the eighth cranial nerve.5 Skull-based computed tomography revealed that most patients had unilateral or bilateral hypoplastic or absent carotid canals, and MR angiograms revealed a variety of internal carotid artery malformations, including bilateral absence of the internal carotid artery defect is referred to as a common cavity deformity and is often accompanied by absence of the eighth cranial nerve.5 Skull-based computed tomography revealed that most patients had unilateral or bilateral hypoplastic or absent carotid canals, and MR angiograms revealed a variety of internal carotid artery malformations, including bilateral absence of the internal carotid artery...
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While we were studying these Middle Eastern families, we recalled an earlier report describing a similar syndrome in 10 Native American children referred to as the Athabaskan brainstem dysgenesis syndrome (ABDS). ABDS demonstrates that this CCDD results from an early loss in the specification of the abducens motoneurons. Reexamination of MR angiograms revealed that patients with ABDS could also have internal carotid artery defects. To determine whether abnormalities are asymptomatic, and there is a compensatory increase in blood flow through enlarged basilar and posterior communicating arteries. A subset of patients has developmental motor delay, and 2 of 9 have autism. While we were studying these Middle Eastern families, we recalled an earlier report describing a similar syndrome in 10 Native American children referred to as the Athabaskan brainstem dysgenesis syndrome (ABDS). All children with ABDS had a sporadic congenital disorder characterized by horizontal gaze restriction, sensorineural hearing loss, mental retardation, and central hypoventilation. In addition, a subset of patients with ABDS had facial weakness and conotruncal heart defects, including the tetralogy of Fallot. Reexamination of MR angiograms revealed that patients with ABDS could also have internal carotid artery defects. To determine whether ABDS was allelic to BSAS, we established a collaboration to study the genetic basis of ABDS as well.

We mapped the BSAS phenotype to chromosome 7p15.3–p14.3 by single nucleotide polymorphism–based linkage analysis of the largest Saudi family and then established that the findings in the remaining families were also consistent with linkage to this locus. Subsequently, we determined that the findings in the children with ABDS also reduced to homozygosity across this critical region. The HOXA1 cluster of genes fell within the BSAS and ABDS critical region. Two Hoxa1 loss-of-function mouse models have been analyzed, and both demonstrated that Hoxa1 is critical in mouse development for proper patterning of the hindbrain, cranial nerves, inner ear, skull, and craniofacial features. Within the developing hindbrain, there are 7 transient divisions referred to as rhombomeres, each of which possesses molecular and cellular properties necessary for its proper patterning. The Hoxa1−/− mice display grossly abnormal hindbrain rhombomere segmentation, with a nearly complete loss of rhombomere 5, partial loss of rhombomere 4, and alterations of rhombomeres 3, 6, and 7. The abducens motoneurons arise primarily in rhombomere 5, and the mice lack the abducens motoneurons. Hence, HOX1A was an excellent candidate gene for BSAS and ABDS.

HOX1A contains 2 coding exons that produce a full-length 335–amino acid protein. The 2 critical functional domains, the 50–amino acid homeobox and the PBX-binding motif, are encoded in exon 2 and the 3′ end of exon 1, respectively. We sequenced HOX1A in the 3 ethnic populations and identified 3 different homozygous truncating HOX1A mutations. The Saudi Arabian patients harbored an insertion of a single nucleotide in exon 1 that resulted in a frameshift before the PBX-binding motif, with premature truncation of the HOX1A protein. The Turkish and the Native American patients harbored nonsense mutations resulting in truncation of HOX1A at amino acid 28 and 26, respectively. The nature of the HOX1A mutations suggests that they likely result in complete loss of function of this gene. The identification of HOX1A mutations in BSAS and ABDS demonstrate that this CCDD results from an early defect in the specification of the abducens motoneurons, and also that HOX1A is indispensable for proper development of the head, nervous system, heart, and cerebrovascular system in humans. These syndromes, BSAS

Figure. The congenital cranial dysinnervation disorders (CCDDs). Schematic representation of extraocular muscle (EOM) innervation in healthy individuals and in those with 3 of the CCDDs. In the normal wild-type state (A), the globe is moved by the 4 recti and 2 oblique EOMs, and the eyelid is elevated by the levator palpebrae superiors (LPS). Throughout the illustration, the oculomotor nucleus (blue) is composed of 5 motor subnuclei that send their axons in the oculomotor nerve (blue) and divide into a superior branch that innervates the LPS and superior rectus (SR) muscles, and an inferior branch that innervates the medial rectus (MR), inferior rectus (IR), and inferior oblique (IO) muscles. The trochlear nucleus (brown) sends its axons in the trochlear nerve (brown) to innervate the superior oblique (SO) muscle. The abducens nucleus (green) is composed of motoneurons and interneurons. The motoneurons send their axons in the abducens nerve (green) to innervate the lateral rectus (LR) muscle. The interneurons send their axons in the medial longitudinal fasciculus (MLF) (green), which crosses the midline to innervate neurons in the MR subnucleus of the contralateral oculomotor nucleus. In addition, crossed input onto the abducens nucleus is shown (magenta). The pathology of the human homeobox A1 (HOXA1) syndromes (BSAS/ABDS [Bosley-Salih-Alorainy syndrome/Athabascan brainstem dysgenesis syndrome]) (B), horizontal gaze palsy with progressive scoliosis (HGPPS) (C), and congenital fibrosis of the extraocular muscles type 1 (CFEOM1) (D) are presented. Aberrant or missing nuclei, nerves, and muscles are shown with hatched vs solid lines. The region of the rhombomere defects in the homeobox

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and ABDS, are the first human mendelian syndromes to result from homozygous mutations in a HOX gene and from mutations in a 3’ HOX gene critical for the development of the head and central nervous system. The internal carotid artery and cardiac outflow defects found in these patients have not been reported in the mouse models; targeted examinations of these mice should reveal whether these were missed or whether the expression of human HOX1 differs from that of mouse Hoxa1. The mental retardation and autism in the patients with ABDS and BSAS is also notable. Expression of HOX1 has not been reported above the developing brainstem and, if this holds true, it suggests that proper brainstem development is essential to later cognitive development.

HORIZONTAL GAZE PALSY WITH PROGRESSIVE SCOLIOSIS

Similar to BSAS/ABDS, HGPPS is an autosomal recessive syndrome most often found in offspring of consanguineous parents, and individuals with this syndrome are born with restricted horizontal gaze. In HGPPS, the congenital gaze restriction is co-inherited with progressive scoliosis, which can begin in the first year of life and typically becomes severe in the first decade.12,13 Horizontal gaze palsy with progressive scoliosis was first mapped to 11q23-q25 in a Saudi Arabian and an Indian pedigree by members of the Jen laboratory at the University of California–Los Angeles.12 We had enrolled a pedigree that also mapped this region and significantly reduced the critical region, and so our 2 laboratories established a collaboration to study HGPPS and identify the mutated disease gene.13

Neuroimaging and electrophysiological studies of HGPPS revealed unexpected findings.13 Magnetic resonance imaging of affected individuals from pedigrees that mapped to the HGPPS locus were found to have a normal-appearing cerebrum, corpus callosum, and cerebellum, but abnormal flattening of the pons and medulla, with an unusual midline medullary cleft. In contrast to the HOX1 syndromes, the abducens nerve was present and the orbital anatomy appeared normal. When children with HGPPS undergo corrective surgery for scoliosis, the integrity of the spinal cord can be monitored by evoked potential studies. Because the descending cortical spinal tracts and the ascending somatosensory tracts normally decussate in the medulla, motor and sensory evoked potentials are monitored contralaterally. The patients with HGPPS were found to have ipsilateral motor and sensory responses, suggesting that both of these tracts were uncrossed. It is likely that the absence of normal decussation of these tracts on the ventral aspect of the hindbrain results in the midline medullary cleft seen by MR imaging in these patients.

Genetic studies led to our identification of homozygous mutations in the transmembrane receptor ROBO3 (a homologue of ROBO1 [roundabout 1 protein]) in patients with HGPPS, and the nature of the mutations suggested complete loss of gene function.13 The human homologue to mouse Rig-1, ROBO3, is a transmembrane cell adhesion molecule that serves as a receptor for neuron and axon guidance molecules during development of the hindbrain. Loss of Rig-1 function was similarly demonstrated to result in a lack of midline axonal crossing in the developing hindbrain and spinal cord of mice.14,15 The absence of horizontal gaze in these patients remains speculative and may arise from aberrant supranuclear input onto the abducens motoneurons by axons from the paramedian pontine reticular formation that cannot cross the midline and inability of the developing axons in the medial longitudinal fasciculus to cross the midline, and/or from lack of midline crossing by developing pontine neurons normally destined to cross.13 Scoliosis may result from lack of normal contralateral cross talk because of the absence of crossing fibers and suggests that scoliosis can, indeed, be neurogenic in etiology. What may be most interesting, however, is that patients with HGPPS are otherwise asymptomatic, despite this extensive hindbrain and spinal cord miswiring; this suggests that these axons find their intended target, albeit on the ipsilateral rather than contralateral side. In summary, HGPPS represents a later developmental defect than the HOX1 syndromes and demonstrates that horizontal gaze abnormalities can result from aberrant axonal targeting of cranial motoneurons.

CFEOM TYPE I

The toddler I met as a neurology resident was born with bilateral ptosis and bilateral ophthalmoplegia with his eyes fixed downward and has a CCDD syndrome we now refer to as CFEOM1. We gained significant insight into CFEOM1 by conducting the postmortem examination of the brain and orbit of an elderly affected family member of the toddler.16 We found that the superior division of the oculomotor nerve and the corresponding motoneurons in the oculomotor nucleus were absent. This branch of the oculomotor nerve innervates the levator palpebrae superioris and superior rectus muscles that elevate the eyelid and eye, respectively, and these muscles were aplastic. This suggested that the etiology of CFEOM1 may be the oculomotor analogue of Duane syndrome, and both may be neurogenic in nature.

We mapped the CFEOM1 gene to the pericentromeric region of chromosome 12 and, after enrolling many additional pedigrees with CFEOM1 from around the world, identified the mutated gene as KIF21A, a member of the kinesin family of molecular motors.17 Kinesins transport cargo along microtubules in an anterograde direction. They are responsible for anterograde axonal transport in neurons, moving cargo from the neuronal cell body to the growing or mature synapse. There are at least 45 human kinesins that transport different cargoes, including mitochondria, vesicles, and protein complexes. The structure of the KIF21A kinesin is predicted to be similar to classic kinesin, with a motor, tail, and stalk domain. The motor domain interacts with tubulin; typically, 2 kinesins homodimerize or heterodimerize, allowing the 2 motor domains to “walk” down the microtubule tract. The tail domain is where the cargo is typically carried, and KIF21A and KIF21B are the only kinesins known to have a series of WD40 repeats in their tails. The cargo of KIF21A, however, is not yet known. The stalk domain is a flexible connection between the
tail and the motor that contains several coiled-coil regions implicated in protein-protein interactions. These domains are likely to be important to KIF21A dimerization, and the coiled-coil domains closer to the tail may also interact with specific cargo.

Remarkably, among the 61 probands with CFEOM1 for whom KIF21A mutations have been published, only 8 different KIF21A mutations, altering only 4 amino acids, have been reported. Fifty-nine of the probands harbor mutations that alter 1 of 3 amino acid residues in the third coiled-coil region of the stalk, and, of these, all but 5 probands have mutations that alter the arginine at amino acid residue 954. The remaining 2 unrelated probands harbor a mutation that alters an amino acid residue at the end of the motor domain.17 The nature of these mutations suggests that the CFEOM1 phenotype results from altered function of KIF21A rather than from loss of function of 1 allele. These mutations may result in an inability of KIF21A to dimerize normally or may disrupt its ability to bind to specific cargo critical to the development of oculomotor axons. Future work should lead to an understanding of how these mutations disrupt KIF21A function to result in the CFEOM1 phenotype. Identification of the KIF21A cargo may provide insight into the normal and abnormal development of the oculomotor nerve. Our current hypothesis, however, is that CFEOM1 results from absent or aberrant delivery of a cargo from the motoneuron cell body to the growth cone that is critical to the development of the oculomotor nerve.

CONCLUSIONS

The identification and analysis of pedigrees with rare congenital oculomotility syndromes has led to the definition of the CCDDs. These disorders appear to result from mutations in genes that are essential to the normal development and/or connectivity of cranial motoneurons. These genetic defects can lead to disruption in early motoneuron development, aberrant axonal targeting onto motoneurons, and aberrant axonal targeting onto the extraocular muscles. The identification and study of additional CCDD genes are likely to continue to provide knowledge about the pathogenesis of oculomotor disease and development of the human brainstem.