Human Genetic Disorders of Axon Guidance

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This article reviews symptoms and signs of aberrant axon connectivity in humans, and summarizes major human genetic disorders that result, or have been proposed to result, from defective axon guidance. These include corpus callosum agenesis, L1 syndrome, Joubert syndrome and related disorders, horizontal gaze palsy with progressive scoliosis, Kallmann syndrome, albinism, congenital fibrosis of the extraocular muscles type 1, Duane retraction syndrome, and pontine tegmental cap dysplasia. Genes mutated in these disorders can encode axon growth cone ligands and receptors, downstream signaling molecules, and axon transport motors, as well as proteins without currently recognized roles in axon guidance. Advances in neuroimaging and genetic techniques have the potential to rapidly expand this field, and it is feasible that axon guidance disorders will soon be recognized as a new and significant category of human neurodevelopmental disorders.

The human brain is highly organized and contains a myriad of axon tracts that follow precise pathways and make predictable connections. Model organism research has provided tremendous advances in our understanding of the principles and molecules governing axon growth and guidance. Remarkably, however, only a handful of human disorders resulting from primary errors in these processes have been identified.

Traditional tools of the physician have limited sensitivity and specificity to detect human disorders of axon guidance. In particular, congenital synkinesis may be the only physical examination finding that has been attributed to such disorders. Synkinesis is the involuntary and pathological contraction of a muscle simultaneously with contraction of the intended muscle, and is typically reported with hand/finger or eye/eyelid movements and confirmed by electrophysiological studies. Mirror movement synkinesis refers to the contraction of homologous hand/finger muscles bilaterally when one attempts to move only one hand (Schott and Wyke 1981). In humans, 75%–90% of corticospinal tract (CST) fibers normally decussate in the lower medulla. Mirror movement synkinesis occurs in several human disorders with pathological, neuroimaging, and/or electrophysiological evidence of reduced CST decussation, including Joubert, Kallmann, and Klippel-Feil syndromes (Vulliez et al. 2005; Cincotta and Ziemann 2008). In some individuals with mirror movements, electrophysiological data are also consistent with
bilateral engagement of the motor corticies (Lein-singer et al. 1997). Ocular synkinesis refers to aberrant patterns of eye movement and accompa-nies various congenital cranial dysinnervation disorders (CCDDs) (Gutowski et al. 2003; Engle 2007), including CFEOM, Duane syndrome, and Marcus Gunn jaw-winking phenomenon (Fig. 1). Finger and ocular movements require precise motor control, and errors in innervation of these muscles may be more easily detected than errors in the wiring of larger muscle groups. If true, this suggests that the clinical exam could fail to recognize many guidance errors in both the peripheral and central nervous system.

The physician’s ability to detect disorders of axon guidance has been augmented by classical pathological, radiological, and electrophysiological techniques. Diagnostic radiologic and postmortem neuropathological studies detect overall changes in white matter volume and major abnormalities of axon tracts demarcated from the background such as the corpus callosum, anterior and posterior commissures, optic chiasm, and cerebellar peduncles. Neuropathological studies can also detect absence of axons that normally cross the midline at many points in the brain stem and spinal cord, which are more difficult to visualize by standard magnetic resonance imaging (MRI). Electrophysiological studies such as evoked potentials can reveal aberrant central connections of peripheral sensory or motor nerves.

The genetic disorders with aberrant axon connectivity presented in this article have been defined primarily using traditional approaches described above. Exciting advances in neuroimaging and genetics, however, are revolution-izing the ability to define axon guidance disor-ders, and it is likely that these syndromes are only the first of an important new category

Figure 1. Ocular synkinesis. (A) Child with CFEOM1 and Marcus Gunn jaw-winking phenomenon harboring a KIF21A mutation. His superior branch of the oculomotor nerve is hypoplastic/absent, resulting in bilateral ptosis from lack of appropriate innervation of the levator palpebrae superioris (LPS) muscle, and a downward position of each eye from absent innervation of the superior rectus muscle (left). Marcus Gunn phenomenon (right) is seen as the synkinetic elevation of the left eyelid with a subtle change in jaw position associated with a volitional increase in pterygoid muscle tension. This results from aberrant innervation of the LPS by axons from the motor branch of the trigeminal nerve that also innervates the intended ipsilateral pterygoid muscle. (B) Adult with Duane retraction syndrome harboring a CHN1 mutation. Central gaze reveals mild exotropia (middle). On attempted right gaze (left) and left gaze (right), there is limited horizontal excursion with globe retraction and secondary palpebral fissure narrowing of the adducting eye. Globe retraction results from synkinesis of the medial and lateral recti muscles. (A) Modified with permission from Yamada et al. 2005. Copyright © (2005) American Medial Association. All rights reserved. (B) Modified from Demer et al. 2007. Copyright © (2007) Association for Research in Vision and Ophthalmology. All rights reserved.
of such human neurodevelopmental disorders. Detailed fiber tract anatomy can now be visualized using noninvasive tractography such as diffusion tensor imaging (DTI) and diffusion spectrum imaging (DSI). These techniques provide tract orientation by determining the anisotropic properties of water diffusion, and can be used to reconstruct the trajectories of fiber systems in three-dimensional space (Tovar-Moll et al. 2007; Wahl et al. 2009). Tractography has successfully confirmed aberrant projections in several of the disorders discussed below (Fig. 2). At the same time, human genetics now provides an unbiased approach to identify the etiologies of disorders with aberrant axon tracts. For some syndromes, animal and in vitro studies have confirmed that the encoded protein has a primary role in axon guidance. For others, such studies reveal a primary role in neuronal specification and/or migration rather than, or in addition to, a role in axon guidance. Finally, some neurodevelopmental disorders without clinical, pathologic, or radiologic evidence of aberrant axon tracts have been found to result from mutations in genes that contribute to axon guidance in animal models.

The major human genetic disorders that result, or are proposed to result, from defective axon guidance are ordered below from rostral to caudal based on the location of the aberrant axon tracts. These include genetic mutations that alter axon growth cone ligands and receptors, downstream signaling molecules, and axon transport, as well as proteins without...
currently recognized roles in axon guidance (Fig. 3) (Table 1).

HUMAN GENETIC DISORDERS OF MIDLINE CROSSING

Corpus Callosum Dysgenesis

Corpus callosum (CC) axons normally connect homologous cortical regions in the left and right hemispheres, and are topographically organized along the anteroposterior axis (Hofer and Frahm 2006) (Fig. 2). CC dysgenesis accompanies a multitude of inherited disorders, and results in a clinical spectrum ranging from normal to severe mental retardation. Both complete and partial agenesis (CCA, pCCA) can likely occur secondary to disruption in any one of the multiple steps in callosal development, including primary defects in cell proliferation and migration, axon growth and guidance, and midline glial development (Kamnasaran 2005; Paul et al. 2007).

In patients with callosal dysgenesis, axons that fail to cross the midline can form longitudinally oriented bundles of Probst located medial to the lateral ventricles (Probst 1901). Notably, Probst bundles may serve as a relatively specific marker of axon guidance defects, and bundle topography may provide mechanistic insights. Probst bundles are common in patients with CCA without other midline, cortical, or posterior fossa anomalies, and are infrequent in patients with CCA and cortical malformations (Hetts et al. 2006). In some individuals, the bundles maintain a well-organized topography, suggesting that the axons remained responsive to guidance cues despite failure to cross the midline (Utsunomiya et al. 2006; Tovar-Moll et al. 2007). Other individuals have highly variable callosal connectivity, including heterotopic tracts not seen in healthy controls (Fig. 2) (Tovar-Moll et al. 2007; Wahl et al. 2009). It is likely that genetic causes of isolated CCA will be elucidated as imaging advances lead to more precise pheno-typing.

L1 Syndrome

The L1 syndrome is a highly variable X-linked neurological disorder resulting from mutations in the \( \text{L1CAM} \) gene and originally recognized as four distinct entities: X-linked hydrocephalus; MASA (mental retardation, aphasia, shuffling gait, adducted thumbs); X-linked complicated spastic paraplegia type 1; and X-linked corpus callosum agenesis. Based on their genetic homogeneity and phenotypic overlap, these disorders are now considered a single disease entity. Boys with L1 syndrome are mildly to severely affected
## Table 1 Summary of major human genetic disorders resulting, or hypothesized to result, from errors in axon growth and guidance

<table>
<thead>
<tr>
<th>Disorder</th>
<th>L1</th>
<th>JSRD</th>
<th>HGPPS</th>
<th>KS</th>
<th>Albinism</th>
<th>CFEOM1</th>
<th>DRS</th>
<th>PTCD</th>
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<td>Inheritance</td>
<td>X-L</td>
<td>AR</td>
<td>AR</td>
<td>X-L, AR</td>
<td>X-L, AR</td>
<td>AD</td>
<td>AD</td>
<td>Sporadic</td>
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<td>Gene(s)</td>
<td>L1</td>
<td>AHI1</td>
<td>ROBO3</td>
<td>KAL1</td>
<td>FGFR1</td>
<td>PROKR2</td>
<td>MATP</td>
<td>KIF21A</td>
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<td>Synkinesis</td>
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<td>Occurs</td>
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<td>Occurs (KAL1)</td>
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<td>CC</td>
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<td>Rarely thin</td>
<td>Small</td>
<td>Absent</td>
<td>Mal-oriented</td>
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<td>Small</td>
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<td>SCP</td>
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<td>Reduced to Absent</td>
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<td>CST-P</td>
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Key: X-L, X-linked; AR, autosomal recessive; AD, autosomal dominant; CC, corpus callosum; SCP, superior cerebellar peduncle; SCP-D, SCP midline decussation; MCP, middle cerebellar peduncle; ICP, inferior cerebellar peduncle; CST-P, corticospinal tract pyramids; CST-D, corticospinal tract midline decussation; CPT-D, central pontine tract decussation; CN I, olfactory nerve; CN II, optic nerve; CN II-D, optic chiasm decussation; CN III, oculomotor nerve; CN VI, abducens nerve; CN VII, facial nerve; CN VIII, vestibulocochlear nerve.
with a combination of macrocephaly, mental retardation, spastic paraparesis, and thumb flexion deformities. Postmortem and neuroimaging studies may reveal agenesis of the corpus callosum and corticospinal tracts in the absence of cortical malformations (Chow et al. 1985; Halliday et al. 1986; Graf et al. 2000), supporting a defect in axon guidance.

L1 is a transmembrane neural adhesion molecule comprised of six immunoglobulin-like and five fibronectin type III-like extracellular motifs and a short cytoplasmic tail. L1 acts as a short-range axon guidance cue and is highly expressed in developing axons and apical dendrites of cortical neurons, and within migratory axons of the corpus callosum and corticospinal tract (Joosten and Gribnau 1989; Demyanenko et al. 1999). L1 has multiple extracellular binding partners, including β1 integrins, NCAM, TAG-1/axonin-1, contactin, neuropilin-1, and L1 itself, through which it potentiates cell adhesion, provides a mechanical link to the actin cytoskeleton, and serves as a coreceptor to assist in intracellular signal transduction. For example, L1 homophilic binding increases cell adhesion and enhances neuronal migration and neurite outgrowth, whereas binding to neuropilin-1 mediates Sema3A-induced growth cone collapse and axon repulsion (Castellani et al. 2002; Wiencken-Barger et al. 2004). Small uncrossed CST may result from disrupted Sema3A signaling (Castellani et al. 2000; Castellani et al. 2002). Consistent with the human phenotype-genotype correlations, a knockin mouse harboring a human mutation in the L1 cytoplasmic tail has disrupted ankyrin binding and a milder phenotype (Buhusi et al. 2008). Finally, the role of L1CAM in neuronal migration and survival, synaptogenesis, and long-term potentiation may also contribute to the L1 syndrome phenotype (Demyanenko et al. 1999; Dihne et al. 2003; Maness and Schachner 2007; Schmid and Maness 2008).

Joubert Syndrome and Related Disorders (JSRD)

Joubert Syndrome (JS) is an autosomal recessive and genetically heterogeneous trait characterized by combinations of congenital hypotonia, ataxia, abnormal respiratory patterns, mental retardation, social disabilities including autism, and synkinesis. JS can also cosegregate with retinopathy, kidney disease, liver disease, polydactyly, obesity, and/or situs inversus. This spectrum is now called Joubert syndrome and related disorders (JSRD) (Joubert et al. 1968; Gleeson et al. 2004; Zaki et al. 2008; Gerdes et al. 2009). Postmortem studies of individuals with genetically
undefined JS have revealed severe cerebellar vermian hypoplasia, dysplasia of the deep cerebellar and inferior olivary nuclei, elongation of the caudal midbrain tegmentum, reduction in pontine neurons, and hypoplasia of the solitary, trigeminal, and dorsal column nuclei and tracts. Reduced decussation of the superior cerebellar peduncles (SCP), CST, and central pontine tracts suggests defective axon guidance. In some cases, the CST is split into many small fascicles and the pyramids appeared flat (Joubert et al. 1968; Friede and Boltshauser 1978; Maria et al. 1999; Quisling et al. 1999; Yachnis and Rorke 1999). It is not known whether these crossing defects result from a defect in axon guidance or occur secondary to a defect in cell fate or survival.

In the current era of MRI, the diagnosis of JSRD is dependent on the presence of the “molar tooth” sign, a tooth-like shape on axial images at the level of the midbrain-hindbrain junction that reflects cerebellar vermian hypoplasia, a deepened interpeduncular fossa, and horizontally oriented and thickened SCP (Chance et al. 1999; Maria et al. 1999; Millen and Gleeson 2008). Multiple studies of genetically undefined patients have reported the failure of these mis-oriented SCP fibers to decussate (Padgett et al. 2002; Lee et al. 2005; Widjaja et al. 2006; Spampinato et al. 2008). In one patient with presumed absence of CST decussation, fMRI revealed aberrant bilateral activation of the cerebellar and sensorimotor cortex (Parisi et al. 2004b).

JSRD is genetically heterogeneous, and at least nine loci and seven genes (AHI1, NPHP1, CEP290, TMEM67, RPGRIP1L, ARL13B, and CC2D2A) have been identified to date (Dixon-Salazar et al. 2004; Ferland et al. 2004; Parisi et al. 2004a; Sayer et al. 2006; Arts et al. 2007; Baala et al. 2007; Delous et al. 2007; Cantagrel et al. 2008; Gorden et al. 2008; Noor et al. 2008). Failure of the SCP and CST to decussate has been documented in patients harboring mutations in AHI1, CEP290, and at least two additional JS genes (Poretti et al. 2007).

JS and JSRD are now classified as ciliopathies because the mutated genes encode signal transduction and scaffolding proteins implicated in the function of the primary cilium or its anchoring structure, the basal body (Badano et al. 2006; Gerdes et al. 2009). Several of the proteins interact (Gorden et al. 2008), suggesting that they may all be part of a signaling complex (Millen and Gleeson 2008). Although a role for cilia in axon guidance has not been elucidated, cilia are similar to growth cones in that they sense environmental cues and mediate signals through receptor-dependent pathways such as sonic hedgehog, noncanonical Wnt, and platelet-derived growth factor receptor (Fliegauf et al. 2007; Gerdes et al. 2009). Future studies will determine whether there is a primary axon guidance defect in JSRD and, if so, if this is mediated through ciliary-dependent or potentially ciliary-independent roles of the JSRD genes in neuro-developement.

### Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS)

HGPPS is a clinically and genetically homogeneous disorder in which hindbrain axons fail to cross the midline. Affected individuals are born with restricted horizontal gaze and develop scoliosis within the first decade of life. HGPPS is an autosomal recessive trait and results from mutations in the ROBO3 gene (Jen et al. 2004). ROBO3 encodes a transmembrane receptor analogous to mouse Rig1/Robo3, with five Ig-like and three fibronectin-like extracellular motifs and three cytoplasmic signaling motifs. Indistinguishable phenotypes result from ROBO3 nonsense, frame-shift, splice-site, or missense mutations spread across the gene, supporting a complete loss of ROBO3 function. Although the disease gene was identified in affected members of consanguineous families harboring homozygous ROBO3 mutations, HGPPS is also present in individuals from non-consanguineous families harboring compound heterozygous mutations (Chan et al. 2006).

Electrophysiological and neuroimaging studies in HGPPS support absence of decussating axons in the pons and medulla. Somatosensory and motor-evoked-potential tests reveal ipsilateral (Jen et al. 2004; Haller et al. 2008) or predominantly ipsilateral (Amoiridis et al. 2006)
rather than normal contralateral responses, reflecting uncrossed ascending dorsal column-medial lemniscal sensory pathways and descending corticospinal motor pathways. MRI reveals ventral flattening and hypoplasia of the hindbrain, and a butterfly-shaped medulla with a midline cleft (Jen et al. 2004). DTI confirms ipsilateral CST and sensory tracts (Haller et al. 2008), as well as failure of the SCP to decussate, absence of the major crossing fibers in the pons, and small cerebellar peduncles (Sicotte et al. 2006). Functional MRI reveals ipsilateral rather than the normal contralateral activation in the primary motor cortex following motor tasks (Haller et al. 2008). The cortex, corpus callosum, and exiting cranial nerves appear structurally normal (Jen et al. 2004; Bosley et al. 2005; Sicotte et al. 2006; Haller et al. 2008).

Robo3 is a divergent member of the Robo family of axon guidance molecules, and studies of the \textit{Robo3} \textsupERS \textit{Rig-1} \textsupERS mouse established that Robo3 is essential for midline crossing of hindbrain and spinal cord commissural (Sabatier et al. 2004) and precerebellar axons (Marillat et al. 2004). Robo3 is also necessary for midline crossing of precerebellar neurons (Marillat et al. 2004), and defects in neuronal migration may also contribute to the HGPPS phenotype. Robo3 alternative splicing produces two functionally antagonistic isoforms with distinct carboxy termini (Chen et al. 2008). Robo3.1 inhibits the responsiveness of commissural axons to Slit repellents and is present on commissural axons before and during midline crossing, whereas Robo3.2 is Slit-responsive and appears on the growth cone postcrossing to block re-crossing (Chen et al. 2008). HGPPS mutations reported to date alter nucleotides common to both isoforms.

Although the mechanism by which loss of \textit{ROBO3} leads to the HGPPS phenotype is not defined, the gaze palsy may result from errors in axon connectivity into and out of the abducens nucleus. The normal contralateral inputs onto the abducens nucleus from the pontine paramedian reticular formation and vestibular nuclei are predicted to be ipsilateral in HGPPS, and this would likely alter the firing patterns of motor and internuclear neurons. Axons of the abducens internuclear neurons would also fail to cross the midline via the medial longitudinal fasciculus to synapse on medial rectus motor neurons in the contralateral oculomotor nucleus, further perturbing horizontal gaze. Although the etiology of scoliosis is also speculative, HGPPS provides the first genetic evidence of a neurogenic cause for this disability. Finally, individuals with HGPPS perform normally on neuropsychological testing and have normal fine motor control without mirror movements (Amoiridis et al. 2006), suggesting that the pathologically ipsilateral corticospinal axons find their appropriate target, albeit on the wrong side.

**HUMAN GENETIC DISORDERS OF CRANIAL NERVE GUIDANCE**

**Kallmann Syndrome**

Individuals with Kallmann syndrome (KS) have congenital anosmia (lack of sense of smell) and hypogonadotropic hypogonadism (HH). In HH, the hypothalamus fails to release gonadotropin-releasing hormone (GnRH) that normally stimulates the pituitary gland to release sex hormones. Often, the lack of smell goes unnoticed and individuals with KS are not diagnosed until they fail to undergo secondary sexual development during their teenage years. It is proposed that errors in growth and guidance of olfactory axons can result in KS.

Both olfactory sensory neurons and GnRH neurons are born in the olfactory placode of the developing nose. Olfactory sensory axons then extend their growth cones through the cribriform plate into the central nervous system where they synapse with second-order mitral neurons within the olfactory bulb glomeruli. Mitral axons then extend in the olfactory tract to the piriform cortex. GnRH neurons migrate across the cribriform plate into the olfactory bulb anlage along a path that colocalizes with olfactory sensory axons (Schwanzel-Fukuda and Pfaff 1989; Wray et al. 1989), then migrate on to the hypothalamus, where they extend their axons to the median eminence, enabling neurosecretion into the hypophyseal portal.

The only KS neuropathology report is of a 19-week male fetus with a family history of X-linked KS (Schwanzel-Fukuda et al. 1989). Although his olfactory axons passed through the cribriform plate, they ended prematurely in a tangle within the meninges, failing to make contact with the brain. Olfactory bulbs and tracts were absent, consistent with the observation that olfactory bulb development requires innervation from olfactory sensory neurons (Graziadei and Monti Graziadei 1986). GnRH expressing cells were not in their appropriate position in the hypothalamus, but instead were found in the nose, along the path of the olfactory axons, and within the tangle of axons in the meninges. Thus, at least the X-linked form of KS may result from defective olfactory axon guidance, with secondary failure in GnRH neuronal migration and olfactory bulb formation.

KS is genetically heterogeneous and can be inherited as an X-linked, autosomal dominant, and possibly autosomal recessive trait. Because affected individuals are often infertile without therapy, pedigrees tend to be small and two-thirds of cases are sporadic. Despite these challenges, six KS genes have been reported, accounting for approximately 30% of cases. These KS genes encode transmembrane receptors and ligands that may be important for growth cone guidance. Some KS proteins also interact with one another and with heparan sulfate proteoglycans to amplify downstream signaling pathways (Hu et al. 2003; LeCouter et al. 2003; Gonzalez-Martinez et al. 2004). Consistent with this, KS can be oligogenic, resulting from combinations of mutations in more than one KS gene (Dode et al. 2006; Pitteloud et al. 2007a; Canto et al. 2009).

X-linked KS is caused by loss-of-function mutations in KAL1 (Franco et al. 1991; Legouis et al. 1991), which is expressed in developing olfactory placode and olfactory bulb (Gonzalez-Martinez et al. 2004). Two-thirds of males harboring KAL1 mutations also have mirror movements and enlarged, aberrant ipsilateral CSTs (Quinton et al. 1996; Mayston et al. 1997; Kraims et al. 1999; Quinton et al. 2001), supporting a role of KAL1 in guidance of CST as well as olfactory axons. KAL1 encodes the secreted glycoprotein anosmin-1 (Hardelin et al. 1999), which has cell adhesion, neurite outgrowth, and axon guidance and branch-promoting activities in vitro (Rugarli et al. 1996; Soussi-Yanicostas et al. 1996; Soussi-Yanicostas et al. 1998; Hardelin et al. 1999; Robertson et al. 2001; Soussi-Yanicostas et al. 2002; Gonzalez-Martinez et al. 2004). Direct studies of the role of KAL1 in axon guidance have been limited by the absence of Kal1 in the mouse genome. KAL3 and KAL4 encode prokineticin-2 receptor and its ligand, PROKR2 and PROK2, respectively (Dode et al. 2006). PROK2 is expressed in the developing olfactory bulb (Ng et al. 2005), whereas the G-protein coupled receptor PROKR2 is expressed along the path of the olfactory axons and migrating GnRH neurons. PROKR2<sup>-/-</sup> and PROK2<sup>-/-</sup> mice have stalled olfactory sensory axons that fail to enter the CNS, arrested GnRH neuron migration, olfactory bulb hypoplasia, and reproductive system atrophy (Ng et al. 2005; Matsumoto et al. 2006; Pitteloud et al. 2007b). It is not yet known whether olfactory sensory neurons express PROKR2 on their growth cones and are attracted toward PROK2 in the olfactory bulb anlage (Pitteloud et al. 2007b).

KAL6 and KAL2 encode fibroblast growth factor receptor 1 and its ligand, FGFR1 and FGF8, respectively (Dode et al. 2003; Falardeau et al. 2008). Following conditional removal of Fgfr1 from mouse telencephalon and olfactory epithelium, the olfactory bulb fails to develop, but olfactory sensory axons successfully enter the forebrain (Hebert et al. 2003). Thus, these genetic forms of KS may not result from errors in olfactory sensory axon development. Notably, however, FGFR1 signaling promotes GnRH neurite outgrowth and may be necessary for GnRH axons to target the median eminence (Gill et al. 2004; Tsai et al. 2005; Gill and Tsai 2006).

Albinism

Individuals with oculocutaneous albinism (OCA) have absent melanin pigment in their eyes, hair, and skin, whereas males with ocular
albinism (OA) lack eye pigment only. Individuals with either OCA or OA have increased contralateral and reduced ipsilateral projecting axons at the optic chiasm as well as hypopigmentation of the retinal pigment epithelium and iris, foveal hypoplasia, loss of binocular vision, reduced visual acuity, and nystagmus.

Melanin is synthesized within intracellular melanosomes, and in the eye is present in optic cup derived retinal pigment epithelial (RPE) cells and neural crest derived melanocytes of the iris. X-linked OA results from mutations in OA1, which encodes a G protein-coupled receptor on the melanosome membrane. Autosomal recessive OCA genes include TYR, encoding the enzyme tyrosinase that catalyzes rate-limiting steps in the melanin biosynthetic pathway; OCA2, encoding a protein regulating melanosome pH; TYRJ1, encoding a tyrosinase-related catalase; and MATP, encoding a transporter mediating melanin synthesis.

During development, retinal ganglion cell (RGC) axons extend toward the optic disc, turn posterior, and exit the eye as the optic nerve (cranial nerve II). Within the middle cranial fossa, the left and right optic nerves join to form the optic chiasm where approximately 40% of the axons cross the midline. Both ipsilateral and contralateral axons then continue posterior to terminate in the lateral geniculate nucleus of the thalamus. There is little direct evidence in albinism for a primary defect in the guidance of axons at the chiasm (Colello and Jeffery 1991; Marcus et al. 1996; Jeffery and Erskine 2005), and instead there may be a defect in cell fate.

The retina contains two populations of sharply demarcated RGC; those positioned in the temporal retina extend axons that project ipsilateral, whereas those positioned nasally extend axons that decussate at the chiasm. In mature albino mammals, this line of demarcation is shifted toward the temporal periphery, corresponding to a decrease in ipsilateral projecting RGC axons (Guillery et al. 1995; Petros et al. 2008). In mouse, RPE melanin formation begins at E11 just before the onset of neuroblast division and proceeds in a graded fashion, and the amount of melanin in the RPE correlates with the percent of ipsilateral axons at the optic chiasm (Guillery et al. 1995; Ray et al. 2007; Petros et al. 2008). This has led to the hypothesis that pigment formation provides positional information to RGC neurons, committing them to ipsilateral or contralateral projecting axons (Ray et al. 2007). Albino mice have disorganized RGC neurons with perturbed proliferation and a reduced number of cells expressing Zic2, the zinc finger transcription factor that directs the uncrossed retinal projection (Rachel et al. 2002; Herrera et al. 2003; Williams et al. 2003; Tibber et al. 2006; Garcia-Frigola et al. 2008; Petros et al. 2008). Thus, although the precise role of melanin in RGC and chiasm development remains to be determined, the reduction in ipsilateral-projecting axons in albinism may result from a developmental shift in RGC specification and fate, rather than a primary defect in axon guidance.

**Congenital Fibrosis of the Extraocular Muscles Type I**

Congenital fibrosis of the extraocular muscles type I (CFEOM1) is a complex strabismus syndrome categorized as one of the congenital cranial dysinnervation disorders (CCDD) (Gutowski et al. 2003; Engle 2007). Affected individuals are born with bilateral blepharoptosis (drooping eyelids) and strabismus, and absence of fusion and binocular vision. The eyes look down at rest and cannot be elevated, whereas horizontal movement can range from absent to full. Affected individuals often have ocular synkinesis, including synergistic convergence, synergistic divergence, and Marcus Gunn jaw-winking phenomenon (Fig. 1A).

Postmortem examination of an individual with CFEOM1 harboring the common KIF21A mutation (see below) revealed absence of the superior division of the oculomotor nerve and marked hypoplasia of the muscles this division innervates, the levator palpebrae superioris and superior rectus, that elevate the eyelid and eye, respectively. The oculomotor inferior division and abducens nerves were also small (Engle et al. 1997). Although the autopsy technique...
did not permit identification of aberrant innervation, subsequent MR imaging confirmed the autopsy findings and noted misinnervation of the lateral rectus muscle by an oculomotor nerve branch (Demer et al. 2005). Despite this strong clinical and radiological data supporting aberrant innervation in CFEOM1, however, it is not yet known if the primary defect is that of axon growth and guidance, pruning, or motor neuron survival.

CFEOM1 is inherited as an autosomal dominant trait and results from heterozygous mutations in \textit{KIF21A}, which encodes a kinesin motor (Yamada et al. 2003). The pattern of \textit{KIF21A} mutations suggests that CFEOM1 results from an alteration in, rather than haploinsufficiency of, \textit{KIF21A} function. Eighty mutation-positive patients of multiple ethnicities reported to date harbor only 11 unique missense mutations, which are often de novo, and 75% harbor 2860C\textsuperscript{T} (R954W). These mutations alter only seven of the 1675 amino acids in \textit{KIF21A}, of which five are located in the third coiled-coil domain of the \textit{KIF21A} stalk and two in the motor domain (Yamada et al. 2003; Ali et al. 2004; Tiab et al. 2004; Lin et al. 2005; Shimizu et al. 2005; Yamada et al. 2005; Zhang et al. 2006; Chan et al. 2007; Lu et al. 2008; Flaherty et al. 2009; Rudolph et al. 2009).

\textit{Kif21a} encodes an anterograde kinesin motor that is broadly expressed in rodent neuronal cell bodies, axons, and dendrites (Marszalek et al. 1999), and may interact with Big1 and Kank1 in vitro (Shen et al. 2008; Kakinuma and Kiyama 2009). Additional studies of wild-type and mutant \textit{KIF21A} are necessary to determine the role of axon guidance in the etiology of CFEOM1.

**Duane Retraction Syndrome**

Duane retraction syndrome (DRS) is a CCDD affecting 1:1000 individuals. Affected individuals have restricted horizontal gaze greatest with attempted abduction (movement away from the midline), and ocular synkinesis resulting in globe retraction with attempted adduction (movement toward the midline) (Fig. 1B). Postmortem examinations of individuals with DRS found absence of abducens motor neurons and nerve, and aberrant innervation of the lateral rectus muscle by axons of the oculomotor nerve (Hotchkiss et al. 1980; Miller et al. 1982). Thus, when an affected individual attempts to adduct their eye, both the intended medial rectus and the pathologically innervated lateral rectus muscles contract, resulting in retraction of the eyeball into the orbit (Duane 1905). Cocontraction of the two muscles can be recorded by electromyography (Gunderson and Zeavin 1956; Huber 1984).

Genetic studies of rare families segregating autosomal dominant DRS led to the identification of \textit{CHN1} as a DRS gene (Miyake et al. 2008). Individuals harboring \textit{CHN1} mutations have a higher incidence of vertical movement abnormalities and bilateral eye involvement when compared to individuals with nonfamilial DRS (Chung et al. 2000; Demer et al. 2007). Consistent with this, MRI of individuals harboring \textit{CHN1} mutations can reveal hypoplasia of the oculomotor nerve and oculomotor-innervated muscles in addition to the expected abducens nerve hypoplasia and aberrant lateral rectus innervation (Demer et al. 2007). Together, these findings suggest that human \textit{CHN1} mutations alter the development of abducens and, to a lesser extent, oculomotor axons.

DRS \textit{CHN1} mutations identified to date are missense, and result in amino acid substitutions that alter \(\alpha_2\)-chimaerin, a Rac guanosine triphosphate-activating signaling protein containing a RacGAP domain, a C1 domain that binds to diacylglycerol, and an amino-terminal SH2 domain (Hall et al. 1993; Hall et al. 2001). \(\alpha_2\)-chimaerin is expressed widely in developing neurons of rodent (Hall et al. 1993; Hall et al. 2001) and human (Miyake et al. 2008). It serves as an effector for axon guidance, and mice with loss of \(\alpha_2\)-chimaerin have elevated RacGTP levels, disrupted ephrin/EphA4 signaling, and pathological midline re-crossing of corticospinal tract axons within the spinal cord (Brown et al. 2004; Beg et al. 2007; Iwasato et al. 2007; Shi et al. 2007; Wegmeyer et al. 2007). In contrast, human DRS \textit{CHN1} mutations are gain-of-function, resulting in hyperactive \(\alpha_2\)-chimaerin and lower
RacGTP levels through several mechanisms including enhanced α2-chimaerin translation to the membrane (Miyake et al. 2008). Moreover, in ovo overexpression of mutant α2-chimaerin results in stalling, aberrant branching, and de-fasciculation of the oculomotor nerve (Miyake et al. 2008). The axon guidance molecules upstream and signaling pathway downstream of α2-chimaerin in the developing abducens and oculomotor axons are not yet known. Understanding why corticospinal and ocular axons are vulnerable to down- and up-regulation of this widely expressed signaling molecule may provide new insights into the regulation of axon guidance.

**Pontine Tegmental Cap Dysplasia**

Pontine tegmental cap dysplasia (PTCD) is a cerebellar, brain stem, and cranial nerve malformation syndrome (Maeba et al. 1997; Ouannounou et al. 2005; Barth et al. 2007; Jissendi-Tchofo et al. 2009). The 12 affected children described to date have mild to severe developmental delay, ataxia, and a combination of restricted horizontal eye movements, ocular apraxia, facial weakness, deafness, and swallowing and feeding impairments. Neuroimaging reveals pontine hypoplasia with ventral flattening and dorsal protrusion of tissue into the fourth ventricle (“tegmental cap”). Cerebellar vermian hypoplasia and elongated and laterally misplaced SCP result in a modified molar-tooth sign. The middle and inferior cerebellar peduncles and cranial nerves VII and VIII are small. DTI reveals failure of the SCP, MCP, and axons of the pontine nuclei to decussate, and defines the tegmental cap as an ectopic dorsal transverse fiber bundle (Barth et al. 2007; Jissendi-Tchofo et al. 2009). Thus, PTCD represents an intriguing new human axon guidance phenotype that shares features with Joubert, HGPPS, and the CCDD syndromes. The reported children have neither a positive family history nor consanguineous parents, so it remains to be proved that PTCD is genetic. It is plausible, however, that it results from de novo dominant mutations or recessive mutations in an unidentified gene.

**CONCLUDING REMARKS**

Only a handful of human disorders have been purported to result from defects in axon guidance and, in most cases, much work remains to understand their molecular etiologies. It seems eminent, however, that advances in neuroimaging and electrophysiology will provide the necessary tools to accurately recognize new patterns of aberrant axon connectivity and permit ascertainment of phenotypically homogeneous patient cohorts for genetic study. Continued advances in genetic linkage analysis, association studies, and next-generation sequencing will then lead to identification of genetic variants among these cohorts that cause, or increase susceptibility to, defects in axon guidance. Additional clinical symptoms and signs resulting from defects in axon guidance may also become apparent. For example, it is intriguing to speculate whether synesthesia, in which a stimulus in one sensory modality triggers an automatic and consistent response in another modality, is a central nervous system parallel of synkinesis (Mattingley 2009).

The combination of these rapidly advancing fields may lead to the definition of more subtle guidance defects and the determination of their potential contribution to human disease, including neurodevelopmental and psychiatric disorders. Hints of advances to come include a recent genetic study of synesthesia (Asher et al. 2009), as well as the association of variants of AHI1 with autism and schizophrenia (Ammann-Zakenberg et al. 2006; Ingason et al. 2007; Alvarez Retuerto et al. 2008), of ROBO3 with autism (Anitha et al. 2008), of ROBO1 with dyslexia (Hannula-Jouppi et al. 2005), and of L1 with schizophrenia and major depression (Kurumaji et al. 2001; Laifenfeld et al. 2005). Finally, one can speculate on the contribution of variable axon guidance and connectivity to the normal spectrum of human cognition and behavior, and to the brain’s default network (Buckner et al. 2008).

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