CHAPTER

31

Lissencephalies and Axon Guidance Disorders

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OUTLINE

Neural Circuit Development and Function in the Brain: Comprehensive Developmental Neuroscience, Volume 3 http://dx.doi.org/10.1016/B978-0-12-397267-5.00128-X

31.1 INTRODUCTION: DISORDERS OF AXON GUIDANCE

During development, neurons extend their axons over long distances to form connections with synaptic targets (Lin et al., 2009; Schmidt et al., 2009). For this process to occur, a choreographed sequence of events must take place (Garbe and Bashaw, 2004; Izzi and Charron, 2011; Lin et al., 2009; O'Donnell et al., 2009). First, neurons and their surrounding target tissues must be specified to express the correct complement of receptors and guidance cues, respectively (Garbe and Bashaw, 2004; Izzi and Charron, 2011; O'Donnell et al., 2009). Second, receptors must be assembled into the appropriate complexes and localized to the axonal or dendritic growth cones, whereas guidance cues must be correctly trafficked to and localized within the extracellular environment (Garbe and Bashaw, 2004; Izzi and Charron, 2011; O'Donnell et al., 2009). Third, signaling mechanisms must be in place to integrate and transmit signals from the surface receptors to changes in the growth cone actin cytoskeleton, resulting in stereotyped steering decisions (Garbe and Bashaw, 2004; Izzi and Charron, 2011; O'Donnell et al., 2009). Each of these steps provides many potential levels for the regulation of axon guidance decisions (Garbe and Bashaw, 2004). These processes of axon guidance are controlled by the so-called axon guidance proteins (Schmidt et al., 2009), and remarkably, only a handful of human disorders resulting from primary errors in these processes have been identified (Engle, 2010).

The physician's ability to detect disorders of axon guidance has been augmented by classic, pathological, radiological, and electrophysiological techniques (Engle, 2010). Diagnostic radiological 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 (Engle, 2010). Neuropathological studies can also detect the 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) (Engle, 2010). Electrophysiological studies such as evoked potentials can reveal aberrant central connections of peripheral sensory or motor nerves (Engle, 2010). Exciting advances in neuroimaging and genetics are revolutionizing the ability to define axon guidance disorders (Engle, 2010). Detailed fiber tract anatomy can now be visualized using noninvasive tractography such as diffusion tensor imaging (DTI) and diffusion spectrum imaging (Engle, 2010). 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). Human genetics also is providing unbiased approaches to identify the etiologies of disorders with aberrant axon tracts (Engle, 2010). For some syndromes, animal and *in vitro* studies have confirmed that the encoded protein has a primary role in axon guidance (Engle, 2010). For others, such studies reveal a primary role in neuronal specification and migration rather than, or in addition to, a role in axon guidance (Engle, 2010). Some neurodevelopmental disorders without clinical, pathological, or radiological evidence of aberrant axon tracts have been found to result from mutations in genes that contribute to axon guidance in animal models (Engle, 2010).

The major human genetic disorders that result, or are proposed to result, from defective axon guidance 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 (Engle, 2010). For example, mutations in the β-tubulin isotype III (*TUBB3*) gene have been identified in a series of autosomal dominant disorders of axon guidance, which are known as the *TUBB3* syndromes (Tischfield et al., 2010). Understanding mechanisms of axon growth and axon guidance are critical to elucidating developmental disorders (Yu et al., 2010).

31.2 LIGAND/RECEPTOR SYSTEMS MEDIATING AXON GUIDANCE

During development, neuronal growth cones, the highly motile, specialized structures at the tips of extending axons, follow specific pathways and navigate series of intermediate choice points to find their correct targets (Allen and Chilton, 2009; Bush and Soriano, 2009; Cooper, 2002; Izzi and Charron, 2011; Kaprielian et al., 2001; Nie et al., 2010; O'Donnell et al., 2009; Richards et al., 2004; Schmidt et al., 2009). Pathway selection by axons is oriented by a large variety of short- and long-range guidance cues distributed along the entire pathway, to which different axons respond differently (Barkovich et al., 2009; Bush and Soriano, 2009; Cooper, 2002; Izzi and Charron, 2011; Kaprielian et al., 2001; Richards et al., 2004; Schmidt et al., 2009). Axon guidance proteins can be either secreted or associated with membranes (Kaprielian et al., 2001; Schmidt et al., 2009). In the case of secreted proteins, gradients are formed that enable them to signal over long distances, whereas membrane-bound molecules are responsible for local signaling (Schmidt et al., 2009). Generating precise patterns of connectivity depends on the regulated action of conserved families of guidance cues and their neuronal receptors (Bashaw and Klein, 2010;

Kaprielian et al., 2001; Schmidt et al., 2009). Cell surface receptors residing on growth cones and their associated axons interpret these signals as positive/attractive or negative/repulsive forces (Bush and Soriano, 2009; Izzi and Charron, 2011; Kaprielian et al., 2001; Schmidt et al., 2009). Local regulation of protein synthesis and degradation in the axon also contribute to the rapid changes in growth cone dynamics that occur during axonal navigation (Nie et al., 2010).

Identifying the molecules responsible for guiding growing axons to their target is only the first step; it is crucial to know how they are localized within the neuron itself (Allen and Chilton, 2009). The intracellular signaling cascade initiated on detection of the guidance cue by the axon-bound receptor triggers dynamic rearrangements of the actin cytoskeleton within the growth cone, promoting cycles of extension and retraction of filopodia at the leading edge (Bashaw and Klein, 2010; Cooper, 2002). This allows continual reassessment of the immediate environment by the growth cone (Cooper, 2002). In the case of chemoattraction, movement along the desired trajectory is achieved by elongation of the actin cytoskeleton, leading to the promotion of filopodia extension toward the source of the guidance cue (Cooper, 2002). In contrast, chemorepulsion promotes actin depolymerization and filopodia retraction, resulting in growth cone collapse and ultimately migration away from the ligand source (Cooper, 2002).

Several phylogenetically conserved families of guidance cues and receptors have been discovered (Chiappedi and Bejor, 2010; Koeberle and Bahr, 2004; O'Donnell et al., 2009; Schmidt et al., 2009). The four classic ligand/receptor systems mediating axon guidance are (1) semaphorins (sema) and their plexin (plex) and neuropilin receptors, (2) netrins and their DCC and UNC5 receptors, (3) slits and their roundabout (Robo) receptors, and (4) ephrins and their Eph receptors (Koeberle and Bahr, 2004; Lin et al., 2009; Nie et al., 2010; O'Donnell et al., 2009; Schmidt et al., 2009). It is the type of receptor, or receptor complex, expressed on the growth cone's surface, rather than a given guidance cue, that determines the direction of axon growth (Cooper, 2002; O'Donnell et al., 2009). Additional protein families previously recognized for other developmental functions have been implicated in growth cone guidance, including sonic hedgehog (SHH), bone morphogenetic proteins (BMPs), and wingless-type (WNT) proteins (Chiappedi and Bejor, 2010; Giger et al., 2010; O'Donnell et al., 2009; Schmidt et al., 2009). For example, WNT proteins have been identified as axon guidance proteins that play a role in guiding commissural axons from the spinal cord to the brain (Giger et al., 2010; Schmidt et al., 2009).

The expression of guidance cues and receptors is exquisitely tailored to allow growth cones to make appropriate path-finding decisions at specific times and places throughout development (O'Donnell et al., 2009). A wide variety of mechanisms are in place to ensure the correct presentation and receipt of guidance signals, ranging from spatially and temporally restricted transcriptional regulation of cues and receptors to their specific posttranslational trafficking (Chiappedi and Bejor, 2010; O'Donnell et al., 2009).

Secreted guidance cues, such as netrins and slits, have been shown to act as long-range cues, secreted from intermediate or final targets, and are presumed to form a chemotactic gradient along the pathway of the exploring growth cone (Cooper, 2002). In other instances, these factors can behave as short-range guidance cues, where they act over a distance of only a few cell diameters to affect changes in the direction of growth cone migration at specific choice points (Cooper, 2002). The molecular mechanisms that can influence the spatial distribution of guidance cues are the interaction with heparin sulfate proteoglycans, protein components of the extracellular matrix or axon-bound proteins, and selective proteolytic cleavage of the guidance protein (Cooper, 2002). Guidance receptors transport their ligands along axons to new locations distant from their points of synthesis, thereby determining their spatial distribution (Cooper, 2002).

Regulating the delivery of guidance receptors to the growth cone plasma membrane can have profound influences on axon growth and guidance; the regulation of receptor expression at the cellular level is not only strictly confined to surface expression but also includes regulated removal by endocytosis (Bashaw and Klein, 2010; O'Donnell et al., 2009). Endocytosis may be a necessary aspect of guidance receptor activation and signaling (Bashaw and Klein, 2010). In several cases, receptor endocytosis seems to be an obligate step in receptor activation that is evoked by ligand binding, whereas other examples point to the modulation of guidance responses by receptor endocytosis that is triggered by an independent pathway (O'Donnell et al., 2009). Endocytosis of guidance receptors also is influenced by other membrane receptor systems and can lead to changes in responsiveness to the guidance cue (Bashaw and Klein, 2010). In addition to contributing to receptor signaling, endocytosis can also modulate axon responses by regulating which receptors are expressed at the surface of the growth cone (O'Donnell et al., 2009).

Similar to endocytosis, proteolytic processing contributes to receptor activation and modulates guidance responses (Bashaw and Klein, 2010; O'Donnell et al., 2009). A role for proteolysis in axon guidance was supported by a number of early studies demonstrating that growth cones secrete proteases, and investigators proposed that cleavage of extracellular matrix components is required to advance through the extracellular

environment (O'Donnell et al., 2009). Later, genetic screens for defects in axonal navigation at the midline in Drosophila implicated the Kuzbanian ADAM (a disintegrin and metalloprotease) family transmembrane metalloprotease in the regulation of axon extension and guidance at the midline (O'Donnell et al., 2009). Several additional studies have implicated ADAM metalloproteases and matrix metalloproteases in contributing to axon guidance *in vivo* in both invertebrate and vertebrate nervous systems (O'Donnell et al., 2009).

ADAM10 forms a stable complex with ephrin-A2, and when EphR interacts with ephrin-A2, the resulting ligand–receptor complex is clipped by selective ADAM10-dependent cleavage of ephrin-A2; cleavage events are restricted to only those ephrin ligands that are engaged by receptors (Bashaw and Klein, 2010; O'Donnell et al., 2009). Ligand/receptor binding and formation of an active complex expose a new recognition sequence for ADAM10, resulting in the optimal positioning of the protease domain with respect to the substrate (Bashaw and Klein, 2010; O'Donnell et al., 2009). The ligand dependence of the cleavage event provides an elegant explanation for how an initially adhesive interaction can be converted to repulsion and offers an efficient strategy for axon detachment and attenuation of signaling (Bashaw and Klein, 2010; O'Donnell et al., 2009). The matrix metalloprotease family can play a similar role in converting ephrinB/EphB adhesion into axon retraction by specific cleavage of the EphB2 receptor (Bashaw and Klein, 2010; O'Donnell et al., 2009). Thus, both ephrin ligands and Eph receptors can be substrates for regulated proteolysis, and these proteolytic events seem to be critical in mediating axon retraction (Bashaw and Klein, 2010; O'Donnell et al., 2009). There also seems to be a common regulatory mechanism for the DCC and a number of ephrin ligands in which metalloprotease-mediated ectodomain shedding is followed by intramembranous gamma-secretase cleavage (Bashaw and Klein, 2010; O'Donnell et al., 2009).

31.2.1 Ephrins and Their Eph Receptors

Ephrins are members of a family of guidance molecules that are either anchored to the cell membrane by a glycosylphosphatidylinositol (GPI) linkage or have a transmembrane domain (Bush and Soriano, 2009; Kaprielian et al., 2001; Koeberle and Bahr, 2004). The ephrin/eph family has a variety of important functions, including axonal outgrowth and pruning, neuronal connectivity, synaptic maturation and plasticity, and neuronal apoptosis (Bush and Soriano, 2009; Lin et al., 2009). The Eph receptors represent the largest family of receptor tyrosine kinases in the mammalian genome and regulate various signaling pathways through a number of downstream effectors, including guanine nucleotide exchange factors (GEFs), guanosine triphosphatase (GTPase)–activating proteins, tyrosine kinases, phosphatases, and adaptor proteins (Nie et al., 2010). Eph/ephrin signaling is important in the formation of retinal connections, other axonal projections, and the corpus callosum (Nie et al., 2010).

The interaction of the Eph receptor tyrosine kinase family with their membrane-bound ligands, the ephrins, drives axon path-finding throughout the developing central and peripheral nervous systems via a chemorepulsive mechanism (Cooper, 2002; Giger et al., 2010). However, it has been shown that chemoattractive functions for ephrins also exist (Koeberle and Bahr, 2004). Eph receptors and their ephrin ligands both are capable of transmitting signals in the cell in which they are expressed: Eph receptor signaling is termed *forward signaling* and ephrin ligand signaling is termed *reversed signaling* (Bashaw and Klein, 2010; O'Donnell et al., 2009). The capacity for bidirectional signaling is a hallmark of the Eph/ephrin signaling system. In addition to their ability to signal through cognate Eph tyrosine kinase receptors, ephrins can also transduce a reverse signal into the cell in which they are expressed (Bush and Soriano, 2009). Binding of ephrin ligands triggers Eph receptor clustering, autophosphorylation, and downstream signaling cascades, which cause cytoskeletal rearrangements and changes in cell adhesion (Nie et al., 2010). Through these mechanisms, Eph receptors control axon turning, retraction, and branching (Nie et al., 2010).

Ephrin ligands and Eph receptors contribute to the guidance of retinal ganglion cell (RGC) axons in the visual system (Koeberle and Bahr, 2004; Nie et al., 2010; O'Donnell et al., 2009). Endocytosis of activated Eph receptors at the growth cone is necessary to allow for proper forward signaling, leading to growth cone retraction (Allen and Chilton, 2009; O'Donnell et al., 2009). In the case of membrane-associated ephrins, endocytosis of the ephrin–Eph complex is required for efficient cell detachment (parallel to proteolytic cleavage; Bashaw and Klein, 2010). Vav family GEFs have been implicated as regulators of Eph receptor endocytosis, signaling, or both (Bashaw and Klein, 2010). Vav proteins may trigger Eph internalization into signaling endosomes from where Eph receptors mediate dynamic changes of the actin cytoskeleton underlying growth cone collapse (Bashaw and Klein, 2010). Alternatively, Vav proteins may act in concert with other regulators of Rho GTPases to regulate Eph repulsive signaling, independent of endocytosis (Bashaw and Klein, 2010). For ephrinB-EphB–induced repulsive guidance, efficient cell detachment requires bidirectional endocytosis (Bashaw and Klein, 2010). Vav proteins may primarily promote cell detachment by mediating local Rac-dependent endocytosis of the ephrin–Eph complex and membrane (Bashaw and Klein, 2010).

Ephrin-A5 has been shown to act as a repulsive cue for somatosensory thalamocortical (TC) axons expressing EphA receptors in the TC system (Torii and Levitt, 2005). It seems to control the topography of TC projections within cortical areas (Torii and Levitt, 2005). Analysis of EphA/ephrin-A mutants suggests that ephrin-A5 signaling may also control the specificity of TC projections into individual cortical areas by regulating the positioning of TC axons within the subcortical telencephalon (ST) (Torii and Levitt, 2005). Gradients of EphA7 and ephrin-A5 exhibit complementary expression patterns from embryonic to postnatal ages, consistent with putative molecular interactions during development (Torii and Levitt, 2005). It has been hypothesized that ephrin-A5 serves as a dominant ligand *in vivo* for EphA7 among combinatorial gradients of ephrin-As (Torii and Levitt, 2005). The gradient of EphA7 receptor levels by neocortical neurons is a critical regulator of the topographic targeting of corticothalamic (CT) axons through local interactions within thalamic nuclei, and this regulation is independent of the relative positioning of CT axons within the ST (Torii and Levitt, 2005). The topography of CT projections can be disrupted while the inter- and intra-areal topography of TC projections develops normally (Torii and Levitt, 2005).

A-type Eph receptors and their ligands, ephrin-As, also have been implicated in controlling cell positioning in a variety of developmental contexts by sorting cell types, restricting their intermingling, or regulating their migration (Torii et al., 2009). EphA and ephrin-A signaling has a proapoptotic effect in the embryonic cortex without affecting the proliferation or cell cycle progression (Torii et al., 2009). EphA and ephrin-A signaling regulates lateral neuronal dispersion and intermingling during the multipolar stage of radial migration, and this mechanism is required to generate cortical columns with appropriate cellular components (Torii et al., 2009).

31.2.2 Semaphorins (Semas) and Their Plexin (Plex) and Neuropilin Receptors

Semaphorins are a large family of secreted and membrane-associated proteins most commonly associated with a role in axon guidance (Parrinello et al., 2008). The most extensively studied biological function of semaphorins is their role in guiding axons to their targets in the developing nervous system by providing repulsive signals (Cooper, 2002; Giger et al., 2010; Koeberle and Bahr, 2004; Parrinello et al., 2008). However, more recently, it has been reported that semaphorins can also act as axonal attractants and mediate adhesive signals in a variety of tissues, both in development and adulthood (Koeberle and Bahr, 2004; Parrinello et al., 2008). For example, semaphorins have also been found to act as chemoattractive guidance cues for cortical dendrites and olfactory bulb axons (Cooper, 2002).

The first semaphorin receptors to be identified were the neuropilins (Np-1 and -2), which recognize only the secreted semaphorins (Cooper, 2002; Koeberle and Bahr, 2004). Semaphorin 3A–Np-1 interactions are required for the fasciculation of the peripheral fibers of the trigeminal and vagal projections (Cooper, 2002). Np-2 is required for the organization and fasciculation of several cranial and spinal nerves (Cooper, 2002). Receptors for membrane-bound semaphorins are members of the family of plexins (Koeberle and Bahr, 2004). Plexins can interact with Np-1, conferring different semaphoring-binding specificities (Koeberle and Bahr, 2004).

Activation of the semaphorin receptor complex can lead to chemoattractive or chemorepulsive responses depending on the molecular composition of the receptor complex (Cooper, 2002). The growth cone response to semaphorins can be modulated by the direct physical interaction between neuropilins and members of the receptor families, highlighting the importance of receptor cross-talk in determining growth cone responses to guidance cues (Cooper, 2002).

The guidance factor semaphorin 3C, which is expressed by corpus callosum neurons, acts through the Np-1 receptor to orient axons crossing through the corpus callosum; transient neurons work together with their glial partners in guiding callosal axons (Niquille et al., 2009). The transducer that mediates the semaphoring 3C attractive response remains so far undefined (Niquille et al., 2009). Midline glial cells are the principal corpus callosum guidepost cells and secrete guidance factors that channel the callosal axons into the correct path (Niquille et al., 2009). These guidance signaling factors include netrin 1/DCC, Slit2/Robo1, ephrins/Eph, semaphorin/Np-1, and WNT (Niquille et al., 2009). Mutant mice for these guidance cues and their receptors exhibit callosal defects that range from minor, with few axons leaving the callosal track, to severe, with complete agenesis of the corpus callosum (ACC) (Niquille et al., 2009). Transmembrane proteins, including the tyrosine kinase receptors MET, ERBB2, OTK, and VEGFR2, participate in semaphorin responses by regulating diverse intracellular signaling events and functional outcomes (Niquille et al., 2009).

The cell adhesion molecule L1 has also been shown to act as a receptor for semaphorins (Koeberle and Bahr, 2004). A dependency on endocytosis to trigger axon retraction is observed in neurons responding to Sema3A, where the L1 IgCAM (immunoglobulin cell adhesion molecules), a component of the sema receptor complex, mediates endocytosis of the Sema3A holoreceptor in response to ligand binding (Bashaw and Klein, 2010; O'Donnell et al., 2009). The cell adhesion molecules L1

and TAG-1 (transient axonal glycoprotein-1) promote Sema3A activity through interaction and coendocytosis with its receptor neurolipin-1 (Bashaw and Klein, 2010).

Semaphorin 5A (Sema5A) is a membrane-bound protein and interacts with the receptor of plexin 3B (Lin et al., 2009). During development, Sema5A functions as an attractive and repulsive guidance molecule (RGM), and altered expression has been linked with aberrant development of axonal connections in the forebrain (Lin et al., 2009).

Semaphorin 4D (Sema4D) and B-type plexins demonstrate distinct expression patterns over critical time windows during the development of the murine cortex (Hirschberg et al., 2010). Sema4D–plexin-B2 interactions regulate mechanisms underlying cell specification, differentiation, and migration during corticogenesis (Hirschberg et al., 2010).

31.2.3 Netrins and Their DCC and UNC5 Guidance System

The DCC axon guidance receptor and its ligands, the netrins, have been shown to play a pivotal role in the guidance of axonal projections toward the ventral midline throughout the developing nervous system (Cooper, 2002; O'Donnell et al., 2009). The DCC belongs to a family of transmembrane proteins that possess four immunoglobulin domains and six fibronectin type III repeats (Kaprielian et al., 2001). Netrins are diffusible bifunctional molecules that can act as chemoattractants or chemorepellents for developing axons (Koeberle and Bahr, 2004). Secreted netrins and their receptors are one of the well-characterized axon guidance pathway families (Lin et al., 2009). The interaction of netrin-1 with DCC results in a chemoattractive response while interaction with the UNC5 family of netrin receptors results in chemorepulsion (Cooper, 2002; Lin et al., 2009). Netrin-1 functions as a floor plate-derived chemoattractant, which directs the pathfinding of commissural axons (Kaprielian et al., 2001). Netrin-1 binds to DCC that is expressed on commissural axons and their associated growth cones (Kaprielian et al., 2001). Mice lacking DCC or netrin-1 exhibit severe defects in commissural axon extension toward the floor plate and also lack several major commissures within the forebrain, including the corpus callosum and the hippocampal commissure (Cooper, 2002; Kaprielian et al., 2001). Studies have also revealed that DCC is crucial for the migration of some neuronal populations (Cooper, 2002).

In the developing mammalian neural tube, the DCC protein is present on the surface of commissural axons, as they migrate toward the floorplate, the source of the netrin gradient (Cooper, 2002). Once these axons have crossed the floorplate, they no longer respond to netrin despite the fact that they still retain expression of DCC on the axonal membrane (Cooper, 2002). Instead, they become responsive to the chemorepellents Slit2 and class 3 semaphorins, which are produced by the floorplate and the ventral neural tube, respectively (Cooper, 2002). This switch in responsiveness to chemorepulsive cues once having crossed the midline is believed to propel axons away from the midline and explains why axons are never seen to recross the midline after reaching the contralateral side (Schmidt et al., 2009). The key to the silencing of the chemoattractive response of the netrin-1-DCC interaction in this context lies in the absence or presence of the Slit receptor, Robo (Cooper, 2002). Axons projecting toward the midline express DCC but not Robo on their surface (Cooper, 2002). When on the ipsilateral side, netrin engagement by DCC homodimers triggers a chemoattractive response (Cooper, 2002). The direct coupling of the DCC and Robo receptors provides a precise temporal and spatial mechanism that accurately controls growth cone responses at a given choice point comprising conflicting directional information (Cooper, 2002). Slit–Robo chemorepulsion overrides netrin–DCC chemoattraction, thus becoming the driving force for that growth cone (Cooper, 2002).

The DCC is subservient to another chemorepulsive guidance receptor, UNC5 (Cooper, 2002). The chemoattractive response of DCC–netrin interactions is converted to chemorepulsion by the direct interaction between the cytoplasmic domains of DCC and UNC5 in the presence of netrin-1 (Cooper, 2002). In the presence of UNC5, the DCC is forced to change its polarity and act as a chemorepulsive netrin receptor (Cooper, 2002). In the presence of netrin-1, the affinity of the DCC is higher for Robo and UNC5 than it is for another DCC receptor (Cooper, 2002).

Modifying serotonin (5-HT) abundance in the embryonic mouse brain disrupts the precision of sensory maps formed by TC axons (TCAs), suggesting that 5-HT influences TCAs during development (Bonnin et al., 2007). 5-HT 1B and 5-HT 1D receptor expression in the fetal forebrain overlaps with that of the DCC and UNC5c expression. 5-HT coverts the attraction exerted by netrin-1 on posterior TCAs to repulsion (Bonnin et al., 2007).

Protein kinase A (PKA) has a role in regulating translocation of the DCC receptor to the growth cone plasma membrane (O'Donnell et al., 2009). Netrin-dependent inhibition of Rho activity also contributes to the DCC mobilization (O'Donnell et al., 2009). Rho GTPases constitute a major signaling output of guidance receptor activation (O'Donnell et al., 2009). The trafficking and polarized localization of netrin and Slit receptors are critical for proper direction of axon outgrowth (O'Donnell et al., 2009). Specifically, mutations in the UNC-73 Trio-family RacGEF or the VAB-8 kinesin-related protein disrupt the normal localization of the SAX-3 (Robo)

and UNC-40 (DCC) receptors, and in the case of UNC-40, regulation of localization also requires the MIG-2 Rac small GTPase (O'Donnell et al., 2009). These perturbations in normal receptor localization lead to significant defects in Slit and netrin-dependent posterior oriented cell and growth cone migration and further emphasize important regulatory roles for Rho GtPases in the control of axon guidance receptor localization (O'Donnell et al., 2009). In addition to these positive regulatory mechanisms, the trafficking of SAX-3 (Robo) and UNC-5 can also be negatively regulated with important outcomes for axon growth (O'Donnell et al., 2009).

In the regulated endocytosis of the repulsive netrin receptor UNC5 in vertebrate neurons, activation of PKC triggers the formation of a protein complex, including the cytoplasmic domain of UNC5H1, proteins interacting with C-kinase 1 (Pick1), and PKC and leads to specific removal of UNC5H1 from the growth cone surface (Bashaw and Klein, 2010; O'Donnell et al., 2009). Furthermore, the PKC activation leads to colocalization of UNC5A with early endosomal markers (Bashaw and Klein, 2010; O'Donnell et al., 2009). Thus, PKC-mediated removal of surface UNC5 provides a means to switch netrin responses from repulsion, mediated by either UNC5 alone or an UNC5-DCC complex, to attraction mediated by DCC (Bashaw and Klein, 2010; O'Donnell et al., 2009). G-protein-coupled adenosine 2b (A2b) receptor is a likely mediator of PKC activation because activation of A2b leads to the PKC-dependent endocytosis of UNC5 (Bashaw and Klein, 2010; O'Donnell et al., 2009). A2b is a netrin receptor that, together with DCC, appears to be required to mediate axon attraction, although this proposal has been controversial, and other evidence indicates that either A2b plays no role in netrin signaling or its role in netrin signaling is to modulate netrin responses (Bashaw and Klein, 2010; O'Donnell et al., 2009). In the context of UNC5 regulation, A2b acts independently of netrin, and its ability to regulate UNC5 surface levels supports its role as a potent modulator of netrin responses (Bashaw and Klein, 2010; O'Donnell et al., 2009).

31.2.4 Slits and Their Robo Guidance System

Slits are secreted proteins that control midline repulsion during development in vertebrates, signaling through receptors that belong to the Robo family (Allen and Chilton, 2009; Cooper, 2002; Kaprielian et al., 2001; Koeberle and Bahr, 2004). Slit can have dual roles, also promoting axonal growth (Koeberle and Bahr, 2004). Slit is a large extracellular matrix protein that is secreted by midline glia and is associated with the surfaces of axons (Kaprielian et al., 2001).

The Robo receptor is the best demonstration of both the importance of receptor trafficking for mediating axon guidance and the complexities yet to be unraveled (Allen and Chilton, 2009). This system is a striking example of the need for the growth cone to change its response as it grows toward, through, and beyond a guidance cue and the fundamental role played by the surface expression of receptors and their associated signaling components (Allen and Chilton, 2009). In both vertebrates and invertebrates, surface expression of the Robo receptor on axons of longitudinally projecting neurons and on precrossed commissural ones prevents them from approaching the embryonic midline (Allen and Chilton, 2009). Robo is the receptor for the chemorepellent protein Slit, which emanates from the midline (Allen and Chilton, 2009). Downregulation of Robo on commissural axons during midline crossing abrogates the repulsive effect of Slit so that a contralateral projection can be formed (Allen and Chilton, 2009). Following crossing, Robo is then restored to the axonal growth cone, which is not proven to prevent recrossing (Allen and Chilton, 2009).

In *Drosophila*, Slit is expressed by glia at the ventral midline, where it acts as a chemorepulsive guidance cue (Allen and Chilton, 2009; Cooper, 2002). The Slit receptor, Robo, is expressed at high levels on those axons that never cross the midline (Allen and Chilton, 2009; Cooper, 2002). In contrast, axons destined to cross the midline express very low levels of Robo when projecting on the ipsilateral side (Allen and Chilton, 2009; Cooper, 2002). Once on the contralateral side, Robo protein is greatly upregulated on the axonal membrane, and these axons never cross the midline again (Allen and Chilton, 2009; Cooper, 2002). Robo loss-of-function mutations result in both the commissural and noncomissural axons crossing the midline multiple times (Cooper, 2002).

Three Slit and three Robo orthologs have been identified in mammals (Cooper, 2002). The ability of mammalian Slits to act as chemorepulsive guidance cues has been demonstrated for a variety of axon populations, including olfactory bulb, hippocampal, and spinal motor axons (Cooper, 2002). The chemorepulsive activity of Slits has also been implicated in the targeted migration of neuroblasts within the rostral migratory stream toward the olfactory bulb and GABAergic neurons from the ganglionic eminence into the cortex (Cooper, 2002). Slit2 has also been shown to induce axon branching in sensory neurons (Cooper, 2002).

Heparan sulfate proteoglycans are essential for Slit-driven chemorepulsion (Cooper, 2002). These proteoglycans may be responsible for establishing effective local Slit concentrations and/or presenting Slit to the receptor in an appropriate format (Cooper, 2002).

Slit2 binds laminin-1, suggesting that the localization of Slit2 to precise choice points may be due to direct

interactions with the laminin isoforms within the surrounding extracellular matrix (Cooper, 2002). Slit2 also interacts directly with netrin-1 (Cooper, 2002). Both Slit and netrin-1 are coexpressed in many regions of the embryonic brain, including the floorplate of the neural tube (Cooper, 2002). Netrin attracts commissural axons toward the floorplate, while Slit acts to repel axons from the floorplate (Cooper, 2002). Once at the floorplate, the chemoattractive response to netrin-1 is silenced by the direct coupling of the netrin receptor, DCC, with the chemorepulsive Slit receptor, Robo, allowing the growth cones to escape the attractive forces of netrin-1 and move away from the floor plate (Cooper, 2002).

31.3 DOWNSTREAM SIGNALING MECHANISMS AND OTHER PROTEINS INVOLVED IN AXON GUIDANCE

On binding of axon guidance proteins to their growth cone receptors, intracellular signaling cascades are activated, resulting in extensive remodeling of the cytoskeleton and subsequent steering of the growth cone (Izzi and Charron, 2011; O'Donnell et al., 2009; Schmidt et al., 2009). Axon guidance is not a one-way process in which the growth cone passively receives information through its growth cone receptor (Schmidt et al., 2009). Instead, neurons can regulate the expression of receptors on the growth cone surface and modulate their response to axon guidance cues through various mechanisms (Schmidt et al., 2009). Also, different axon guidance cues can influence each other by binding to the same binding partner and/or through interaction of different signaling cascades (Schmidt et al., 2009). Furthermore, axon guidance receptor activity can be regulated by physical interactions between different guidance receptors (Schmidt et al., 2009).

Activation of specific signaling pathways can promote attraction or repulsion, result in growth cone collapse, or affect the rate of axon extension (Izzi and Charron, 2011; O'Donnell et al., 2009). The way a given guidance signal is interpreted also depends on the activities of a number of second-messenger pathways within the cell, and these pathways are potent modulators of axon responses *in vivo* (O'Donnell et al., 2009).

Calcium and cyclic nucleotides (cAMP and cGMP) can act *in vitro* to directly mediate guidance receptor signaling and also can modulate the strength and valence of guidance responses (Bashaw and Klein, 2010; O'Donnell et al., 2009). Disruption of calcium and cyclic nucleotide signaling leads to guidance defects in many systems, and in some cases, direct links have been made to specific guidance receptor signaling pathways (Bashaw and Klein, 2010). These two signaling systems show extensive cross talk in the regulation of growth cone guidance: Calcium signaling can promote the production of cyclic nucleotides through activation of soluble adenylyl cyclases and nitric oxide synthase, and cyclic nucleotides can regulate cellular calcium concentration by controlling the activity of plasma membrane calcium channels as well as through the regulation of calcium-induced calcium release (CICR) from internal stores (Bashaw and Klein, 2010). This positive feedback could potentially play a role in amplifying responses to shallow gradients of guidance cues (Bashaw and Klein, 2010). High cyclic nucleotide levels favor attraction, whereas low levels favor repulsion (O'Donnell et al., 2009). It is the ratio of cAMP/cGMP that is important in determining the polarity of the guidance response and has implicated calcium channel modulation in the control of guidance responses (O'Donnell et al., 2009). A clear demonstration that cyclic nucleotides and their downstream effectors can convert responses from attraction to repulsion and vice versa during axon guidance *in vivo* is lacking; however, a growing body of evidence supports a potent role for cyclic nucleotide signaling in modulating the strength of receptor responses (O'Donnell et al., 2009).

The signals mediating changes in cyclic nucleotide levels are thought to be independent of the guidance receptors whose responses they modulate; however, a more direct role of guidance receptor signaling in activating cAMP signaling has been suggested in the case of netrin (Ming et al., 1997; O'Donnell et al., 2009). Netrin acting through DCC (or A2b) leads to the elevation of cAMP and activation of PKA, and these events have been proposed to be instrumental in promoting netrin-mediated axon outgrowth and attraction (O'Donnell et al., 2009). There is an important role for cyclic nucleotides in modulating the strength of guidance responses *in vivo* rather than switching the polarity of responses (O'Donnell et al., 2009). The challenge now is to define the signals and receptors that regulate cyclic nucleotide signaling *in vivo* and to define specific contexts where their modulatory effects influence axon guidance (O'Donnell et al., 2009).

Exposure of growth cones to *in vitro* gradients of guidance cues can induce a corresponding gradient of calcium elevation (Bashaw and Klein, 2010). These asymmetric changes in calcium concentrations appear to be instructive signals to direct growth cone turning, because focal elevation of calcium is sufficient to induce turning responses (Bashaw and Klein, 2010). Increases in calcium influx and CICR can be triggered by guidance cues, and the outcome for growth cone behavior (either attraction or repulsion) can be influenced by the magnitude of the calcium elevation, the slope of the calcium gradient, and potentially the specific source of the calcium as well (Bashaw and Klein, 2010). In general, moderate amplitude increases in calcium (often involving CICR) favor attraction, whereas high- or low-amplitude

increases favor repulsion, although differences in neuron type, growth substrate, and resting calcium concentrations can affect growth cone responses (Bashaw and Klein, 2010).

Electrophysiological recordings from growth cones indicate that attractive and repulsive guidance cues trigger rapid and reciprocal changes in membrane potential; attractants such as brain-derived neutrophic factor (BDNF) and netrin lead to membrane depolarization and repellents, such as Slit and semaphorin, lead to hyperpolarization (Bashaw and Klein, 2010). Moreover, the polarity of the change in membrane potential determines attraction versus repulsion (Bashaw and Klein, 2010). For netrin- and BDNF-mediated attraction, transient receptor potential (TRP) calcium channels contribute to membrane depolarization, and calcium influx through these channels is required for chemoattraction (Bashaw and Klein, 2010). BDNF and netrin through engagement of their respective TrkB and DCC receptors lead to calcium release from internal stores and activation of TRP channels: The subsequent TRP channel-dependent membrane depolarization is sufficient to activate voltage-dependent calcium channels (VDCCs), and the resulting calcium influx is essential for attractive turning (Bashaw and Klein, 2010). A role for semaphorins in the activation of cyclic nucleotide gated (CNG) calcium channels strengthens the case for the specific regulation of calcium influx through plasma membrane channels and points to the importance of cross regulation of cyclic nucleotide and calcium signaling (Bashaw and Klein, 2010). Here, Sema signaling through plexin receptors stimulates the production of cGMP, which in turn is required for membrane hyperpolarization, activation of CNG channels, and growth cone repulsion (Bashaw and Klein, 2010).

Similar to calcium signaling, cyclic nucleotides (cAMP or cGMP) can have profound effects on growth cone responses to guidance cues (Bashaw and Klein, 2010). The levels of cyclic nucleotides, specifically the ratio of cAMP to cGMP, can determine whether the response to a guidance cue will be attractive or repulsive, with high cyclic nucleotide levels (or high cAMP/cGMP ratios) favoring attraction and low levels (or low cAMP/cGMP ratios) favoring repulsion; cyclic nucleotide signaling can clearly modulate the strength of receptor responses (Bashaw and Klein, 2010).

Sema–plexin signaling leads to the production of cGMP, and cGMP plays an instructive role in promoting repulsion by regulating membrane hyperpolarization and the influx of calcium through CNG channels (Bashaw and Klein, 2010). Netrin outgrowth and attraction require DCC (or A2b)-mediated elevation of cAMP and activation of PKA (Bashaw and Klein, 2010).

Rho-family GTPases, a subgroup of the Ras superfamily of small GTPases, have been extensively studied for their role in cell motility and regulation of cytoskeletal structures (O'Donnell et al., 2009). Members of the Rho (Rac homology) family include Rac, Cdc42, and RhoA (O'Donnell et al., 2009). Rho-family GTPases catalyze the hydrolysis of bound GTP to GDP, switching from active (GTP-bound) and inactive (GDP-bound) states (O'Donnell et al., 2009). The activity of these GTPases has profound effects on actin cytoskeletal and microtubule dynamics (O'Donnell et al., 2009). Rho-family GTPases are very important in mediating axon guidance receptor signaling output (O'Donnell et al., 2009). Guidance cues including slits, netrins, ephrins, and semaphorins can all influence the activity of Rho-family GTPases (Bashaw and Klein, 2010; O'Donnell et al., 2009). Slits, acting through Robo receptors, lead to decreased levels of active Cdc42 and increased RhoA and Rac activity (Bashaw and Klein, 2010; O'Donnell et al., 2009). Ephrins, through Eph receptors, result in increased RhoA activity as well, but they can also cause transient, decreased Rac activity in RGCs, whereas Eph–ephrin reverse signaling activates Rac and Cdc43 to direct repulsive axon pruning (Bashaw and Klein, 2010; O'Donnell et al., 2009). There is no clear consensus for how Rho GTPases mediate repulsion, because these repulsive guidance pathways each influence RhoA, Rac, and Cdc42 activity in distinct ways (Bashaw and Klein, 2010; O'Donnell et al., 2009).

The primary regulators of Rho GTPase cycling and activity are the Rho-family GEFs and GAPs (GTPase-activating proteins) (O'Donnell et al., 2009). Upstream regulation is likely to provide tissue specific as well as temporal control of Rho GTPase signaling during growth cone guidance (O'Donnell et al., 2009). Guidance receptors can directly regulate Rho GTPases (O'Donnell et al., 2009). Identifying the GEFs and GAPs that function downstream of a given guidance receptor is critical to understanding the mechanism of guidance receptor signal transduction (O'Donnell et al., 2009).

Identification of individual Rho GTPase regulators that are essential mediators of guidance receptor signaling pathways is complicated by at least three major factors: (1) redundancy can obscure important functions, (2) individual GEFs and GAPs can act in multiple signaling pathways, (3) GEFs and GAPs often contribute to only part of any given signaling output (O'Donnell et al., 2009).

Ras-GAP α-chimaerin is an essential mediator of the ephrinB3/EphA4 guidance pathway (Bashaw and Klein, 2010; O'Donnell et al., 2009). The α2-chimaerin isoform contains two interaction domains for EphA4, the N-terminal SH2 domain, which can interact with phosphorylated juxtamembrane tyrosines of EphA4, and a second region in the C-terminus that constitutively interacts with the kinase domain of EphA4 (O'Donnell et al., 2009). The association of α2-chimaerin with Eph

receptors appears to be direct or mediated by the Nck2 (Grb4) adaptor protein (Bashaw and Klein, 2010). EphA4-dependent tyrosine phosphorylation of α2-chimaerin occurs in response to ephrinB3, and this treatment increases the Ras-GAP activity of α-chimaerin (O'Donnell et al., 2009). The interaction with EphA4 activates the intrinsic GAP activity of α2-chimaerin, and this leads to inactivation of Rac1 (Bashaw and Klein, 2010). The cooperative action of α2-chimaerin in reducing Rac1-mediated actin polymerization and ephexin1 in enhancing RhoA-mediated actin depolymerization appears to induce efficient axon retraction (Bashaw and Klein, 2010). In addition, α-chimaerin's diacylglyceron (DAG)-binding C1 domain is very likely to regulate the GAP activity of α2-chimaerin (O'Donnell et al., 2009). The GAP domain in β2-chimaerin is occluded by the N-terminal SH2 motif, mediated by intramolecular interactions with the C1 domain, and ligand binding to the C1 domain is predicted to result in exposure of the Rac-GAP domain (O'Donnell et al., 2009). Increases in DAG production would be expected to increase the Rac-GAP activity of α2-chimaerin (O'Donnell et al., 2009). Similarly, SH2-mediated interactions with receptors may free the GAP domain for Rac inhibition (O'Donnell et al., 2009). Although a reduction in Rac activity is required to mediate ephrin-A-induced collapse, Rac activation also appears to be necessary for responses to ephrins (O'Donnell et al., 2009). Interference with Rac signaling blocks growth cone collapse in response to both semaphorins and ephrins (O'Donnell et al., 2009). Although decreases in Rac activity are observed following ephrin stimulation, reactivation of Rac is temporally correlated with growth cone collapse (O'Donnell et al., 2009).

Rac activity appears to be required for endocytosis; semaphorin 3A or ephrin treatment of retinal growth cones results in Rac-dependent endocytosis, which appears to mediate contact repulsion (O'Donnell et al., 2009). Specifically, for class B Eph/ephrins, bidirectional endocytosis occurs as the ephrin ligand and the Eph receptor are each internalized in trans to neighboring cells in a process that depends on their cytoplasmic domains and Rac activity (O'Donnell et al., 2009). The conserved Vav subfamily of Dbl GEFs plays a central role in this process, which appears to be instrumental for growth cone retraction (O'Donnell et al., 2009). Vav2/3 and α2-chimaerin have opposing effects on Rac1, and yet both are mediators of EphA forward signaling (Bashaw and Klein, 2010). Unlike Vav2/3, α2-chimaerin does not influence Eph receptor endocytosis (Bashaw and Klein, 2010). It is possible that the activated Eph receptor first activates α2-chimaerin to induce axon retraction and then activates Vav2/3 to locally activate Rac1-dependent endocytosis to allow cell detachment (Bashaw and Klein, 2010). Ephrins also function through Rho activation, and this activation appears to be mediated by the Dbl-family Rho GEF ephexin (O'Donnell et al., 2009). Ephexin activates RhoA, Rac, and Cdc42, but activation of the EphA receptor results in preferential activity toward RhoA (O'Donnell et al., 2009).

Genetic analysis of motor axon guidance in *Drosophila* indicates that plexin-B mediates repulsion in part by binding to active Rac (Bashaw and Klein, 2010). In contrast to plexin-Bs, plexin-A-induced growth cone repulsion requires the activation of Rac (Bashaw and Klein, 2010). The FERM domain-containing GEF protein, FARP2, associates with the plexin-A1/Npn-1 complex (Bashaw and Klein, 2010). Sema3A binding to Npn-1 causes FARP2 to dissociate from plexin-A1, activating FARP2's GEF activity and raising the levels of Rac-GTP in the cell (Bashaw and Klein, 2010). Activation of Rac triggers Rnd1 binding to plexin-A1, thereby activating plexin-A1's intrinsic Ras-GAP activity (Bashaw and Klein, 2010). Activated plexin-A1 downregulates R-Ras activity, which may lead to inhibition of integrin function and growth cone repulsion (Bashaw and Klein, 2010).

In contrast to the mechanism of plexin-B1 activation via Rac sequestration and RhoA activation, growth cone collapse induced by Sema3A requires activation of Rac (O'Donnell et al., 2009). Plexin-A1, together with neuropilin, transduces guidance signals from class 3 semaphorins, leading to Rac activation, Rnd1 recruitment, and reduction in R-Ras activity (O'Donnell et al., 2009).

In the absence of ephrin stimulation, nonphosphorylated ephexin1 is bound to EphA4 and activates RhoA, Rac1, and Cdc42, leading to a balance of GTPase activation that promotes axonal growth (Bashaw and Klein, 2010). Eph tyrosine kinase activity is required, but not sufficient to promote ephexin1 phosphorylation; instead, ephrin-induced clustering of Ephs appears to promote ephexin1 phosphorylation, probably involving Src tyrosine kinase (Bashaw and Klein, 2010). Tyrosine phosphorylation of ephexin1 shifts its exchange activity toward RhoA, thereby causing growth cone collapse *in vitro* (Bashaw and Klein, 2010). When Ephs are activated in a portion of the growth cone, tyrosine phosphorylated ephexin1 may tip the local balance of GTPase activity toward RhoA, thereby causing actin depolymerization and local retraction (Bashaw and Klein, 2010). In other regions of the growth cone that have not made contact with ephrins, ephexin1 promotes growth by activating RhoA, Rac1, and Cdc42 (Bashaw and Klein, 2010).

In motile cells, activation of Rho GTPases results in modulation of cytoskeletal dynamics via effector proteins, and one of the best characterized of these is the dual Cdc42/Rac effector, p21-activated kinase (O'Donnell et al., 2009). A well-established pathway of PAK activation via Cdc42 or Rac results in inhibition of the actin depolymerizing factor cofilin by activating

its inhibitor, LIM kinase (O'Donnell et al., 2009). Other notable targets of PAK include the myosin activator, myosin light chain (MLC) kinase, and the microtubule destabilizing protein, Op18/stathmin, which are each inhibited by PAK phosphorylation (O'Donnell et al., 2009). GTP-bound Rac and Cdc42 regulate Pak activity through binding to its Cdc42/Rac interactive binding domain, relieving autoinhibition of Pak by its N-terminal domain (O'Donnell et al., 2009). Pak likely functions downstream of Rac/Cdc42 in axon guidance (O'Donnell et al., 2009). *Drosophila pak*, *dock*, and *rac* each functions in midline axon repulsion and interacts genetically with the Slit/Robo pathway (O'Donnell et al., 2009). Expression of a constitutively membrane-targeted Pak suppresses defects caused by *rac* loss of function, which suggests that these Rac-dependent defects likely occur through loss of PAK regulation (O'Donnell et al., 2009). Both Cdc42 and Rac likely also function through pathways independently of PAK, particularly in axon growth (O'Donnell et al., 2009). Regulation of outgrowth via Rac can occur through a PAK-independent mechanism; however, guidance mediated through Rac and Cdc42 at least partly involves PAK function (O'Donnell et al., 2009).

Regulation of Pak leads to modulation of actin dynamics in axon growth and guidance by regulating the actin depolymerizing factor, cofilin, by modulating the activity of the serine/threonine kinase, LIMK (lin-11 and Mec-3 kinase) (O'Donnell et al., 2009). Cofilin destabilizes F-actin through pointed-end severing of actin filaments, although this activity may be necessary to maintain the supply of monomeric G-actin, thus promoting actin polymerization (O'Donnell et al., 2009). This activity is inhibited by phosphorylation at the N-terminal Ser3: Phosphorylation at this site is reciprocally regulated by the LIM and TES (testis-derived transcript) kinases and by the Slingshot phosphatase (Shh) (O'Donnell et al., 2009). In some cases, the rate of cycling between phosphorylated and nonphosphorylated states, rather than the absolute level of either species, can determine the influence of cofilin on actin dynamics (O'Donnell et al., 2009). How the LIM kinase and slingshot function in concert to regulate growth cone dynamics by regulating cofilin is of great interest in understanding receptor-mediated guidance (O'Donnell et al., 2009). Cycling of cofilin is required for promoting axon growth (O'Donnell et al., 2009). The LIMK also appears to mediate both axon outgrowth and attraction in certain contexts (O'Donnell et al., 2009). A gradient of phosphorylated cofilin accompanies the attractive response to BMP7, and repulsive responses from the same ligand are mediated by Ssh activity, demonstrating that distinct responses can be generated through activities converging on a single actin regulator (O'Donnell et al., 2009). The LIMK is required for growth cone attraction in certain contexts (O'Donnell et al., 2009).

Reverse signaling by receptorlike ephrinB proteins has been implicated in axon guidance (Bashaw and Klein, 2010). Following interactions with cognate Ephs, ephrinB proteins become clustered, and signaling is initiated either by Src-mediated tyrosine phosphorylation of the ephrinB cytoplasmic tail or by recruitment of PDZ domain-containing effectors (Bashaw and Klein, 2010). The NCK2 (NCK adaptor protein 2) is recruited to the phosphorylated ephrinB protein and is essential for several ephrinB-mediated processes, including spine formation (Bashaw and Klein, 2010). Tyrosine phosphorylation-dependent ephrin-B3 reverse signaling controls the stereotyped pruning of exuberant mossy fiber axons in the hippocampus, and NCK2 has been implicated in this process (Bashaw and Klein, 2010). The NCK2 appears to couple ephrinB3 with DOCK180 and PAK, leading to the activation of RAC1 (ras-related C3 botulinum toxin substrate 1) and CDC42 (cell division cycle 42) and downstream signaling that results in axon retraction/pruning (Bashaw and Klein, 2010).

Although inhibition of RAC can accompany repulsive guidance decisions, activation of RAC may also be involved in mediating responses to repulsive cues exemplified by Eph-dependent growth cone retraction and Ephrin-dependent axon pruning (Bashaw and Klein, 2010; O'Donnell et al., 2009). In the context of Slit–Robo-mediated repulsion, activation of Robo receptors by Slit leads to the activation of RAC, and limiting RAC function reduces the efficiency of Slit–Robo signaling (Bashaw and Klein, 2010; O'Donnell et al., 2009). The conserved RAC GAP, Vilse/CrGAP, contributes to Slit-dependent guidance decisions and antagonizes RAC function (Bashaw and Klein, 2010; O'Donnell et al., 2009). In axons, high levels of Vilse overexpression causes similar defects to Robo loss of function, and low levels of overexpression cause dosage-dependent defects in Slit–Robo repulsion similar to loss of function of Vilse (Bashaw and Klein, 2010; O'Donnell et al., 2009). The consequences of increasing or decreasing Vilse function are similar: Both lead to a compromise in the efficiency of Slit–Robo midline repulsion, suggesting that the precise levels of spatial distribution of Vilse RAC GAP activity may be instructive for Robo repulsion (Bashaw and Klein, 2010; O'Donnell et al., 2009). The interaction of Vilse/crGAP with Robo may be regulated in different subcellular contexts or during distinct stages of Slit–Robo repulsion (O'Donnell et al., 2009). Both the Slit–Robo and the forward Eph pathway use RAC GAP (Vilse for Robo and α-chimaerin for Ephs) and a Rac GEF (Sos for Robo and Vav for Ephs) to mediate repulsion (Bashaw and Klein, 2010; O'Donnell et al., 2009). A complex of proteins, including the adapters Nick, Pak, and Rac, are recruited to the receptors to mediate repulsive signaling (Bashaw and Klein, 2010). Coordinated action of GEFs and GAPs may promote the cycling of Rac

activity, which may be more important than the overall levels of Rac-GTP in a responding growth cone (Bashaw and Klein, 2010; O'Donnell et al., 2009). Alternatively, these GAPs and GEFs may represent distinct steps in the repulsive signal transduction output (Bashaw and Klein, 2010; O'Donnell et al., 2009). Rac activation in the case of Robo receptors may precede internalization as well (Bashaw and Klein, 2010; O'Donnell et al., 2009). Parallel mechanisms of repulsion may exist in these distinct ligand–receptor systems (O'Donnell et al., 2009).

Src family kinases (Src, Fyn, Yes, and others; collectively known as SFKs) are nonreceptor protein tyrosine kinases that have emerged as essential mediators of various guidance receptors (Bashaw and Klein, 2010). SFKs appear to be required in motor (LMC; lateral motor column) axons for limb trajectory selection and are critical for Eph forward signaling (Bashaw and Klein, 2010). Recruitment and activation of SFKs have been documented downstream of reverse signaling via GPI-anchored ephrinA ligands (Bashaw and Klein, 2010). The link between GPI-anchored ephrinAs and SFKs may be provided by transmembrane proteins such as p75 and TrkB (Bashaw and Klein, 2010). SFKs and focal adhesion kinase (FAK) are also essential mediators of netrin signaling (Bashaw and Klein, 2010).

The morphogen SHH mediates cell fate and axon guidance in the developing nervous system by two distinct pathways (Bashaw and Klein, 2010). Cell fate specification by Shh is mediated by the receptor Patched (Ptc) via the canonical pathway requiring the Gli family of transcription factors (Bashaw and Klein, 2010). In contrast, axon guidance by Shh is mediated by SFK in a smoothened-dependent manner via a rapidly acting, noncanonical signaling pathway not requiring transcription (Bashaw and Klein, 2010).

In addition to their critical contributions to downstream signaling, second messengers and Rho GTPases can also influence guidance responses by regulating the surface localization and activation of guidance receptors (Bashaw and Klein, 2010). Netrins induce outgrowth and attractive turning via the DCC family of receptors at least in part by regulating Rho GTPases (O'Donnell et al., 2009). Outgrowth of commissural axons in response to netrin requires Rho GTPase activity (O'Donnell et al., 2009). Netrin induces rapid activation of RAC1, CDC42, and PAK1 (p21 protein-activated kinase 1), which may occur in a complex containing the constitutive components DCC and NCK-1, as well as netrin-induced components, RAC1, CDC42, PAK1, and N-WASP (O'Donnell et al., 2009). Activation of this complex by netrin causes profound changes in growth cone morphology (O'Donnell et al., 2009). Netrin stimulation leads to an increase in the DCC surface levels, and this effect is enhanced by PKA activation (Bashaw and Klein, 2010). Blocking adenylate cyclase, PKA activity, or exocytosis prevents the increase in the DCC surface levels and blunts netrin-induced axon outgrowth (Bashaw and Klein, 2010). In addition to PKA's role in regulating DCC, netrin-dependent inhibition of Rho activity also contributes to DCC membrane localization (Bashaw and Klein, 2010). The trafficking and polarized localization of netrin and Slit receptors are critical for proper direction of axon outgrowth (Bashaw and Klein, 2010). There are important upstream regulatory roles of Rho GTPases in the control of axon guidance receptor localization (Bashaw and Klein, 2010). Trio is an important regulator of axon guidance decisions in several contexts (O'Donnell et al., 2009). Trio contains two Rho GEF domains, one with specificity for Rac and RhoG and the other that activates RhoA (O'Donnell et al., 2009).

Stimulation of RhoA results in activation of Rho kinase (O'Donnell et al., 2009). Rho kinases (ROCK or Rok) are serine/threonine kinases that, similar to PAK, regulate LIMK (O'Donnell et al., 2009). In addition, Rho kinases can regulate myosin activity through the phosphorylation of MLC, which results in activation and increases actin–myosin contractility (O'Donnell et al., 2009). Rho kinase also indirectly regulates myosin activity by phosphorylating and inhibiting MLC phosphatase (O'Donnell et al., 2009). ROCK activity is necessary for RhoA-induced retraction, likely through regulation of myosin II (O'Donnell et al., 2009). Inhibition of ROCK prevents the stability and contraction of actin arcs, which are filamentous actin structures that form in the transition zone of growth cones and affect microtubule bundling and dynamics (O'Donnell et al., 2009). ROCK can phosphorylate LIMK to regulate cofilin activity (O'Donnell et al., 2009). ROCK also phosphorylates the colapsin response mediator protein-2 (CRMP-2) after LPA (lipoprotein, Lp) or ephrin-A5 stimulation inhibits its ability to bind tubulin heterodimers (O'Donnell et al., 2009). CRMP-2 normally promotes axon outgrowth and branching, presumably by nucleating and promoting microtubule assembly (O'Donnell et al., 2009). The same residue in CRMP-2 that is targeted by ROCK downstream of LPA and ephrinA5 is phosphorylated by Cdk5 downstream of Sema3a-induced growth cone collapse, suggesting that independent signaling pathways can converge on the regulation of CRMP-2 phosphorylation (O'Donnell et al., 2009).

The RGM gene family consists of three members, RGMa, RGMb, and RGMc (Severyn et al., 2009). Each gene encodes a protein whose expression is restricted to a small number of tissues and is hypothesized to be involved in distinct biological functions ranging from control of iron metabolism to regulation of axonal guidance and neuronal survival in the developing nervous system (Severyn et al., 2009). RGMa is a cell

membrane-associated GPI-linked two-chain axonal guidance protein found primarily in the developing and adult central nervous system (Severyn et al., 2009). It has been shown that RGMa regulates repulsive guidance of retinal axons via binding to neogenin, a transmembrane protein that is also a receptor for netrins (Severyn et al., 2009).

Nervous system development is highly dependent on the microtubule cytoskeleton (Tischfield et al., 2010). Microtubules are copolymers assembled from tubulin heterodimers, which contain several different α- and β-isotypes (Tischfield et al., 2010). Each isotype may have properties necessary for specific cellular functions (Tischfield et al., 2010). TUBB3, one of at least six β-tubulins found in mammals, is distinct because purified microtubules enriched in TUBB3 are considerably more dynamic than those composed from other β-tubulin isotypes and because its expression is primarily limited to neurons (Tischfield et al., 2010). TUBB3 expression is greatest during periods of axon guidance and maturation; the level decreases in the adult central nervous system but remains high in the peripheral nervous system (Tischfield et al., 2010). The neuronal β-tubulin isotype is required in axon guidance and normal brain development (Tischfield et al., 2010).

During neurite extension, various families of microtubule-associated proteins (MAPs) bind to microtubules to regulate their stability, and hence the rate and direction of process outgrowth (Allen and Chilton, 2009). Some of these proteins appear to be specifically localized in either axons or dendrites (Allen and Chilton, 2009). Understanding how these MAPs become recruited to different compartments could shed light on how this occurs with axon guidance molecules (Allen and Chilton, 2009). The stability of microtubules will directly affect the facility with which motor proteins can convey their cargoes to the growing axon tip (Allen and Chilton, 2009).

Following axogenesis, the proximal section of the axon forms a diffusion impermeable barrier termed the axon initial segment (AIS) (Allen and Chilton, 2009). The AIS, microtubule polarity, and motor protein affinities collectively provide a series of filters to selectively transport necessary proteins to the axonal tip (Allen and Chilton, 2009). The growth cone autonomously executes intrinsic mechanisms to sort and retain from these deliveries those that are required (Allen and Chilton, 2009). The interplay between the cell adhesion molecule L1 and the neuropilin–semaphorin signaling axis provides the best current insight into how the turnover of axon guidance receptors is regulated at the growth cone (Allen and Chilton, 2009). L1 and other members of its family of cell adhesion molecules are important for the overall adhesion of a developing axon to its substrate (Allen and Chilton, 2009). By regulation, the distribution and turnover of semaphorin signaling complexes, L1 and maybe other cell adhesion molecules such as neuronal cell adhesion molecule, required for the signaling of Sema3B and Sema3F through Np-2, may play a key role in determining which receptors reach and stay at the developing axon tip (Allen and Chilton, 2009).

L1 is expressed along the length of the axon, but it is preferentially inserted into the growth cone membrane (Allen and Chilton, 2009). It functions as a critical component of the Sema3A receptor complex by binding Np-1 and determining whether a repulsive or attractive response is generated (Allen and Chilton, 2009). L1 also regulates the turnover of neuropilin receptors by controlling its endocytosis following semaphoring binding (Allen and Chilton, 2009). At the growth cone, L1 interacts with Ezrin–Radixin–Moesin proteins to influence the actin cytoskeleton and axon outgrowth (Allen and Chilton, 2009). L1 is anchored to the axonal membrane via ankyrinB, which is colocalized with L1 along the axonal shaft (Allen and Chilton, 2009). A mechanism is suggested whereby L1 could carry neuropilin to the growth cone and modulate its signaling once there (Allen and Chilton, 2009). Given the large turnover of plasma membrane occurring in developing growth cones, association with L1 in its cycle of surface renewal would be a very efficient means of locally recycling neuropilin, obviating the need to continually transport fresh receptor down the growing axon (Allen and Chilton, 2009). Following a change in its phosphorylation, L1 can subsequently associate with different cytoskeletal components and assume a more passive, adhesive role in the axon (Allen and Chilton, 2009). The interaction between L1 and neuropilin provides a means for both selective targeting of the latter to the developing growth cone and regulation of its endocytic turnover once there (Allen and Chilton, 2009).

There is another mechanism to retain guidance receptors at the axonal tip (Allen and Chilton, 2009). For neuropilin and the receptors for ephrin and netrin guidance molecules, local variations in the composition of the plasma membrane play an important role (Allen and Chilton, 2009). In the axon, glycolipid-enriched complexes, also known as lipid rafts, mediate the sorting of GPI-anchored proteins (Allen and Chilton, 2009). The responses induced by semaphorin and netrin are both dependent on the integrity of lipid rafts within the growth cone (Allen and Chilton, 2009). The GPI-anchored ephrinA and the transmembrane ephrinB families of molecules are also present in lipid rafts (Allen and Chilton, 2009).

Palmitoylation of proteins is a posttranslational modification, which can enhance the association of proteins with lipid rafts, and is a critical component of the nervous system development (Allen and Chilton, 2009). This modification occurs with the netrin receptor, DCC

(Allen and Chilton, 2009). This is required for its proper function in promoting both axonal growth and turning (Allen and Chilton, 2009). Lipid modification and microenvironments could be crucial for getting receptors to the developing growth cone (Allen and Chilton, 2009). Restriction within the growth cone of enzymes, which regulate palmitoylation, could then locally modulate retention of guidance receptors and associated signaling components (Allen and Chilton, 2009).

31.3.1 Agenesis of the Corpus Callosum

The corpus callosum is the largest fiber tract in the central nervous system and the major interhemispheric fiber bundle in the brain (http://www.ncbi.nlm.nih.gov/omim) (Bloom and Hynd, 2005; Donahoo and Richards, 2009; Paul et al., 2007; Richards et al., 2004). It consists of over 190 million axons, connects neurons in the left and right cerebral hemispheres, and is essential for the coordinated transfer of information between them (Bloom and Hynd, 2005; Chiappedi and Bejor, 2010; Donahoo and Richards, 2009; Paul et al., 2007; Richards et al., 2004). The corpus callosum contains homotopic and heterotopic interhemispheric connections (Paul et al., 2007).

Formation of the corpus callosum begins as early as 6 weeks of gestation, with the first fibers crossing the midline at 11–12 weeks of gestation and completion of the basic shape by 18–20 weeks (http://www.ncbi.nlm.nih.gov/omim) (Bloom and Hynd, 2005; Chiappedi and Bejor, 2010). The corpus callosum first enlarges caudally then develops rostrally (Bloom and Hynd, 2005). Myelination occurs slowly over the lifespan, with the process completing in puberty (Bloom and Hynd, 2005). Myelination progresses caudally to rostrally, much as the corpus callosum develops, from the splenium to the genu and rostrum (Bloom and Hynd, 2005; Richards et al., 2004). The most anterior portion of the callosum is the genu, which connects the prefrontal cortices on either hemisphere (Bloom and Hynd, 2005; Richards et al., 2004). The middle portions of the corpus callosum connect the motor and somatosensory regions (Bloom and Hynd, 2005). The caudal part of the body of the corpus callosum connects the cortex from the temporoparietal–occipital junction, as do portions of the splenium, the most posterior section of the corpus callosum (Bloom and Hynd, 2005; Richards et al., 2004). The splenium also connects the dorsal parietal and occipital regions (Bloom and Hynd, 2005).

For the corpus callosum to form, several critical developmental events must occur in sequence (Donahoo and Richards, 2009; Paul et al., 2007). These include correct midline patterning, formation of telencephalic hemispheres, birth and specification of commissural neurons, and axon guidance across the midline to the final target in the contralateral hemisphere (Donahoo and Richards, 2009; Paul et al., 2007; Richards et al., 2004).

Np-1 has been shown to be involved in corpus callosum formation (Donahoo and Richards, 2009). The first axons to cross the midline arise from neurons in the cingulated cortex (Paul et al., 2007). Cingulate pioneering axons of the corpus callosum express Np-1 at a crucial temporal stage for callosal development (Donahoo and Richards, 2009; Paul et al., 2007). Callosal axons are guided medially to the midline, where they are channeled into the contralateral hemisphere (Donahoo and Richards, 2009). Midline cellular populations that have been shown to assist in the formation of the corpus callosum include the midline zipper glia, the glial wedge, the indusium griseum glia, and the subcallosal sling (Bush and Soriano, 2009; Donahoo and Richards, 2009; Paul et al., 2007; Richards et al., 2004). The glial wedge has been shown to express molecules such as Slit2 required for callosal axons to cross the midline (Richards et al., 2004). The indusium griseum also expresses the guidance molecule Slit2 and resides directly above the corpus callosum (Richards et al., 2004).

After crossing the midline, callosal axons grow into the contralateral hemisphere toward their designated target region, usually homotopic to their region of origin, and then innervate the appropriate cortical layer (Paul et al., 2007). Such processes probably involve both molecular recognition of the appropriate target region and activity-dependent mechanisms that regulate axon targeting to the correct layer and the subsequent refinement of the projection (Paul et al., 2007).

ACC encompasses a broad range of diagnoses and is one of the most frequent malformations in the brain with a reported incidence ranging between 0.5 and 70 in 10 000 births (http://www.ncbi.nlm.nih.gov/omim) (Chiappedi and Bejor, 2010; Richards et al., 2004). Both complete and partial ACC can result from disruption in any one of the multiple steps of callosal development, such as cellular proliferation and migration, axonal growth, or glial patterning at the midline (Bush and Soriano, 2009; Engle, 2010; Paul et al., 2007; Richards et al., 2004). ACC is a clinically and genetically heterogeneous condition, which can be observed as either an isolated condition or a manifestation in the context of a congenital syndrome (http://www.ncbi.nlm.nih.gov/omim. Further heterogeneity in ACC can arise from concomitant abnormalities in the anterior commissure (Paul et al., 2007). Malformation of the corpus callosum can result in either the complete absence of the corpus callosum or partial absence (hypogenesis) or thinning of the corpus callosum (Donahoo and Richards, 2009; Paul et al., 2007; Richards et al., 2004).

The wide range of disorders in which callosal abnormalities are found underscores the importance of

understanding the basic nature of the development and function of the corpus callosum (Bloom and Hynd, 2005).

31.3.1.1 *Clinical Characteristics*

Corpus callosum dysgenesis accompanies a multitude of inherited disorders and results in a clinical spectrum ranging from normal to severe mental retardation (Engle, 2010; Richards et al., 2004). The most frequent clinical findings in patients with ACC are mental retardation (60%), visual problems (33%), speech delay (29%), seizures (25%), and feeding problems (20%) (http://www.ncbi.nlm.nih.gov/omim).

Isolated ACC, even when not ascertained clinically, still causes behavioral and cognitive impairment (Paul et al., 2007). As more individuals with primary ACC are identified and assessed with sensitive standardized neuropsychological measures, a pattern of deficits in higher order cognition and social skills has become apparent (Chiappedi and Bejor, 2010; Paul et al., 2007). The major anatomical feature of primary ACC is the absence of the corpus callosum, and it is presumed to be the cause of the cognitive and behavioral changes in these individuals (Paul et al., 2007). However, colpocephaly (abnormal enlargement of the occipital horns of the brain) and Probst bundles (large, bilateral, barrel-shaped axonal structures that do not cross the midline) are common in people with primary ACC and together with other subtle anatomical changes probably also affect behavior (Paul et al., 2007).

The functional consequences of structural changes in brain connectivity contribute to cognitive impairment (Paul et al., 2007). Primary ACC has a limited impact on general cognitive ability (Paul et al., 2007). Although the full-scale intelligence quotient (IQ) can be lower than expected based on family history, scores frequently remain within the average range (Paul et al., 2007). In an unexpectedly large number of persons with primary ACC (as many as 60%), performance IQ and verbal IQ are significantly different (Paul et al., 2007). However, there is no consistency with respect to which of the two is more affected (Paul et al., 2007). Impairments in abstract reasoning, problem solving, generalization, and category fluency have all been consistently observed in patients with primary ACC (Chiappedi and Bejor, 2010; Paul et al., 2007). Data from large sample sizes suggest that problem-solving abilities become more impaired as task complexity increases (Paul et al., 2007).

The most comprehensively examined higher cognitive domain in patients with ACC is language (Paul et al., 2007). Overall, individuals with primary ACC have intact general naming, receptive language, and lexical reading skills (Paul et al., 2007). However, impairments have been reported in the comprehension of syntax and linguistic pragmatics, and in phonological processing and rhyming (Paul et al., 2007). With respect to linguistic pragmatics, persons with primary ACC are impaired in the comprehension of idioms, proverbs, vocal prosody, and narrative humor (Paul et al., 2007). Patients with primary ACC also show marked difficulty with expressive language (Paul et al., 2007).

Parents of individuals with primary ACC consistently describe impaired social skills and poor personal insight as the features that interfere most prominently with the daily lives of their children (Chiappedi and Bejor, 2010; Paul et al., 2007). Also, parents frequently report health concerns such as feeding or sleeping issues, elimination problems, and unusual tolerance for pain (Chiappedi and Bejor, 2010). Specific traits include emotional immaturity, lack of introspection, impaired social competence, general deficits in social judgment and planning, and poor communication of emotions (Chiappedi and Bejor, 2010; Paul et al., 2007). Consequently, patients with primary ACC often have impoverished and superficial relationships, suffer social isolation, and have interpersonal conflict both at home and at work because of misinterpretation of social cues (Chiappedi and Bejor, 2010; Paul et al., 2007).

Responses of adults with primary ACC on self-report measures also suggest diminished self-awareness (Paul et al., 2007). The patients' self-reports are often in direct conflict with observations from friends and family (Paul et al., 2007). One potential factor contributing to poor self-awareness may be a more general impairment in comprehension and description of social situations (Paul et al., 2007).

Taken together, the neuropsychological findings in primary ACC highlight a pattern of deficits in problem solving, social pragmatics of language and communication, and processing emotion (Chiappedi and Bejor, 2010; Paul et al., 2007). Behavioral and emotional factors are frequently associated: A tendency for deficient social cognition in individuals with ACC seems to stem from a combination of difficulty integrating information from multiple sources, using paralinguistic cues for emotion, and understanding nonliteral speech (Chiappedi and Bejor, 2010).

The deficits in social communication and social interaction in patients with primary ACC overlap with the diagnostic criteria for autism (Chiappedi and Bejor, 2010; Paul et al., 2007). Furthermore, people with primary ACC may display a variety of other social, attentional, and behavioral symptoms that can resemble those of certain psychiatric disorders (Paul et al., 2007). Examination of symptom overlap between psychiatric disorders and ACC may help to isolate the symptoms that are directly caused by a dysfunction in corticocortical connectivity (Paul et al., 2007).

There are also structural similarities between ACC and some psychiatric disorders (Paul et al., 2007). Structural correlates of abnormal brain connectivity are

evident in essentially every psychiatric disorder that has been examined (Paul et al., 2007). For example, several studies have found altered morphology of the corpus callosum in schizophrenia patients, including changes in size and shape, as well as microstructural changes in callosal regions that are revealed by diffusion MRI (Bloom and Hynd, 2005; Paul et al., 2007). There are also a number of reports of complete ACC in patients with schizophrenia, underscoring a direct connection between ACC and schizophrenia (Paul et al., 2007). Corpus callosum size, especially its anterior sectors, is also decreased in some cases of autism (Bloom and Hynd, 2005; Chiappedi and Bejor, 2010; Paul et al., 2007). Microstructural changes in the corpus callosum have also been found in patients with Tourette's syndrome (a disorder characterized by repetitive, stereotyped, involuntary movements and vocalizations called tics) and attention deficit hyperactivity disorder (Bloom and Hynd, 2005; Paul et al., 2007). Abnormalities in the size of the corpus callosum have also been found in patients diagnosed with mental retardation, Down syndrome, developmental dyslexia, and developmental language disorders (Bloom and Hynd, 2005). Deviant asymmetry of cortical areas, possibly related to callosal abnormalities, has been found in developmental dyslexia and specific language impairment (Bloom and Hynd, 2005).

31.3.1.2 Genetics

The genetics of ACC is variable and reflects the underlying complexity of callosal development (Bush and Soriano, 2009; Chiappedi and Bejor, 2010; Paul et al., 2007). A combination of genetic mechanisms, including single-gene Mendelian mutations, single-gene sporadic mutations, and complex genetics, might have a role in the etiology of ACC (Paul et al., 2007). Retrospective chart reviews and cross-sectional cohort studies report that 30–45% of cases of ACC have identifiable causes (Chiappedi and Bejor, 2010; Paul et al., 2007). Approximately 10% have chromosomal anomalies, and the remaining 20–35% have recognizable genetic syndromes (Paul et al., 2007). However, if only individuals with complete ACC are considered, then the percentage of patients with recognizable syndromes drops to 10–15%, and thus 75% of cases of complete ACC do not have an identified cause (Chiappedi and Bejor, 2010; Paul et al., 2007).

One example of ACC associated with a Mendelian disorder is X-linked lissencephaly with ACC and ambiguous genitalia (XLAG), which results from a mutation in the aristaless-related homeobox gene (*ARX*) (Chiappedi and Bejor, 2010; Paul et al., 2007).

Another syndrome caused by a single-gene mutation is CRASH syndrome (corpus callosum agenesis, retardation, adducted thumbs, spastic paraplegia, and hydrocephalus), which is accompanied by diminutive corticospinal tracts within the brainstem (Paul et al., 2007). CRASH is caused by mutations in the L1 cell adhesion molecule (*L1CAM*) gene that codes for a transmembrane cell adhesion protein broadly expressed in the central nervous system (Paul et al., 2007).

Andermann syndrome, an autosomal recessive condition prevalent in the Saguenay-Lac-St-Jean region of Quebec, presents with callosal hypoplasia or ACC, cognitive impairment, episodes of psychosis, and a progressive central and peripheral neuropathy (Chiappedi and Bejor, 2010; Paul et al., 2007). It is caused by mutation of the KCl cotransporter *KCC3* (*potassium chloride cotransporters 3*) (Paul et al., 2007).

Mowat–Wilson syndrome (MWS) in addition to ACC causes Hirschsprung disease, congenital heart disease, genitourinary anomalies, microcephaly, epilepsy, and severe cognitive impairment (Chiappedi and Bejor, 2010; Paul et al., 2007). The MWS is caused by heterozygous inactivating mutations in the gene zinc finger homeobox 1b on chromosome 2q22, which codes for SMAD-interacting protein 1 (Paul et al., 2007). ACC is not observed in all MWS cases (Paul et al., 2007).

Aicardi syndrome is another disorder probably caused by sporadic mutations on the X chromosome (Chiappedi and Bejor, 2010; Paul et al., 2007). It is only observed in females and XXY males with Klinefelter syndrome (Paul et al., 2007). It consists of ACC, infantile spasms and chorioretinal lacunae, and additional cerebral and ophthalmological abnormalities (Paul et al., 2007).

ACC is a notable facet of craniofrontonasal syndrome (CFNS) (Bush and Soriano, 2009). Mutations in the *EPHRIN-B1* gene result in a wide spectrum of developmental abnormalities that constitute this syndrome (Bush and Soriano, 2009). This syndrome includes cleft palate, craniofrontonasal dysplasia, craniosynostosis, axial skeletal defects such as asymmetry of the thoracic skeleton and limb abnormalities, and neurological defects such as mental retardation (Bush and Soriano, 2009). It is an X-linked condition (Bush and Soriano, 2009).

Some other syndromes associated with ACC are ACC with fatal lactic acidosis, HSAS/MASA syndromes (X-linked hydrocephalus) (*L1CAM*), acrocallosal syndrome, Chudley–MCCullough syndrome, Donnai–Barrow syndrome, FG syndrome, gentiopatellar syndrome, Temtamy syndrome, Toriello–Carey syndrome, Vici syndrome, ACC with spastic paraparesis (SPG11), CFNS, Fryns syndrome, Marden–Walker syndrome, Meckel–Gruber syndrome, microphthalmia with linear skin defects, Opitz G syndrome, orofaciodigital syndrome, pyruvate decarboxylase deficiency, Rubinstein–Taybi syndrome, septo-optic dysplasia (DeMorsier syndrome), Sotos syndrome, Warburg micro syndrome, and Wolf–Hirschhorn syndrome (Bush and Soriano, 2009; Chiappedi and Bejor, 2010; Paul et al., 2007).

In most individuals with ACC, there is no clearly inherited cause or a recognized genetic syndrome, suggesting that ACC can be caused by sporadic (*de novo*) genetic events (Paul et al., 2007). It is likely that some cases of ACC are caused by haploinsufficiency at other genetic loci (Paul et al., 2007). This is supported by many reports of patients with ACC who have sporadic chromosomal mutations with particular loci identified repeatedly (Paul et al., 2007).

The number of patients with ACC in which chromosomal rearrangements are found has increased following technical improvements, from conventional karyotyping to subtelomeric and array-CGH (comparative genomic hybridization) analysis (Chiappedi and Bejor, 2010). Candidate genes have been located especially on chromosome 1 and also on 3, 7, 8, 13, 15, 18, and 21 (Chiappedi and Bejor, 2010). Data obtained using microarray-based comparative genomic hybridization demonstrate that patients with ACC have chromosomal deletions or duplications that are smaller than those that can be detected using conventional cytogenetics (Paul et al., 2007). The risk of having a child with ACC is nearly threefold higher for mothers aged 40 and above, which is consistent with causal sporadic chromosomal changes (Paul et al., 2007).

Many cases of ACC might be caused by polygenic and other complex interactions (Paul et al., 2007). The abundance of case reports of ACC associated with specific diseases probably also reflects complex underlying mechanisms (Paul et al., 2007).

Environmental factors might contribute to ACC (Chiappedi and Bejor, 2010; Paul et al., 2007). One example of environmental influences on callosal development is provided by fetal alcohol syndrome (FAS) (Chiappedi and Bejor, 2010; Paul et al., 2007). Alcohol exposure *in utero* decreases gliogenesis and glial–neuronal interactions, processes that are vital for corpus callosum development (Paul et al., 2007). Ethanol disrupts the transcription and biochemical function of L1CAM, implicating an interplay of environment and genetics in ACC (Paul et al., 2007). The incidence of ACC in FAS is approximately 6.8% (Paul et al., 2007). In many FAS cases, the corpus callosum is hypoplastic; this may result not only from the disruption of early events in callosal formation but also from later dysregulation of axonal pruning (Paul et al., 2007). Such mechanisms might also cause callosal hypoplasia in other disorders such as schizophrenia and autism (Paul et al., 2007). Other environmental factors may also influence postnatal and prenatal callosal development, including musical training, hypothyroidism, and enrichment or deprivation of experience (Paul et al., 2007).

31.3.1.3 Neuroradiological Findings

Consistent with the broad range of genetic factors involved in ACC, the cognitive, behavioral, and neurological consequences of ACC are wide ranging (Paul et al., 2007; Richards et al., 2004). One approach to defining clinical subsets of the ACC patient population is to categorize individuals according to specific neuroanatomical findings and subsequently relate these to the behavioral symptoms within these groups (Paul et al., 2007). The presence of polymicrogyria (PMG) (an excessive number of gyri on the surface of the brain), pachygyria (too few gyri on the surface of the brain), and heterotopia, detected using MRI, correlate with moderate to severe developmental delay (Chiappedi and Bejor, 2010; Paul et al., 2007).

In ACC, the superomedial aspects of the lateral ventricles are deformed by the fibers of the cerebral hemispheres that were destined to cross in the corpus callosum and that, with agenesis, course instead longitudinally as the bundles of Probst (Chiappedi and Bejor, 2010). Crescentic lateral ventricles result from the impression of medial ventricular wall by these bundles (Chiappedi and Bejor, 2010). The other relevant neuroradiological sign is the eversion of the cingulated gyri which can be seen in coronal scans (Chiappedi and Bejor, 2010).

Ultrasonography can be helpful, even if MRI is thought to be far superior at least for partial agenesis (Chiappedi and Bejor, 2010). Morphologically, two types of ACC can be distinguished: In type 1, axons are present but unable to cross the midline, forming large aberrant fiber bundles (Probst bundles), while in the less frequent type 2, axons fail to form (Chiappedi and Bejor, 2010).

The most relevant sonographic sign in sagittal views is the superior displacement of the third ventricle, while parasagittal views show that the medial cortical sulci radiates superiorly instead of horizontally and the absence of the normally echogenic pericallosal sulcus (Chiappedi and Bejor, 2010). Coronal scans show absence of callosum and Probst longitudinal bundles indenting the dorsomedial aspect of lateral ventricles (Chiappedi and Bejor, 2010).

With partial agenesis, the posterior portion is nearly always affected, with the notable exception of the anterior involvement that occurs when partial agenesis is associated with the holoprosencephalies (Chiappedi and Bejor, 2010).

In MRI, the four components of the corpus callosum are best viewed on sagittal imaging, although its relationship to the cerebral hemispheres is best shown on coronal images (Chiappedi and Bejor, 2010). The corpus callosum is a densely packed white matter structure, with high signal on T_1-weighted and low signal in T_2-weighted images after the age of 24 months (Chiappedi and Bejor, 2010). MRI imaging shows that myelination is more advanced in the posterior parts of the corpus callosum when compared to the anterior regions (Chiappedi and Bejor, 2010).

Prenatal diagnosis of complete callosal agenesis is feasible from the midtrimester onward by expert

sonography (Chiappedi and Bejor, 2010). In the axial view, suspicious findings are absent cavum septi pellucid and teardrop configuration of the lateral ventricles with possible ventriculomegaly; the nonvisualization of the corpus callosum at transfontanellar ultrasound in either the sagittal or coronal plane is diagnostic (Chiappedi and Bejor, 2010). More subtle findings, such as hypoplasia and partial ACC, may also be recognized antenatally (Chiappedi and Bejor, 2010). Fetal MRI is worth doing in order to reinforce a difficult sonographic diagnosis and, at the same time, to exclude possible additional cerebral anomalies, which may be overlooked at ultrasound (Chiappedi and Bejor, 2010).

Malformations of the brainstem and cerebellum have been increasingly recognized in patients with malformations of the cerebrum, including callosal anomalies (Barkovich et al., 2009).

31.3.2 L1 Syndrome

The L1 syndrome is a highly variable X-linked neurological disorder (Bertolin et al., 2010; Engle, 2010; Schafer et al., 2010). It results from mutations in the *L1CAM* gene (Bertolin et al., 2010; Engle, 2010; Schafer et al., 2010; Vos et al., 2010). L1 is a transmembrane neural adhesion molecule that 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 (Bertolin et al., 2010; Engle, 2010; Schrander-Stumpel and Vos, 1993).

The L1CAM is one of a subgroup of structurally related integral membrane glycoproteins belonging to a large class of immunoglobulin superfamily cell adhesion molecules that mediate cell-to-cell adhesion at the cell surface (http://www.ncbi.nlm.nih.gov/omim) (Bertolin et al., 2010; Schrander-Stumpel and Vos, 1993; Vos et al., 2010). The various functions of L1CAM include guidance of neurite outgrowth in development, neuronal cell migration, axon bundling, synaptogenesis, myelination, neuronal cell survival, and hippocampal long-term potentiation (http://www.ncbi.nlm.nih.gov/omim) (Bertolin et al., 2010; Schafer et al., 2010; Schrander-Stumpel and Vos, 1993).

L1 has multiple extracellular binding partners, including β1 integrins, neuronal cell adhesion molecule, TAG-1/axonin-1, contactin, Np-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 (Engle, 2010). L1 homophilic binding increases cell adhesion and enhances neuronal migration and neurite outgrowth, whereas binding to Np-1 mediates Sema3A-induced growth cone collapse and axon repulsion (Engle, 2010).

L1 has also multiple intracellular binding partners; L1 links to the actin cytoskeleton through interactions with ankyrin or FERM domain-containing proteins, and the interaction of L1 with adaptor protein 2 is required for sorting of L1 to the axonal growth cone (Engle, 2010).

31.3.2.1 Clinical Characteristics

The L1 syndrome was originally recognized as four distinct entities: X-linked hydrocephalus due to stenosis of the aqueduct of Sylvius, MASA syndrome (mental retardation, aphasia, shuffling gait, adducted thumbs), X-linked complicated spastic paraplegia type I, and X-linked corpus callosum agenesis (Bertolin et al., 2010; Engle, 2010; Vos et al., 2010). On the basis of their genetic homogeneity and phenotypic overlap, these disorders are considered a single entity (Engle, 2010; Schafer et al., 2010).

This X-linked syndrome comprises a broad phenotypic spectrum, including hydrocephalus, mental retardation, aphasia, spastic paraplegia, and adducted thumbs (Engle, 2010; Schafer et al., 2010; Schrander-Stumpel and Vos, 1993).

X-linked hydrocephalus with stenosis of the aqueduct of Sylvius is the most common genetic form of congenital hydrocephalus, with a prevalence of approximately 1 in 30 000 (Schrander-Stumpel and Vos, 1993). This accounts for approximately 5–10% of males with nonsyndromic hydrocephalus (Schrander-Stumpel and Vos, 1993). Hydrocephalus may be present prenatally and result in stillbirth or death in early infancy (Schrander-Stumpel and Vos, 1993). Males with hydrocephalus with stenosis of the aqueduct of Sylvius are born with severe hydrocephalus and adducted thumbs (Schrander-Stumpel and Vos, 1993). Seizures may occur (Schrander-Stumpel and Vos, 1993). In less severely affected males, hydrocephalus may be subclinically present and documented only because of developmental delay (Schrander-Stumpel and Vos, 1993). Mild-to-moderate ventricular enlargement is compatible with long survival (Schrander-Stumpel and Vos, 1993).

In hydrocephalus with stenosis of the aqueduct of Sylvius, intellectual disability is usually severe and is independent of shunting procedures in individuals with severe hydrocephalus (Schrander-Stumpel and Vos, 1993). In MASA syndrome, intellectual disability ranges from mild (IQ of 50–70) to moderate (IQ of 30–50) (Schrander-Stumpel and Vos, 1993).

Boys initially exhibit hypotonia of the legs, which evolves into spasticity during the first years of life (Schrander-Stumpel and Vos, 1993). In adult males, the spasticity tends to be somewhat progressive (Schrander-Stumpel and Vos, 1993). Spasticity usually results in atrophy of the muscles of the legs and contractures that together cause the shuffling gait (Schrander-Stumpel and Vos, 1993).

Carrier females may manifest minor features such as adducted thumbs and/or subnormal intelligence (Schrander-Stumpel and Vos, 1993). Rarely do females manifest the complete L1 syndrome phenotype (Schrander-Stumpel and Vos, 1993).

31.3.2.2 Genetics

The *L1CAM* gene is located on the long arm of X-chromosome (Xq28) and is comprised of 29 exons, the first being noncoding, spanning about 16 kb (Bertolin et al., 2010; Schrander-Stumpel and Vos, 1993; Vos et al., 2010). The L1 syndrome results from missense, nonsense, splice site, and frameshift mutations scattered throughout the exons and intron–exon boundaries of the *L1CAM* gene (Bertolin et al., 2010; Engle, 2010; Schrander-Stumpel and Vos, 1993). Since the known *L1CAM* mutations are scattered over the whole coding region, the entire gene has to be sequenced in order to achieve molecular diagnosis (Bertolin et al., 2010). To date, more than 240 different mutations have been reported in the *L1CAM* gene (Bertolin et al., 2010). Most *L1CAM* mutations are unique to each family, that is, they appear to be private mutations (Vos et al., 2010). Only a few families harbor the same recurrent mutation (Vos et al., 2010).

Missense mutations account for over one-third of pathological L1 mutations described, and those affecting extracellular domains result in more severe clinical consequences than those affecting the cytoplasmic part of L1 (Schafer et al., 2010). Some missense mutations affect structurally important amino acid sites in the extracellular domains, which may cause protein misfolding (Schafer et al., 2010). Such missense mutations interfere with homo- and heterophilic ligand binding, intracellular trafficking, neurite growth, and neurite branching (Bertolin et al., 2010; Engle, 2010; Schafer et al., 2010). Children with a truncating mutation are more likely to die before the age of 3 than children with a missense mutation (Vos et al., 2010).

Axon guidance defects occur with both extra- and intracellular mutations (Engle, 2010). The role of L1CAM in neuronal migration and survival, synaptogenesis, and long-term potentiation may also contribute to the phenotype (Engle, 2010).

L1CAM mutation analysis is offered to patients suspected of having L1 syndrome (Vos et al., 2010). Once a mutation has been established, prenatal testing can be performed in subsequent pregnancies, and carriership testing can be carried out to determine the potential presence of an *L1CAM* mutation in female relatives (Vos et al., 2010).

31.3.2.3 Neuroradiological Findings

Neuroimaging reveals hydrocephalus with or without stenosis of the aqueduct of Sylvius in combination with corpus callosum agenesis/hypogenesis and/or cerebellar hypoplasia, small brainstem, and agenesis of the pyramids (corticospinal tracts) (Engle, 2010; Schrander-Stumpel and Vos, 1993). Bilateral absence of the pyramids detected by MRI is an almost pathognomonic inding (Schrander-Stumpel and Vos, 1993). Aqueductal stenosis is not a constant feature of L1 syndrome (Schrander-Stumpel and Vos, 1993).

L1 syndrome cannot be reliably diagnosed on the basis of prenatal ultrasound only (Schrander-Stumpel and Vos, 1993). A diagnosis of hydrocephalus often requires serial ultrasound examination and cannot be guaranteed before 20–24 weeks of gestation or even the third trimester of pregnancy (Schrander-Stumpel and Vos, 1993). Apparently, normal ultrasound findings in a pregnancy with a priori increased risk are not reliable in ruling out L1 syndrome in the fetus (Schrander-Stumpel and Vos, 1993).

31.3.3 Joubert Syndrome and Related Disorders

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 synkinetic mirror movements (Engle, 2010; Parisi, 2009; Valente et al., 2008). The JS can also cosegregate with retinopathy, kidney disease, liver disease, polydactyly, obesity, and/or situs inversus (Engle, 2010; Valente et al., 2008). This spectrum is now called JS and related disorders (JSRD) (Engle, 2010).

The prevalence of JSRD has been estimated as approximately 1:100 000 in the United States, but this is likely an underestimate given by the broad spectrum of features particularly in those with milder manifestations (Parisi, 2009).

JS and JSRD are 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 (Engle, 2010; Valente et al., 2008). Cilia sense environmental cues and mediate signals through receptor-dependent pathways such as SHH, noncanonical Wnt, and platelet-derived growth factor (PDGF) receptor (Engle, 2010; Parisi, 2009).

The JSRD, similar to many of the disorders considered ciliopathies, shows considerable heterogeneity in clinical features and on a molecular basis (Parisi, 2009; Valente et al., 2008). The clinical features of JSRD are shared by many ciliary disorders and typically involve the renal epithelium, retinal photoreceptor cells, central nervous system, body axis, sensory organs, and others (Parisi, 2009; Valente et al., 2008).

31.3.3.1 Clinical Characteristics

The features necessary for a diagnosis of classic JB include the following: The molar tooth sign on axial views from cranial MRI studies comprised of these three

findings: cerebellar vermis hypoplasia, deepened interpendicular fossa, and thick, elongated superior cerebellar peduncles; intellectual impairment/developmental delay of variable degree; hypotonia in infancy; one or both of the following (not required but supportive of the diagnosis): irregular breathing pattern in infancy (episodic apnea and/or tachypnea, sometimes alternating) and abnormal eye movements (nystagmus and/or oculomotor apraxia (OMA)) (Parisi, 2009; Valente et al., 2008).

Many children with JS exhibit dysmorphic facial features that include a broad forehead, arched eyebrows, eyelid ptosis, wide-spaced eyes, open mouth configuration, and facial hypotonia (Parisi, 2009). Some individuals also have polydactyly of the hands and/or feet, which can take many forms (Parisi, 2009).

The JSRD encompasses classic JS as described previously, as well as conditions with other features such as central nervous system anomalies (including occipital encephalocele, corpus callosal agenesis), ocular coloboma, retinal dystrophy, renal disease (including cystic dysplasia or nephronophthisis (NPHP) (cystic kidney disease), and hepatic fibrosis (Parisi, 2009; Valente et al., 2008). When the ocular and renal systems are involved, the syndromes are sometimes described as cerebello-oculo-renal syndromes (Parisi, 2009; Valente et al., 2008). An association between kidney disease and retinal involvement has been observed, with specific findings of NPHP plus retinal dystrophy known as Senior–Loken syndrome (Parisi, 2009; Valente et al., 2008).

One JSRD is the condition known as COACH syndrome (coloboma, oligophrenia/developmental delay, ataxia, cerebellar vermis hypoplasia, hepatic fibrosis) (Parisi, 2009; Valente et al., 2008). Liver involvement, when coupled with renal cystic disease, has prompted the inclusion of JSRD as a congenital hepatorenal fibrocystic disease (Parisi, 2009; Valente et al., 2008).

Cognitive impairment in JSRD is highly variable, with many children exhibiting moderately severe disability (Parisi, 2009). The average age of independent sitting has been reported to be 19 months, and the average of walking is reported to be 4 years for those who developed these skills (Parisi, 2009).

The clinical features related to the complex hindbrain malformation include ataxia, which typically becomes apparent as children develop ambulation, and ocular, oral-motor, and speech dyspraxia (Parisi, 2009). Some children require assistive devices or use sign language to communicate given expressive language impairment (Parisi, 2009).

Seizures have been reported in some children with JSRD (Parisi, 2009). Some children with JSRD and autistic features have been described (Parisi, 2009). Behavioral problems, typically impulsivity, perseveration, and temper tantrums, appear to be relatively common particularly with increasing age (Parisi, 2009).

There is a broad spectrum of ocular findings in JSRD (Parisi, 2009; Valente et al., 2008). Abnormalities of ocular motility are very common, particularly nystagmus, which can be horizontal, vertical, and/or torsional, and typically has a pendular or sometimes see-saw pattern, and OMA, which is characterized by difficulty in smooth visual tracking, dysconjugate eye movements, and head thrusting to compensate for poor saccade initiation (Parisi, 2009; Valente et al., 2008). Nystagmus and OMA are often present at birth and may improve with age (Parisi, 2009; Valente et al., 2008).

Other common ocular anomalies that may require medical or surgical treatment include strabismus, amblyopia, and ptosis (Parisi, 2009). Third nerve palsy, Duane anomaly (unilateral or bilateral restriction in the ability to move the affected eye outward, and when attempting to move the affected eye inward, it retracts into the orbit, resulting in narrowing of the width of the palpebral fissure) and optic disc drusen have also been observed (Parisi, 2009).

Coloboma, a congenital ocular developmental defect, is present in a subset of individuals with JSRD and typically involves the choroids and retina, but rarely the iris (Parisi, 2009). Many children with unilateral or bilateral colobomas also develop liver disease, as in COACH syndrome, but colobomas are not a necessary feature of this disorder (Parisi, 2009). However, retinal dystrophy is not typical in COACH syndrome, in contrast to other JSRDs (Parisi, 2009).

Kidney disease is relatively common on JSRD, with a prevalence of up to 30% of subjects in early surveys; this estimate may be even higher with long-term follow-up given its age-dependent penetrance (Parisi, 2009; Valente et al., 2008). Two different forms of kidney disease have been described: cystic dysplasia and juvenile NPHP (Parisi, 2009).

Cystic dysplasia may be identified prenatally or congenitally by ultrasound findings of multiple cysts of many different sizes in immature kidneys with fetal lobulations (Parisi, 2009; Valente et al., 2008). This finding is the characteristic of Dekaban-Arima syndrome, a JSRD that includes congenital blindness and occasional encephalocele (Parisi, 2009; Valente et al., 2008).

The other, more common renal disorder in JSRD is juvenile NPHP, characterized by tubulointerstitial nephritis and cysts concentrated at the corticomedullary junction (Parisi, 2009; Valente et al., 2008). Most children present with urine-concentrating defects in the first or second decade of life as manifested by polydipsia, polyuria, anemia, and growth failure, with a rise in serum creatinine around 9 years and progression to the end-stage renal disease by approximately 13 years of age (Parisi, 2009; Valente et al., 2008). Mutations in at least nine ciliary genes have been identified in individuals with NPHP, about 20% of whom have extrarenal

manifestations, including cerebellar malformations, OMA, and retinal dystrophy (Vos et al., 2010). It is possible that the renal disease in JSRD is part of a continuum of findings with the common etiology involving abnormal ciliary proteins leading to tubular dysfunction (Parisi, 2009).

In rare cases, the congenital renal disease in JSRD consists of enlarged kidneys, microscopic cysts distributed throughout the cortex and medulla, and infantile hypertension, similar to the renal disease of autosomal recessive polycystic kidney disease (Parisi, 2009; Valente et al., 2008). Several of these patients have *MKS3* (Meckel–Gruber syndrome Gene 3) mutations similar to those with COACH syndrome (Parisi, 2009).

Hepatic involvement in JSRD is likely underreported, as manifestations of liver disease are usually not apparent at birth (Parisi, 2009). However, since current management guidelines recommend routine screening for liver dysfunction in all children with JSRD, hepatic involvement is being identified presymptomatically (Parisi, 2009). The liver disease in COACH syndrome has demonstrated variable progression (Parisi, 2009; Valente et al., 2008). Some JSRD/COACH patients present with evidence of portal hypertension, including hematemesis, esophageal varices or portosystematic shunting, and occasionally life-threatening bleeding events (Parisi, 2009; Valente et al., 2008); others present with elevated or fluctuating levels of serum transaminases (ALT or AST) or gamma-glutamyl transferase (Parisi, 2009). Physical examination findings may include hepatomegaly with or without splenomegaly (Parisi, 2009).

The skeletal findings in JSRD include cone-shaped epiphyses and polydactyly (Parisi, 2009). Cone-shaped epiphyses have been most observed in children with Mainzer–Saldino syndrome (cerebellar ataxia with NPHP and retinal dystrophy) (Parisi, 2009). Polydactyly is often postaxial, although preaxial polydactyly of the hands or great toes has been observed (Parisi, 2009). Mesaxial polydactyly has been described in individuals with the oral-facial-digital type VI syndrome, a JSRD with oral frenulae, lingual tumors or hamartomas, and craniofacial findings that include wide-spaced eyes and a midline lip groove (Parisi, 2009). With age, some children with JSRD develop scoliosis related to abnormal tone (Parisi, 2009).

Endocrine abnormalities are not uncommon in JSRD, and some children exhibit pituitary hormone dysfunction such as isolated growth hormone or thyroid hormone deficiency, or even more extensive panhypopituitarism, with some males demonstrating micropenis (Parisi, 2009).

The vast majority of infants and children diagnosed with JSRD survive the neonatal period and many demonstrate improvement with time in their tone, respiratory function, and feeding behaviors (Parisi, 2009). Because of the risk of later development of retinal, renal, and hepatic complications, ongoing monitoring is essential (Parisi, 2009).

31.3.3.2 Genetics

The JSRD is genetically heterogeneous, and at least nine loci and eight genes *INPP5E* (inositol polyphosphate-5-phosphatase), *AHI1* (Abelson helper integration site 1), *NPHP1*, *CEP290* (centrosomal protein 290 kDa), *TMEM67* (transmembrane protein 67), *RPGRIP1L* (RPGR-interacting protein-1-like protein), *ARL13B* (ADP-ribosylation factorlike 13B), and *CC2D2A* (coiled-coil and C2 domain containing 2A) have been identified (Engle, 2010).

The *INPP5E* gene was identified as causative, and the retinal phenotype is predominant (Parisi, 2009). Also, it is possible that this gene represents another COACH gene (Parisi, 2009). This gene encodes an inositol polyphosphatase-5-phosphatase E necessary for cilia stability and indicates a link between phosphotidylinoditol signaling and ciliary function (Parisi, 2009).

Mutations in the 29-exon gene, *AHI1*, have been identified in JSRD (Valente et al., 2008). This gene encodes a protein of unknown function named jouberin, containing several protein–protein interaction domains (Valente et al., 2008). The most common clinical association in *AHI1*-related JSRD is retinal dystrophy, occurring in ~80% of those with mutations (Parisi, 2009; Valente et al., 2008). Renal disease has been observed in some subjects (Parisi, 2009). However, no subjects with *AHI1* mutations have had features of encephalocele, polydactyly, or liver fibrosis (Parisi, 2009). Other central nervous system anomalies, including PMG, corpus callosum anomalies, and frontal lobe atrophy, have been described in some individuals with *AHI1* mutations (Parisi, 2009). Single heterozygous mutations in the *AHI1* gene have been identified in a number of JSRD patients within the reported screenings (Valente et al., 2008). The vast majority of identified mutations are nonsense (truncating, frameshift, or splice-site mutations) that cluster mainly in the first half of the gene (exons 7–16) and in exons encoding the functional domains (Valente et al., 2008). Missense mutations are rare and are predicted to affect amino acid residues that are crucial for the correct functioning of such domains (Valente et al., 2008). No correlation can be drawn between the type or site of mutations and the associated phenotype (Valente et al., 2008).

The first gene associated with JSRD, the 30-exon *NPHP1* gene, was identified as causing juvenile NPHP (Parisi, 2009). *NPHP* resides within a ~290 kb region of genomic DNA flanked by large inverted repeat elements on chromosome 2q13 that is homozygously deleted in JSRD or NPHP; a few individuals are

compound heterozygotes for a deletion and a point mutation in *NPHP1* (Parisi, 2009; Valente et al., 2008). The *NPHP1* mutation detection rate for the purely renal disorder is ~20–30%, whereas the mutation rate is only about 1–3% in individuals with JSRD (Parisi, 2009). Some individuals with the common deletion have congenital OMA known as Cogan syndrome, and others have Senior–Loken syndrome with retinal impairment, but in general, the neurologic symptoms tend to be milder than in many children with JSRD (Parisi, 2009; Valente et al., 2008).

The large, 54-exon *CEP290* gene, mapping to the long arm of chromosome 12, has been associated with multiple clinical disorders ranging from isolated Leber congenital amaurosis to JSRD, MKS, and BBS (Parisi, 2009; Valente et al., 2008). Most subjects with JSRD due to *CEP290* mutations have retinal dystrophy or congenital blindness, and many also develop renal disease consistent with NPHP or renal cortical cysts (Parisi, 2009; Valente et al., 2008). Findings in some affected individuals have included ocular colobomas, encephaloceles, septal heart defects, hepatic disease, and situs anomalies (Parisi, 2009). The most frequent *CEP290* mutation, and the one that holds the strongest genotype–phenotype correlation, is the intronic mutation c.2991 + 1655>G that creates a splice-donor site and inserts a cryptic exon in the *CEP290* messenger RNA between exons 26 and 27, introducing a stop codon immediately downstream of exon 26 (Valente et al., 2008).

The 28-exon *TMEM67/MKS3* gene, mapping to chromosome 8q22, encodes a 995-amino acid protein called meckelin, which plays a role in primary cilium formation and interacts with other known ciliary proteins (Parisi, 2009; Valente et al., 2008). Originally identified as a causative for MKS, mutations in this gene have been reported in JSRD (Parisi, 2009; Valente et al., 2008). The *MKS3* mutations identified in MKS are typically compound heterozygous missense and truncating mutations or homozygous splice-site mutations that are found across the whole gene length, whereas the disease-associated mutations in JSRD/COACH tend to be missense mutations or the combination of a missense mutation and a splice-site or nonsense mutation, with very few mutations overlapping with those seen in MKS (Parisi, 2009; Valente et al., 2008).

Mutations in the *RPGRIP1L* gene were first identified in patients with the renal form of JSRD (Parisi, 2009). This gene has 26 exons encoding for a 1315 amino acid protein containing several coiled-coil domains, required for protein–protein interactions (Valente et al., 2008). The phenotype spectrum includes renal disease with some affected individuals manifesting occipital encephaloceles and polydactyly, and rarely, retinal disease or colobomas; a few have scoliosis, clubfoot, or pituitary hormone deficiency (Parisi, 2009). Hepatic fibrosis and COACH syndrome have been described in individuals with JSRD due to *RPGRIP1L* (Parisi, 2009). Overall, estimates of the prevalence of *RPGRIP1L* mutations in the cerebello-renal form of JSDR range from ~9 to 12% (Parisi, 2009).

ARL13B, a 10-exon gene, encodes a protein that is a member of the Ras GTPase family and localizes to the primary cilia of cerebellar neurons, kidney, and retina (Parisi, 2009).

The phenotype in patients with mutations in the 38-exon *CC2D2A* gene has ranged from classic JS to JS with encephalocele to the COACH phenotype with coloboma, liver, and kidney involvement (Parisi, 2009). *CC2D2A* has been shown to interact with *CEP290* (Parisi, 2009). This gene is estimated to cause almost 10% of JSRD (Parisi, 2009).

The genes mentioned previously account for an estimated 50% of causative mutations in JSRD (Parisi, 2009). The same JSRD gene can cause multiple different phenotypes, and several different genes can be associated with the same clinical features (Parisi, 2009; Valente et al., 2008). In addition, clinical features can vary between affected siblings within the same family (Parisi, 2009). This intrafamilial variability supports the existence of genetic modifiers and epistatic effects (Parisi, 2009; Valente et al., 2008). Commercial clinical testing is available for many of the JSRD genes (Parisi, 2009). Additional JSRD genes remain to be identified (Parisi, 2009).

The gene products associated with JSRD are known to localize to the primary cilium and/or basal body and the centrosome apparatus, which has been identified in almost all cell types, and many of these proteins are important for the structure, function, and/or stability of this organelle and its related structures (Parisi, 2009; Valente et al., 2008).

Primary cilia play a role in intraflagellar transport, cell division, tissue differentiation, establishment of body axis, growth, and mechanosensation involved in cellular signaling processes and are essential for signal transduction processes that underlie many aspects of vertebrate development and morphogenesis, including the SHH, Wnt/β-catenin, PDGF receptor alpha signaling pathways, Ras-GTP, and phosphotidylinositol signaling (Parisi, 2009; Valente et al., 2008).

In the developing brain, primary cilia have been involved in regulating some of the most powerful pathways active in the early embryo, such as those of Wnt and Sonic hedgehog (Valente et al., 2008). It appears that cilia can function as a sort of cellular sensor, picking up environmental signals and transducing them to the nucleus, regulating cell cycle and proliferation (Valente et al., 2008).

Also, the proper cell polarity and orientation of tubular structures in tissues require normal ciliary function

(Parisi, 2009). The expanding group of human disorders known as ciliopathies shares overlapping clinical manifestations that reflect the critical role cilia play in the growth and differentiation of various tissues (Parisi, 2009).

31.3.3.3 Neuroradiological Findings

The diagnosis of JSRD is dependent on the presence in MRI of the 'molar tooth' sign, a toothlike 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 superior cerebellar peduncle (SCP) (Engle, 2010; Parisi, 2009; Valente et al., 2008). When a diagnosis of JSRD is suspected, a detailed cranial MRI to evaluate the molar tooth sign is essential (Parisi, 2009).

The peculiar neuroradiological malformations seen in JS have their correspondence at the neuropathological level, with various abnormalities of the midbrain and hindbrain being consistently identified in brains from JS patients (Valente et al., 2008). Postmortem studies of individuals with genetically undefined JS have revealed severe cerebellar vermian hypoplasia or absence, with midline clefting, dysplasia of the deep cerebellar and inferior olivary nuclei, elongation of the caudal midbrain tegmentum, reduction in pontine neurons, hypoplasia of the solitary, trigeminal and dorsal column nuclei and tracts, and occasional heterotopia of Purkinje-like neurons (Engle, 2010; Valente et al., 2008).

Approximately 10% of individuals with JSRD demonstrate fluid collections in the posterior fossa resembling the Dandy–Walker malformation (agenesis of the cerebellar vermis, cystic dilation of the fourth ventricle, and enlargement of the posterior fossa (Parisi, 2009). Although hydrocephalus is uncommon in JSRD, rare patients have required a shunt for symptomatic elevations of intracranial pressure (Parisi, 2009).

In addition, occipital encephaloceles or meningoceles have been observed, suggesting overlap with Meckel syndrome, a typical prenatal or perinatal lethal ciliopathy characterized by brain anomalies (especially encephalocele), cystic renal dysplasia, and the hepatic ductal plate malformation (Parisi, 2009). Other brain anomalies in JSRD have included PMG, ACC, and cerebellar heterotopias (Parisi, 2009).

Prenatal imaging via ultrasound and/or fetal MRI is the best and most practical diagnostic option (Parisi, 2009). Extracranial anomalies such as polydactyly or renal cysts and major structural brain malformations such as encephalocele may facilitate prenatal diagnosis of JSRD as early as the first trimester or may suggest the diagnosis (Parisi, 2009). Early diagnosis is more difficult when extracranial findings are not present and because the molar tooth sign has not been reported before 27 weeks of gestation (Parisi, 2009).

31.3.4 Horizontal Gaze Palsy with Progressive Scoliosis

Horizontal gaze palsy with progressive scoliosis (HGPPS) is a rare, clinically and genetically homogeneous disorder in which hindbrain axons fail to cross the midline (Amouri et al., 2009; Avadhani et al., 2010; Bomfim et al., 2009; Engle, 2010). This disorder is characterized by congenital absence of conjugate horizontal eye movements and preservation of vertical gaze and convergence, which is associated with progressive scoliosis developing in childhood and adolescence (Amouri et al., 2009; Avadhani et al., 2010; Bomfim et al., 2009; Otaduy et al., 2009).

31.3.4.1 Clinical Characteristics

Clinical findings characteristic of HGPPS include absence of all horizontal gaze reflexes, conjugate pendular nystagmus, and progressive scoliosis (Bomfim et al., 2009; Engle, 2010; Otaduy et al., 2009). Affected individuals are born with restricted horizontal gaze and develop scoliosis within the first decade of life (Engle, 2010; Otaduy et al., 2009).

The gaze palsy may result from errors in axon connectivity into and out of the abducens nucleus (Bomfim et al., 2009; Engle, 2010). 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 (Bomfim et al., 2009; Engle, 2010). 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 (Bomfim et al., 2009; Engle, 2010).

Although the etiology of scoliosis is also speculative, HGPPS provides the first genetic evidence of a neurogenic cause for this disability (Bomfim et al., 2009; Engle, 2010). It has been suggested that the pathogenesis of progressive idiopathic scoliosis involves a primary neurological dysfunction involving the proprioceptive inputs mediated by the posterior column pathways of the spinal cord and medial lemniscus (Bomfim et al., 2009).

Individuals with HGPPS perform normally on neuropsychological testing and have normal fine motor control without mirror movements, suggesting that the pathologically ipsilateral corticospinal axons find their appropriate target, albeit on the wrong side (Engle, 2010).

The differential diagnosis of HGPPS embraces several genetic disorders of eye movement, such as Duane retraction syndrome (DRS), Möbius syndrome, and others (Bomfim et al., 2009; Otaduy et al., 2009). Clinical and

neuroimaging findings can differentiate these entities from HGPPS (Bomfim et al., 2009).

31.3.4.2 Genetics

HGPPS is an autosomal recessive trait and results from mutations in the *ROBO3* gene (http://www.ncbi.nlm.nih.gov/omim) (Amouri et al., 2009; Avadhani et al., 2010; Bomfim et al., 2009; Engle, 2010; Otaduy et al., 2009). *ROBO3* encodes a transmembrane receptor, and it is a divergent member of the Robo family of axon guidance molecules (Amouri et al., 2009; Engle, 2010; Otaduy et al., 2009). Robo 3 is essential for midline crossing of hindbrain and spinal cord commissural and precerebellar axons (Avadhani et al., 2010; Engle, 2010). Robo 3 is also necessary for midline crossing of precerebellar neurons, and defects in neuronal migration may also contribute to the HGPPS phenotype (Amouri et al., 2009; Avadhani et al., 2010; Engle, 2010).

ROBO3 alternative splicing produces two functionally antagonistic isoforms with distinct carboxy termini (Engle, 2010). *ROBO3.1* inhibits the responsiveness of commissural axons to Slit repellents and is present on commissural axons before and during midline crossing, whereas *ROBO3.3* is Slit responsive and appears on the growth cone postcrossing to block the recrossing (Engle, 2010). HGPPS mutations reported to date alter nucleotides common to both isoforms (Engle, 2010).

Indistinguishable phenotypes result from *ROBO3* nonsense, frameshift, splice-site, or missense mutations spread across the gene, supporting a complete loss of *ROBO3* function (Engle, 2010). Over ten different mutations located in different domains of the encoded protein have been identified and are thought to diminish the function of this receptor (Amouri et al., 2009).

31.3.4.3 Neuroradiological Findings

Electrophysiological and neuroimaging studies in HGPPS support the absence of decussating axons in the pons and medulla (Engle, 2010). MRI reveals ventral flattening and hypoplasia of the hindbrain, absence of facial colliculi, and a butterfly-shaped medulla with a midline pontine cleft (Avadhani et al., 2010; Bomfim et al., 2009; Engle, 2010; Otaduy et al., 2009). The unusual appearance of the medulla and abnormal functional results suggest that sensorimotor projections do not cross the midline in HGPPS (Amouri et al., 2009). Functional MRI reveals ipsilateral rather than the normal contralateral activation in the primary motor cortex following motor tasks (Engle, 2010).

The split pons sign is attributable to abnormal development of the abducens nuclei and medial longitudinal fasciculus occurring between 5 and 8 weeks of gestation (Bomfim et al., 2009). Hypoplasia of the medial lemniscus, which is located posterior to the pyramids, is thought to explain why the inferior olivary nuclei are unusually more prominent than the pyramids (Bomfim et al., 2009). The deep midline cleft along the ventral aspect of the medulla oblongata has been described as the result of uncrossed corticospinal tracts (Bomfim et al., 2009).

DTI, with its ability to demonstrate white matter tracts, is a very suitable technique to further evaluate the abnormalities underlying this disease (Otaduy et al., 2009). Previous DTI studies have described the absences of superior cerebellar and pyramidal decussations, major pontine fibers, and decussation of the superior cerebellar peduncles, with fMRI combined study confirming the ipsilateral sensorimotor findings (Avadhani et al., 2010; Engle, 2010; Otaduy et al., 2009). The cortex, corpus callosum, and exiting cranial nerves appear structurally normal (Engle, 2010).

31.3.5 Kallmann Syndrome

Individuals with Kallman syndrome (KS) have congenital anosmia and hypogonadotropic hypogonadism (HH) (http://www.ncbi.nlm.nih.gov/omim) (Engle, 2010; Fechner et al., 2008; Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). Anosmia is related to the absence or hypoplasia of the olfactory bulb and tracts (Hardelin and Dode, 2008). Hypogonadism is due to gonadotropin-releasing hormone (GnRH) deficiency, which presumably results from a failure of the embryonic migration of neuroendocrine GnRH cells from the olfactory epithelium to the forebrain (Engle, 2010; Fechner et al., 2008; Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). This failure could be a consequence of the early degeneration of olfactory nerve and terminal nerve fibers, because the latter normally act as guiding cues for the migration of GnRH cells (Engle, 2010; Hardelin and Dode, 2008). Defects in GnRH cell fate specification, differentiation, axon elongation, or axon targeting to the hypothalamus median eminence may, however, also contribute to GnRH deficiency, at least in some genetic forms of the disease (Hardelin and Dode, 2008). Both olfactory sensory neurons and GnRH are born in the olfactory placode of the developing nose (Engle, 2010; Fechner et al., 2008). It is proposed that errors in growth and guidance of olfactory axons can result in KS (Engle, 2010).

The prevalence of the disease has been estimated at 1 out of 8000 in boys (Hardelin and Dode, 2008). In girls, the prevalence might be five times lower (Hardelin and Dode, 2008).

31.3.5.1 Clinical Characteristics

Transmitting females have partial or complete anosmia (http://www.ncbi.nlm.nih.gov/omim). Often, the lack of smell goes unnoticed, and individuals with KS

are not diagnosed until they fail to undergo secondary sexual development during the teenage years (Engle, 2010; Fechner et al., 2008; Kaplan et al., 2010; Kim et al., 2008). The KS may also be suspected as early as in infancy in boys, in the presence of cryptorchidism or a micropenis, combined with subnormal LH (luteinizing hormone) and FSH (follicle-stimulating hormone) concentrations (Hardelin and Dode, 2008; Kaplan et al., 2010). Microphallus has been noted in up to 65% of individuals with KS, and cryptorchidism has been reported in up to 73% of males with KS (Kaplan et al., 2010). The postnatal surge in FSH, LH, and testosterone in the male infant, as a consequence of the continued function of the fetal GnRH pulse generator, provides a 6-month window of opportunity to establish the diagnosis of HH (Hardelin and Dode, 2008).

Other frequently occurring features in this syndrome include characteristic face and hand dysmorphia, hypotonia, arhinencephaly, semicircular canal agenesis or hypoplasia, deafness, urinary tract anomalies, orofacial clefting, dysphagia, and tracheo-esophagial anomalies (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008).

The renal abnormality most frequently associated with KS is unilateral renal aplasia, but other anomalies such as renal diverticulum, horseshoe kidney, malrotated kidney, multicystic dysplastic kidney, and vesicoureteral reflux have been reported (Kaplan et al., 2010; Kim et al., 2008). Unilateral renal aplasia is more often right sided in KS patients, while renal agenesis in individuals without KS is more likely to be left sided (Kaplan et al., 2010). All patients with suspected KS should have a renal ultrasound to rule out a renal anomaly (Kaplan et al., 2010; Kim et al., 2008).

Cleft lip ± palate has been noted in up to 13–14% of individuals with KS (Kaplan et al., 2010; Kim et al., 2008). The incidence of clefting in KS patients is significant, as the incidence of cleft lip ± palate in the general population is only 0.1–0.2% (Kaplan et al., 2010). In addition to cleft lip ± palate, cleft palate alone and dental agenesis have been reported in several patients with KS (Kaplan et al., 2010).

The incidence of hearing loss in patients with KS has been reported to be as high as 28% (Kaplan et al., 2010; Kim et al., 2008). Both sensorineural and conductive hearing loss have been described (Kaplan et al., 2010). Hearing loss is commonly unilateral (Kaplan et al., 2010). Individuals in whom KS is suspected should undergo formal auditory evaluation (Kaplan et al., 2010). Abnormalities of the inner ear have been noted on CT scan (Kaplan et al., 2010).

Other less-common findings associated with KS include musculoskeletal anomalies, such as clinodactyly, camptodactyly, and fusion of the fourth and fifth metacarpal bones; oculomotor anomalies, such as ptosis, iris coloboma, and nystagmus; high-arched palate, unilateral nasal cartilage agenesis; malrotation of the gut; visual attention defects; and cardiac defects (Kaplan et al., 2010; Kim et al., 2008). Cardiac defects reported include atrial septal defect, ventricular septal defect, right-sided aortic arch, double-outlet right ventricle, transposition of the great arteries, and arrhythmias (Kaplan et al., 2010). X-linked ichthyosis, mental retardation, chondrodysplasia punctata, and short stature can also occur, usually as part of a contiguous gene syndrome (Kim et al., 2008).

Studies of X-linked Kallmann syndrome have found instances of renal agenesis and also pointed to mirror movements of the hands (synkinesia), pes cavus, high-arched palate, and cerebellar ataxia (http://www.ncbi.nlm.nih.gov/omim) (Fechner et al., 2008; Kim et al., 2008).

The KS is diagnosed when low-serum gonadotropins and gonadal steroids are coupled with a compromised sense of smell (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008). The latter should be ascertained by the means of detailed questioning and olfactory screening tests (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008).

The treatment of KS is that of the hypogonadism (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008). It aims first to initiate virilization or breast development and second to develop fertility (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008). Hormone replacement therapy, with testosterone for males and combined estrogen and progesterone for females, is the treatment to stimulate the development of secondary sexual characteristics (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008).

31.3.5.2 Genetics

The KS is genetically heterogeneous (Engle, 2010). Most KS patients present as sporadic cases (two thirds of cases), but many cases are clearly familial and can be inherited as an X-linked, autosomal dominant, and autosomal recessive trait (Engle, 2010; Fechner et al., 2008; Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). In the autosomal dominant form, incomplete penetrance has been emphasized, which makes it difficult to identify affected patients (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008). Overall, the autosomal dominant mode of inheritance seems to account for 86% of inherited cases of KS (Fechner et al., 2008).

Six genes have been reported, accounting for approximately 30% of cases: *KAL1* (Kallmann syndrome 1 sequence), *FGFR1* (fibroblast growth factor receptor 1), *PROK2* (prokineticin 2), *PROKR2* (prokineticin receptor 2), *FGF8* (fibroblast growth factor 8), and *CHD7* (chromodomain helicase DNA-binding protein-7) (Engle, 2010;

Kaplan et al., 2010; Kim et al., 2008). These KS genes encode transmembrane receptors and ligands that may be important for growth cone guidance (Engle, 2010). Some KS proteins also interact with one another and with heparan sulfate proteoglycans to amplify downstream signaling pathways (Engle, 2010).

The KS can be oligogenic, resulting from combinations of mutations in more than one KS gene (Engle, 2010; Hardelin and Dode, 2008; Kim et al., 2008). It is possible that oligogenic inheritance accounts in part for the long recognized incomplete penetrance of the disease, at least in some cases (Hardelin and Dode, 2008; Kim et al., 2008).

X-linked KS is caused by loss-of-function mutations in *KAL1*, which is expressed in developing olfactory bulb (http://www.ncbi.nlm.nih.gov/omim) (Engle, 2010; Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). Two-thirds of males harboring *KAL1* mutations also have mirror movements and enlarged, aberrant ipsilateral CSTs, supporting a role of *KAL1* in the guidance of CST and olfactory axons (http://www.ncbi.nlm.nih.gov/omim) (Engle, 2010; Hardelin and Dode, 2008; Kaplan et al., 2010).

The *KAL1* gene is found in the pseudoautosomal region of the X-chromosome and encodes the secreted glycoprotein anosmin-1, which has cell adhesion, neurite outgrowth, and axon guidance and branch-promoting activities (http://www.ncbi.nlm.nih.gov/omim) (Engle, 2010; Fechner et al., 2008; Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). Anosmin-1 requires heparin sulfate for its functions (Kim et al., 2008). Anosmin is required for the normal migration of olfactory and GnRh neurons from the olfactory placode to the hypothalamus (Fechner et al., 2008; Kim et al., 2008).

The vast majority of *KAL1* mutations reported so far are nonsense mutations, frameshift mutations, or large gene deletions, which are expected to inactivate protein synthesis, and are apparently sufficient to produce the abnormal phenotype in males (Fechner et al., 2008; Hardelin and Dode, 2008; Kim et al., 2008). The *KAL1* gene is also expressed in developing Purkinje cells of the cerebellum, meso- and metanephros, oculomotor nucleus, and facial mesenchyme (Fechner et al., 2008).

Mutations in *FGFR1*, also known as *KAL2*, underlie an autosomal dominant form of the disease (Fechner et al., 2008; Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). This gene is localized on chromosome 8p11.2–p12 (Fechner et al., 2008; Kim et al., 2008). *FGFR1* is a member of the receptor tyrosine kinase superfamily (Hardelin and Dode, 2008). FGF signaling controls cell proliferation, migration, differentiation, and survival and thus, plays essential roles in various processes of embryonic development (Hardelin and Dode, 2008; Kim et al., 2008). Mutations in this gene are associated with failed morphogenesis of the olfactory bulbs (Fechner et al., 2008). Mutations of *KAL1* and *KAL2* account for less than 20% of clinical cases (Kim et al., 2008). Anosmin-1 and *FGFR1* are involved in the same cellular signaling pathway (Kim et al., 2008).

Mutations in *PROKR2* and *PROK2* have been found in heterozygous, homozygous, or compound heterozygous states (Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). Most of these mutations are missense mutations, and many are also present in apparently unaffected individuals (Hardelin and Dode, 2008). For most of the mutations, however, deleterious effects on prokineticin signaling have been shown (Hardelin and Dode, 2008). These two genes are likely to be involved in both monogenic recessive and digenic or oligogenic KS transmission modes (Hardelin and Dode, 2008). *PROKR2* belongs to the G-protein-coupled seven transmembrane domain receptor family (Hardelin and Dode, 2008; Kim et al., 2008).

Other patients carrying heterozygous mutations in *PROKR2*, *PROK2*, or hypomorphic mutations in *KAL1* are expected to carry additional mutations in other, as yet unknown, KS genes (Hardelin and Dode, 2008). For each genetic form of KS identified so far, the clinical heterogeneity of the disease within affected families clearly indicates that the manifestation of KS phenotypes is dependent on factors other than the mutated gene itself, probably factors such as modifier genes and epigenetic factors (Hardelin and Dode, 2008; Kim et al., 2008).

Some of the possible modifier genes and candidate genes that may account for the remaining KS cases are the nasal embryonic LHRH factor (*NELF*), *CHD7*, and early B-cell factor 2 (Kim et al., 2008). *NELF* has been shown to serve as a common guidance molecule for the olfactory axon and GnRH neurons across the nasal region during mouse embryonic development (Kim et al., 2008). *CHD7* is the only known locus associated with CHARGE syndrome (Kim et al., 2008). CHARGE syndrome is a developmental disorder defined by iris coloboma, congenital heart disease, choanal atresia, mental and growth retardation, genital hypoplasia, and ear malformations and/or deafness; it sometimes includes HH associated with a defective sense of smell and abnormal olfactory bulbs development (Kim et al., 2008). *EBF* genes encode a family of transcription factors, and they have been implicated in various neural developmental processes. Their function includes axon navigation and migration (Kim et al., 2008).

A greater variability in the degree of hypogonadism has been observed in patients carrying mutations in *FGFR1*, *FGF8*, *PROKR2*, or *PROK2* than in *KAL1* patients (Hardelin and Dode, 2008). Unilateral renal agenesis occurs in approximately 30% of *KAL1* patients but has not been reported in patients with *FGFR1*, *FGF8*, *PROKR2*, or *PROK2* mutations (Hardelin and Dode, 2008; Kaplan

et al., 2010). If a renal anomaly is present, FISH analysis and/or sequencing of *KAL1* should be the first line genetic test (Kaplan et al., 2010).

Tooth agenesis and hearing impairment are common to several genetic forms of KS, although the mechanism of the hearing impairment could vary between different genetic forms (Hardelin and Dode, 2008; Kaplan et al., 2010). Palate defects should also be considered as one of these shared traits, even though the severity differs between *KAL1* and other genetic forms (Hardelin and Dode, 2008; Kaplan et al., 2010; Kim et al., 2008). Clefting has been associated with mutations in *FGFR1*, *FGF8*, and *CHD7* but not with *KAL1*, *PROK2*, or *PROKR2* (Kaplan et al., 2010). Microphallus and cryptorchidism are more common in patients with *KAL1* mutations versus *FGFR1* mutations (Kaplan et al., 2010). Hearing loss has been reported in patients with *KAL1*, *FGFR1*, *FGF8*, *PKOKR2*, and *CHD7* mutations (Kaplan et al., 2010).

31.3.5.3 *Neuroradiological Findings*

MRI of the forebrain can be carried out to show hypoplasia or aplasia of the olfactory bulbs and tracts (Fechner et al., 2008; Hardelin and Dode, 2008).

A hypoplastic olfactory bulb seen on cerebral MRI does not always correlate with the degree of olfactory deficit, and normal images can also be found in KS (Kim et al., 2008).

31.3.6 Albinism

Albinism is an autosomal recessive inherited condition present at birth (Renugadevi et al., 2010; Summers, 2009). The phenotype ranges from a complete lack of pigmentation in the skin, hair, and iris, called oculocutaneous albinism (OCA), or a lack of pigmentation in the iris alone, termed ocular albinism (OA) (Gronskov et al., 2007; Renugadevi et al., 2010; Summers, 2009). Several defects can cause albinism, including a complete lack of melanocytes or few pigment cells, interference in the migration of cells to their proper location during embryo development, and failure of the cells to produce melanin because of a lack of tyrosinase or abnormalities within the cells (Renugadevi et al., 2010; Summers, 2009).

The prevalence of albinism in the United States is estimated to be 1 in 18 000 (Summers, 2009). Albinism can affect people of all ethnic backgrounds (Gronskov et al., 2007). Prevalence of the different forms of albinism varies considerably worldwide, partly explained by the different founder mutations in different genes and the fact that it can be difficult clinically to distinguish between the different subtypes of albinism among the large normal spectrum of pigmentation (Gronskov et al., 2007).

31.3.6.1 *Clinical Characteristics*

OCA is a group of four autosomal recessive disorders caused by either a complete lack or a reduction of melanin biosynthesis in the melanocytes resulting in hypopigmentation of the hair, skin, and eyes (Gronskov et al., 2007; Summers, 2009). Reduction of melanin in the eyes results in reduced visual acuity caused by foveal hypoplasia and misrouting of the optic nerve fibers (Gronskov et al., 2007; Summers, 2009). The clinical spectrum of OCA varies, with OCA1A being the most severe type characterized by a complete lack of melanin production throughout life, while the milder forms OCA1B, OCA2, OCA3, and OCA4 show some pigment accumulation over time (Gronskov et al., 2007). The different types of OCA are caused by mutations in different genes, but the clinical phenotype is not always distinguishable, making molecular diagnosis a useful tool and essential for genetic counseling (Gronskov et al., 2007).

All types of OCA and OA have similar findings, including various degrees of congenital nystagmus, hypopigmentation of the iris leading to iris translucency, reduced pigmentation of the retinal pigment epithelium, foveal hypoplasia, reduced visual acuity and refractive errors, and sometimes a degree of color vision impairment (Gronskov et al., 2007; Renugadevi et al., 2010; Summers, 2009). Photophobia may be prominent (Gronskov et al., 2007; Renugadevi et al., 2010). A characteristic finding is misrouting of the optic nerves, consisting of an excessive crossing of the fibers in the optic chiasma, which can result in strabismus and reduced stereoscopic vision (Gronskov et al., 2007; Summers, 2009). Absence of misrouting excludes the diagnosis of albinism (Gronskov et al., 2007).

A few patients with albinism who have vision 20/50 or better have some rudimentary foveal development, and some thinning of the retina in the foveal area has been demonstrated with optical coherence tomography (Summers, 2009). Careful inspection can show granular melanin pigment in the macula in a few patients with albinism and occasionally finely granular pigment beyond the macula (Summers, 2009). The presence of melanin pigment in the macula correlates with better visual acuity (Summers, 2009). Recognition of visual acuity among persons with albinism varies from 20/20 to 20/400 but is commonly close to 20/80 (Gronskov et al., 2007; Summers, 2009).

Nystagmus typically develops by 6–8 weeks of age (Summers, 2009). Nystagmus is initially slow and has a large amplitude, but the amplitude typically decreases within the first year of life (Summers, 2009). Delayed visual maturation has been reported in albinism (Summers, 2009). Parents with an infant with albinism have noted poor fixation on faces and objects and a delay in visual development (Summers, 2009).

Pattern visual-evoked potentials performed with monocular visual stimulation demonstrate the excessive retinostriate decussation that is characteristic of albinism (Gronskov et al., 2007; Summers, 2009). In individuals with a questionable phenotype for albinism, the visual-evoked potentials can be useful in identifying those with the disorder (Summers, 2009). This abnormal decussation may account for absent stereoacuity that is often found in albinism (Summers, 2009).

Disorders in which albinism is part of a larger syndrome include Hermansky–Pudlak syndrome (HPS), Chediak–Higashi syndrome, Griscelli syndrome, and Waardenburg syndrome type 2 (WS2) (Gronskov et al., 2007; Summers, 2009). All, except WS2, are inherited as autosomal recessive traits and can be distinguished on the basis of clinical and biochemical criteria (Gronskov et al., 2007). Also, an association of hypopigmentation in Prader–Willi syndrome and Angelman disease with a deletion on 15q11 has been found (Gronskov et al., 2007).

Lifespan in patients with OCA is not limited, and medical problems are generally not increased compared to those in the general population (Gronskov et al., 2007). Skin cancers may occur, and regular skin checks should be offered (Gronskov et al., 2007). Development and intelligence are normal (Gronskov et al., 2007).

31.3.6.2 Genetics

The OCA is a group of congenital heterogeneous disorders of melanin biosynthesis in the melanocytes (Gronskov et al., 2007). At least four genes are responsible for different types of OCA (Gronskov et al., 2007; Summers, 2009). The current classification of albinism is determined by the affected gene (Summers, 2009). Most patients are compound heterozygotes (Gronskov et al., 2007). Siblings with albinism can show variable expression in visual function and clinical phenotype, suggesting that other genes modify the classical phenotype (Summers, 2009).

The OCA1 is caused by mutations in the tyrosinase gene (*TYR*) on chromosome 11q14–21, encoding the enzyme tyrosinase that catalyzes rate-limiting steps in the melanin biosynthetic pathway (Engle, 2010; Gronskov et al., 2007; Renugadevi et al., 2010; Summers, 2009). Mutations completely abolishing tyrosinase activity result in OCA1A, while mutations rending some enzyme activity result in OCA1B, allowing some accumulation of melanin pigment over time (Engle, 2010; Gronskov et al., 2007; Summers, 2009). Almost 200 mutations in *TYR* are known (Gronskov et al., 2007).

Mutations in the *OCA2* gene (formerly known as the P-gene) cause the OCA2 phenotype (Gronskov et al., 2007; Renugadevi et al., 2010; Summers, 2009). This gene maps to chromosome 15q11.2 and encodes an integral melanosomal protein (Engle, 2010; Gronskov et al., 2007; Summers, 2009). OCA2 protein is important for normal biogenesis of melanosomes and for normal processing and transport of melanosomal proteins such as TYR and tyrosinase-related protein1 (TYRP1) (Gronskov et al., 2007; Renugadevi et al., 2010). Seventy-two mutations in *OCA2* are listed as causes of OCA (Gronskov et al., 2007).

The OCA3 is caused by mutations in *TYRP1* (Gronskov et al., 2007; Renugadevi et al., 2010). It maps to chromosome 9p23 (Renugadevi et al., 2010). TYRP1 is an enzyme in the melanin biosynthesis pathway, catalyzing the oxidation of 5,6-dihydroxyindole-2-carboxylic acid monomers into melanin (Gronskov et al., 2007). Tyrp1 functions to stabilize Tyr (Gronskov et al., 2007).

Mutations in the membrane-associated transporter protein gene (*MATP*, also known as *SCL45A2*) cause OCA4 (Gronskov et al., 2007; Renugadevi et al., 2010; Summers, 2009). *MATP* maps to 5p13.3 and encodes a protein expressed in the melanosomal membrane; it may function as a membrane transporter directing melanosomal traffic and other substances to melanosomes (Gronskov et al., 2007; Renugadevi et al., 2010).

Another type of albinism caused by mutations on *OA1* (Xp22.3), OA (OA1), affects males because of X-linked inheritance (Engle, 2010; Gronskov et al., 2007; Renugadevi et al., 2010; Summers, 2009). *OA1* encodes a G-protein-coupled receptor on the melanosome membrane (Engle, 2010; Renugadevi et al., 2010).

31.3.6.3 Neuroradiological Findings

Among disorders where albinism is part of a larger syndrome, such as HPS, cerebral atrophy, most marked in the occipital lobes, can be seen (Budisteanu et al., 2010; Gronskov et al., 2007).

31.3.7 Congenital Fibrosis of the Extraocular Muscles Type I

Congenital fibrosis of the extraocular muscles type 1 (CFEOM1) is a complex strabismus syndrome categorized as one of the congenital cranial dysinnervation disorders (Andrews et al., 1993; Engle, 2010; Heidary et al., 2008). It is the most common form of CFEOM (Andrews et al., 1993; Yamada et al., 2005). The minimum prevalence of CFEOM1 has been estimated to be 1/230 000 (Andrews et al., 1993; Heidary et al., 2008).

31.3.7.1 Clinical Characteristics

Affected individuals are born with congenital bilateral nonprogressive blepharoptosis and strabismus as well as congenital bilateral nonprogressive external ophthalmoplegia with the eyes fixed in an infraducted position approximately 20–30° below the horizontal midline (Andrews et al., 1993; Engle, 2010; Heidary

et al., 2008; Kakinuma and Kiyama, 2009; Khan et al., 2010; Yamada et al., 2005). The eyes look down at rest and cannot be elevated, whereas horizontal movement can range from absence to full (Andrews et al., 1993; Engle, 2010). These patients lack binocular vision (Andrews et al., 1993; Yamada et al., 2005). Forced duction testing is positive for marked restriction of extraocular motility (Andrews et al., 1993; Heidary et al., 2008). Patients typically assume a compensatory 'chin up' head posture to fixate on objects (Heidary et al., 2008).

Affected individuals often have ocular synkinesis (aberrant patterns of eye movement), including synergistic convergence, synergistic divergence, Marcus Gunn jaw-winking phenomenon (congenital synkinetic movement due to synkinesis between the upper eyelid and the pterygoids), and no pupillary involvement (Engle, 2010; Heidary et al., 2008; Khan et al., 2010; Yamada et al., 2005). Synkinetic eye movements are thought to result from aberrant axonal routing (Heidary et al., 2008).

The Marcus Gunn jaw-winking phenomenon is categorized clinically as an ocular miswiring syndrome (Yamada et al., 2005). Affected individuals have ptosis accompanied by elevation of the ptotic eyelid on movement of the lower jaw (Andrews et al., 1993; Yamada et al., 2005). It is first noted in young infants when they are being fed (Andrews et al., 1993). This syndrome is proposed to result from misdirection of axons intended to travel within the motor branch of the trigeminal nerve to innervate the ipsilateral pterygoid muscle (Andrews et al., 1993; Yamada et al., 2005). Instead, these axons aberrantly innervate myofibers of the levator palpebrae superioris muscle, which is normally innervated by a branch of the oculomotor nerve (Andrews et al., 1993; Yamada et al., 2005).

There is phenotypic heterogeneity with respect to involvement of the horizontal extraocular muscles (Heidary et al., 2008). Horizontal movements may be severely restricted or absent, and horizontal strabismus may be present with an increased incidence of exotropia versus esotropia (Heidary et al., 2008). As a consequence of the marked limitation of eye movements and the blepharoptosis, many CFEOM patients develop strabismic and deprivation amblyopia (Andrews et al., 1993; Heidary et al., 2008; Yamada et al., 2005). Among families with CFEOM1, the vertical strabismus is quite uniform, but the horizontal strabismus can vary (Andrews et al., 1993). CFEOM1 patients generally show normal cognitive and physical development (Heidary et al., 2008).

31.3.7.2 Genetics

CFEOM1 is inherited as an autosomal dominant trait with complete penetrance and minimal variation in expression (Andrews et al., 1993; Khan et al., 2010; Yamada et al., 2005). It results from heterozygous mutations in *KIF21A* (kinesin family member 21A), which encodes a kinesin motor and maps to 12q12 (http://www.ncbi.nlm.nih.gov/omim) (Andrews et al., 1993; Engle, 2010; Heidary et al., 2008; Kakinuma and Kiyama, 2009; Khan et al., 2010; Yamada et al., 2005). 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>T (R954W) (Andrews et al., 1993; Engle, 2010). These mutations alter only 7 of the 1675 amino acids in *KIF21A* (Engle, 2010).

The KIF21A is a neuronally expressed protein, which is important in axonal maintenance (Heidary et al., 2008; Yamada et al., 2005). It has been characterized as a member of the Kif4-class superfamily of kinesin motors and acts as a plus-end kinesin motor (Kakinuma and Kiyama, 2009). This gene encodes a developmental motor kinesin responsible for anterograde axonal transport of cargo along neurons such as that of the superior division of cranial nerve III (Engle, 2010; Heidary et al., 2008; Khan et al., 2010).

31.3.7.3 Neuroradiological Findings

Central nervous system maldevelopment, including cortical dysplasia and basal ganglia abnormalities, has been reported (Heidary et al., 2008). Hypoplasia of the superior rectus and levator palpebrae superioris is a common feature of CFEOM1 patients (Heidary et al., 2008). Orbital MRI has demonstrated an absent or severely hypoplastic superior division of the oculomotor nerve (Heidary et al., 2008). Imaging of the skull base has confirmed hypoplasia of the oculomotor nerve as it exited the brain stem (Heidary et al., 2008).

31.3.8 Duane Retraction Syndrome

The DRS is a unilateral or bilateral congenital anomaly of the sixth cranial nerve nuclei with aberrant innervations by supply from the third cranial nerve (Gabay et al., 2010; Yuksel et al., 2010). This syndrome accounts for 1–5% of all cases of strabismus (Zanin et al., 2010). Unilateral Duane syndrome, which accounts for 85% of all cases of DRS, is predominantly sporadic (90%), more prevalent in females (60%), and mainly affects the left eye (Gutowski, 2000; Yuksel et al., 2010; Zanin et al., 2010). It is characterized by marked limitation or absence of abduction, variable limitation of adduction, palpebral fissure narrowing, and globe retraction on attempted adduction (Gabay et al., 2010; Zanin et al., 2010).

Disturbance between the fourth and tenth weeks of embryogenesis seems most obvious and could explain the various nonocular and ocular abnormalities in combination with the Duane's syndrome (Yuksel et al., 2010).

A teratogenic event during the second month of gestation seems to cause most ocular and extraocular abnormalities observed in combination with DRS (Yuksel et al., 2010). Thalidomide has been reported as having a teratogenic effect (Yuksel et al., 2010).

31.3.8.1 Clinical Characteristics

Affected individuals have restricted horizontal gaze greatest with attempted abduction and ocular synkinesis resulting in globe retraction with attempted adduction (Engle, 2010; Gutowski, 2000). When an affected individual attempts to adduct his/her eyes, both the intended medial rectus and the pathologically innervated lateral rectus muscle contract, resulting in retraction of the eyeball into the orbit (Engle, 2010; Gutowski, 2000). Electromyography shows increased electrical activity in a paretic lateral rectus muscle (Engle, 2010; Gutowski, 2000).

Binocular vision is preserved in DRS (Yuksel et al., 2010). The compensation by abnormal head posture allows binocularity in one field of gaze despite the severe eye motility deficit in the other field of gaze (Yuksel et al., 2010). The degree of sensorial binocular status plays an important role in the conjugacy of saccades (Yuksel et al., 2010).

The DRS has been associated with several other conditions where anomalous axonal guidance occurs, such as OCA (Gutowski, 2000; Yuksel et al., 2010). Some other syndromes associated with DRS are Okihiro syndrome (forearm malformation and hearing loss), Wildervanck syndrome (fusion of neck vertebrae and hearing loss), Holt–Oram syndrome (abnormalities of the upper limbs and heart), morning-glory syndrome (abnormalities of the optic disc or blind spot), and Goldenhar syndrome (malformations of the jaw, cheek, and ear, usually on one side of the face) (Yuksel et al., 2010).

Three types of DRS are recognized, depending on the amount of aberrant innervation present (Gutowski, 2000). Palpebral fissure narrowing and retraction of the affected eyeball on adduction tend to be constant findings (Gutowski, 2000). Palpebral fissure narrowing is due to recti contraction and mechanical factors (Gutowski, 2000).

DRS type I consists of defective abduction with normal or minimally defective adduction (Gutowski, 2000; Yuksel et al., 2010). In DRS type II, adduction is defective, and there is exotropia of the affected eye and normal or minimally defective abduction (Gutowski, 2000; Yuksel et al., 2010). Both adduction and abduction are defective in type III (Gutowski, 2000; Yuksel et al., 2010). All three types of DRS frequently produce additional vertical eye movement anomalies, which are characterized by changes in the ocular axes (Gutowski, 2000).

Eye movement recordings are an additional tool for understanding the underlying pathogenesis of DRS (Yuksel et al., 2010). This is a noninvasive technique that has given valuable information about the neural control of movement (Yuksel et al., 2010). Most DRS patients compensate well for the disorder and do not require further management (Yuksel et al., 2010). Standard management of DRS may, in some cases, involve eye muscle surgery (Yuksel et al., 2010).

31.3.8.2 Genetics

The majority of DRS cases are sporadic, with only 2–5% of patients showing a familial pattern (Gutowski, 2000; Yuksel et al., 2010). A high prevalence of DRS has been noted in individuals with thalidomide embryopathy (Gutowski, 2000; Yuksel et al., 2010). Both genetic and environmental factors are likely to play a role in the development of DRS (Yuksel et al., 2010; Zanin et al., 2010).

Studies of sporadic forms of DRS showed 10–20 times greater risk for having other congenital malformations divided in mainly four categories: skeletal, auricular, ocular, and neural (Yuksel et al., 2010). The skeletal abnormalities involved the palate and vertebral column (Yuksel et al., 2010). The auricular malformations included the external ear, the external auditory meatus, and the semicircular canals (Yuksel et al., 2010). Ocular defects concerned the extraocular muscles and the eyelids, including ocular dermoids (Yuksel et al., 2010). Neural defects involved the third, fourth, and sixth cranial nerves (Yuksel et al., 2010).

Most cases of familial DRS are autosomal dominant without associated abnormalities (Gutowski, 2000; Yuksel et al., 2010). There are also several autosomal dominant syndromes in which DRS is a recognized feature (Gutowski, 2000). In some families with dominant DRS, the disease shows reduced penetrance and variable expressivity (Yuksel et al., 2010). An autosomal recessive pattern of inheritance has also been suggested in several reports of DRS (Gutowski, 2000; Yuksel et al., 2010). Autosomal recessive DRS can occur either in isolation or in association with other abnormalities (Gutowski, 2000).

Several chromosomal loci for genes contributing to DRS have been suggested (Gutowski, 2000; Yuksel et al., 2010; Zanin et al., 2010). *CHN1* (chimerin 1) is one of the genes responsible for DRS (Engle, 2010; Zanin et al., 2010). Individuals harboring *CHN1* mutations have a higher incidence of vertical movement abnormalities and bilateral eye involvement when compared to individuals with nonfamilial DRS (Engle, 2010). *CHN1* mutations alter the development of abducens and, to a lesser extent, oculomotor axons (Engle, 2010).

Deletions of chromosomal material on chromosomes 4 and 8 and the presence of an extra marker chromosome, thought to be derived from chromosome 22, has been documented in DRS individuals (Yuksel et al., 2010).

SALL4 (sal-like 4) and *HOXA1* (*homeobox A1*) have been found to be associated also with syndromic forms (Zanin et al., 2010). However, patients with isolated DRS have not been found to carry these mutations (Zanin et al., 2010).

31.3.8.3 Neuroradiological Findings

Motion-encoded MRI has been used for the study of human extraocular muscle function; local physiological contraction and elongation have been quantified (Yuksel et al., 2010). The visualization of the abducens nucleus itself at a neural level remains unfeasible, but the nerve can be explored at the pontomedullar level (Yuksel et al., 2010).

MRI of individuals harboring *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 (Engle, 2010).

MRI has confirmed the maldevelopment of the abducens nerve in DRS and has showed the compensatory innervation by the third nerve at a peripheral level (Yuksel et al., 2010). MRI in cases of DRS type I has demonstrated the absence of the abducens nerve (Yuksel et al., 2010).

31.3.9 Pontine Tegmental Cap Dysplasia

Pontine tegmental cap dysplasia (PTCD) is a cerebellar, brain stem, and cranial nerve malformation syndrome (Engle, 2010). It was first reported in four patients, in 2007 (Barth et al., 2007; Macferran et al., 2010). Pathomechanism of the condition involves a defect in the migration or navigation of axons of rhombencephalic neurons (Szczaluba et al., 2010).

PTCD belongs to a group of related conditions that share in common malformations of the midbrain hindbrain (Macferran et al., 2010). Under this category are those conditions that have in common the molar tooth sign (Macferran et al., 2010).

Conditions described in this group include the following syndromes: Joubert, Senior–Loken, COACH, Dekaban-Arima, oro-facial-digital type VI, and encephalocele with renal cysts (Engle, 2010; Macferran et al., 2010).

It is suggested that many cases of pontocerebellar hypoplasia or Moebius syndrome should be revised for the features of PTCD (Szczaluba et al., 2010). To date, no known etiology for PTCD has been identified (Macferran et al., 2010).

31.3.9.1 Clinical Characteristics

The 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 (Engle, 2010; Macferran et al., 2010). Also, they have shown bilateral trigeminal nerve dysfunction, seizures, and central ventilation abnormalities (Macferran et al., 2010). Commonly reported somatic abnormalities include vertebral anomalies and craniofacial dysmorphism (Macferran et al., 2010).

31.3.9.2 Genetics

The reported children had neither a positive family history nor consanguineous parents, so it remains to be proved that PTCD is genetic (Engle, 2010). It is plausible that it results from *de novo* dominant mutations or recessive mutations in an unidentified gene (Engle, 2010).

A very small (96 kb) 2q13 deletion has been identified as the first case of a molecular genetic abnormality as the identified cause of the condition (Macferran et al., 2010). The deleted region encompasses the *NPHP1* gene (Macferran et al., 2010). This gene has been reported in association with JS (Macferran et al., 2010).

31.3.9.3 Neuradiological Findings

Neuroimaging reveals pontine hypoplasia with ventral flattening and dorsal protrusion of tissue into the fourth ventricle ('tegmental cap') (Engle, 2010; Macferran et al., 2010). Cerebellar vermian hypoplasia and elongated and laterally misplaced SCP result in a modified molar tooth sign (Engle, 2010; Macferran et al., 2010). The middle and inferior cerebellar peduncles and cranial nerves VII and VIII are small (Engle, 2010; Macferran et al., 2010). 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 (Engle, 2010).

31.4 INTRODUCTION: NEURONAL MIGRATION

Development of central nervous system is a highly complicated process, and it is organized in the following steps (Spalice et al., 2009; Verrotti et al., 2010):

1. Primary neurulation (3–4 weeks of gestation), beginning of neuronal migration (fifth week of gestation)
2. Prosencephalic development (2–3 months of gestation)
3. Neuronal proliferation (3–4 months of gestation)
4. Neuronal migration (1–5 months of gestation)
5. Organization (5 months of gestation to after birth)
6. Myelination (after the birth)

Cell migration has an essential role in the developing cerebral cortex because all neurons that eventually

populate the six-layered cerebral cortex and other brain regions undergo mitosis in distant compartments and then migrate great distances to achieve final positioning (Kerjan and Gleeson, 2007).

Neuronal migration consists of nerve cells moving from their original site in the ventricular and subventricular zones to their final location (Kerjan and Gleeson, 2007; Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). Regulation of timing and direction of these simultaneous migrations are highly ordered (Spalice et al., 2009; Verrotti et al., 2010).

Instructed by extracellular cues, the activation of guidance receptors and their downstream signaling pathways enable newborn neurons to migrate through the developing nervous system until they reach their destination (Valiente and Marin, 2010). On arrival to their final destination, neurons cancel their migratory program and continue their differentiation into mature neurons (Valiente and Marin, 2010). It has been suggested that early patterns of activity generated in the target region may influence this process (Valiente and Marin, 2010).

Neurons originating in the cortical ventricular zone migrate radially to form the cortical plate (CP) and mainly become projecting neurons (Spalice et al., 2009; Verrotti et al., 2010).

Migration of neocortical neurons occurs mostly between the 12th and the 24th weeks of gestation (Spalice et al., 2009; Verrotti et al., 2010). The first postmitotic neurons produced in the periventricular germinative neuroepithelium will migrate to form a subpial preplate or primitive plexiform zone (Spalice et al., 2009). Subsequently, produced neurons, which will form the CP, migrate into the preplate and split it into the superficial molecular layer (or layer I or the marginal zone containing Cajal–Retzius neurons) and the deep subplate (Spalice et al., 2009).

Schematically, the successive waves of migratory neurons will pass the subplate neurons and finish their migratory pathway below layer I, forming successively (but with substantial overlap) cortical layers VI, V, IV, III, and II (Spalice et al., 2009). This means that the neurons that migrate first will stop in the deepest cortical layers, and those that migrate afterward pass through the layers formed previously to form the outer cortical layers according to a migration scheme defined 'inside-out' (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010).

Neocortical migrating neurons can adopt different types of trajectories: A large proportion of neurons migrate radially, along radial glial guides, from the germinative zone to the CP (Spalice et al., 2009; Verrotti et al., 2010). Radial glial cells are specialized glial cells present in the neocortex during neuronal migration (Spalice et al., 2009; Verrotti et al., 2010).

Another important group of neuronal precursors initially adopts a tangential trajectory at the level of the ventricular or subventricular germinative zones before adopting a classic radial migrating pathway along radial glia (Spalice et al., 2009; Verrotti et al., 2010). Tangentially, migrating neurons have also been located at the intermediate zone level (prospective white matter) (Spalice et al., 2009; Verrotti et al., 2010).

The phenotype of radial glial seems to be determined by both migrating neurons and intrinsic factors expressed by glial cells (Spalice et al., 2009; Verrotti et al., 2010). Among the latter, the transcription factor Paired Box Gene (*PAX6*), which is specifically localized in radial glia during cortical development, is critical for the morphology, number, function, and cell cycle of radial glia (Spalice et al., 2009; Verrotti et al., 2010).

There are several molecules involved in the control of neuronal migration and in targeting the exact destination of the neurons (Spalice et al., 2009; Verrotti et al., 2010). These molecules can be divided into four broad categories: molecules of the cytoskeleton, which play an important role in the initiation and ongoing progression of neuronal migration, such as Filamin A, ARFGEF2, doublecortin, LIS1, TUBA1A; signaling molecules playing a role in lamination, such as reelin and some reelin receptors such as p35, cdk5, and Brn1/Brn2; molecules modulating glycosylation, which seem to provide stop signs for migrating neurons, such as, POMT1, POMGnT1, fukutin, and FAK; and other factors including neurotransmitters such as glutamate and GABA, trophic factors such as brain-derived neurotrophic factor and thyroid hormones, molecules deriving from peroxisomal metabolism, and environmental factors such as ethanol and cocaine (Spalice et al., 2009; Verrotti et al., 2010).

Reelin is crucial for the lamination of cortical structures, but the molecular mechanisms underlying its action remain unclear (Valiente and Marin, 2010). Genetic studies have positioned Reelin, apolipoprotein E receptor 2 (ApoER2), Vldlr, and Dab1 into a common signaling pathway that leads to the phosphorylation of Dab1 in migrating neurons, an event that is required for normal layering of the cortex (Valiente and Marin, 2010). Both structural barriers at the pial surface of the brain and molecular stop signals are involved in mediating neuronal migration arrest (Pang et al., 2008).

Cell migration requires the dynamic regulation of adhesion complexes between migrating cells and the surrounding extracellular matrix proteins (Valiente and Marin, 2010). In many cell types, this process involves integrin-mediated adhesion, but the function of this signaling system in neuronal migration has remained controversial (Valiente and Marin, 2010). The striking morphology of neurons, as they migrate, extends dendrites and axons and connects with other cells, implying

a strictly regulated program of cytoskeletal organization (Kerjan and Gleeson, 2007).

31.5 OVERVIEW OF NEURONAL MIGRATION DISORDERS

Malformations of cortical development are an important cause of epilepsy and development delay (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). It is estimated that up to 40% of children with refractory epilepsy have a cortical malformation (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). These malformations have also been associated with mental retardation and cerebral palsy (Mochida, 2009). Malformations of cortical development encompass a large spectrum of disorders related to abnormal cortical development with varied genetic etiologies, anatomic abnormalities, and clinical manifestations (Mochida, 2009; Pang et al., 2008).

Cerebral cortical development requires orchestrated movement of cells arising from different regions within the brain, and born at different times, to achieve specific laminar position, orientation, and connections with other cells (Kerjan and Gleeson, 2007; Pang et al., 2008). Disruptions at these various stages may result in malformations of cortical development (Pang et al., 2008). Although cortical development has been separated into various stages, there is a significant overlap between the stages, and many abnormalities may cause dysfunction at more than one level (Pang et al., 2008).

Malformation syndromes are typically classified based on the earliest disruption of development (Barkovich et al., 2005; Pang et al., 2008). This classification system divides brain malformations into disorders of cell proliferation, neuronal migration, and cortical organization (Barkovich et al., 2005; Mochida, 2009). Neuronal migration disorders include lissencephaly, heterotopia, focal cortical dysgenesis, PMG, and schizencephaly (SCZ) (Verrotti et al., 2010).

The pathogenesis of these malformations is multifactorial: Genetic mutations or environmental insults, whether acquired *in utero* at different stages of brain development, or during the perinatal or postnatal period after corticogenesis, may all contribute to the development of these disorders (Jaglin and Chelly, 2009; Pang et al., 2008). The timing, severity, and type of environmental influences, as well as genetic factors, will ultimately determine the type and extent of the malformation (Pang et al., 2008).

Genetic studies in humans and mice have identified a spectrum of mutations in genes involved in a large array of crucial processes such as cell proliferation, cell adhesion, cell migration, chemoattraction and repulsion, posttranslational modifications, and dynamics of the cytoskeleton that often disrupts the development of the cerebral cortex and can lead to severe cortical malformations (Jaglin and Chelly, 2009).

Following neurogenesis, the disruption of neuronal migration resulting from genetic mutations represents a major cause of cortical dysgenesis and encompasses a large variety of malformations (Jaglin and Chelly, 2009). Many of these genes encode important effectors that modulate cytoskeletal dynamics during the migration of neuronal cells (Jaglin and Chelly, 2009). Mutations in *DCX* (*doublecortin*) and *LIS1* genes, which encode MAPs, have been shown to be associated with a large spectrum of neuronal migration disorders (Jaglin and Chelly, 2009).

Despite the significant progress over the past few years, many cases of cortical dysgenesis are still unexplained (Jaglin and Chelly, 2009). The identification of further genes is important for the transfer to the clinic and genetic counseling, as well as to have a better understanding of the physiopathology of human cortical dysgenesis (Jaglin and Chelly, 2009).

31.5.1 Lissencephaly

Lissencephaly is a group of disorders that is characterized by an abnormally smooth surface of the cerebral cortex (Mochida, 2009; Pang et al., 2008; Vallee and Tsai, 2006; Verrotti et al., 2010). It is a severe brain malformation characterized by agyria (absence of gyri) and pachygyria (reduced number of broadened gyri), thickened cortex, abnormal cortical layering, enlarged ventricles, and neuronal heterotopias (abnormal positioning of neurons) (Ghai et al., 2006; Pang et al., 2008; Reiner et al., 2006; Verrotti et al., 2010). The lifespan of patients with these disorders is short and most of them die within the first year of life, usually because of aspiration pneumonia and sepsis (Reiner et al., 2006).

Lissencephaly is a neuronal migration disorder that results from impaired migration of postmitotic neurons from the ventricular zone to the developing CP (Ghai et al., 2006; Reiner et al., 2006).

On the basis of etiologies and associated malformations, five groups of lissencephaly can be identified: classical lissencephaly, cobblestone lissencephaly, X-linked lissencephaly with ACC, lissencephaly with cerebellar hypoplasia (LCH), and microlissencephaly (Verrotti et al., 2010). The onset of lissencephaly is considered to occur no later than the 12th–16th week of gestation (Verrotti et al., 2010).

Functions of some lissencephaly genes are closely related to microtubules (Mochida, 2009). The network of microtubules and molecular motor, dynein, are critical to this movement of the centrosome and nucleus in migrating neurons (Mochida, 2009). Some lissencephaly

genes are associated with specific neuropathology of the cerebral cortex (Mochida, 2009). Mutations of six genes have been associated with lissencephaly, including *LIS1*, *DCX*, *TUBA1A*, *RELN*, very low-density lipoprotein receptor (*VLDLR*), and *ARX*, whereas codeletion of *αWHAE* with *LIS1* appears to act as a modifier locus (Spalice et al., 2009).

31.5.1.1 Classical Lissencephaly

Classical lissencephaly, previously known as type 1 lissencephaly, causes a combination of agyria and pachygyria (Kerjan and Gleeson, 2007; Verrotti et al., 2010). This disorder represents one of the most severe disorders of neocortical neuronal migration (Kerjan and Gleeson, 2007). It is distinguished from the other forms of lissencephaly based on the absence of additional characteristic features (Kerjan and Gleeson, 2007). This type of lissencephaly occurs in Miller–Dieker syndrome (MDS) and in isolated lissencephaly sequence (ILS) (Reiner et al., 2006). The incidence of classical lissencephaly has been estimated to be 1.2 in 100 000 births (Verrotti et al., 2010).

Microscopically, the cortex appears poorly structured with only four immature layers of neurons instead of the normal six highly organized layers present in a well-developed brain (Kerjan and Gleeson, 2007; Pang et al., 2008; Verrotti et al., 2010). This disorder is derived from both tangential and radial migration disorders of neurons (Spalice et al., 2009). The pathogenesis of classic lissencephaly is unlikely to be due to defective neuronal migration alone but may include an aspect of abnormal proliferation (Mochida, 2009).

31.5.1.1.1 CLINICAL CHARACTERISTICS

Children with classical lissencephaly are diagnosed in the first few months of life (Kerjan and Gleeson, 2007). ILS is clinically characterized by early hypotonia, which may evolve to limb spasticity, seizures, psychomotor retardation, and often microcephaly (Jaglin and Chelly, 2009; Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). Seizures are present in almost the totality of children with onset in early age, mostly in the first 6–12 months (Spalice et al., 2009; Verrotti et al., 2010). High prevalence (80%) of infantile spasms with or without typical hypsarrhythmia on EEG has been reported (Spalice et al., 2009; Verrotti et al., 2010). Later, most children have a more complex epileptic syndrome, including atypical absences, drop attacks, and myoclonic, partial complex, tonic, and tonic–clonic seizures (Spalice et al., 2009; Verrotti et al., 2010). The EEG demonstrated diffused fast rhythms with high amplitude, considered peculiar of this condition (Spalice et al., 2009).

Children with MDS have a severe form of ILS with facial dysmorphisms, including a prominent forehead, bitemporal hollowing, a short nose with upturned nares, and a long and protuberant upper lip with thin vermillion border and small jaw (Mochida, 2009; Verrotti et al., 2010). Other children with MDS might also present cardiac and renal abnormalities, cryptorchidism, sacral dimple, omphaloceles, and clinodactyly (Verrotti et al., 2010).

31.5.1.1.2 GENETICS

Lissencephaly associated with *RELN*, *VLDLR*, and *ARX* are pathologically and radiologically distinct from lissencephaly because of *LIS1*, *DCX*, and *TUBA1A* (Mochida, 2009). The LIS1 gene is the first gene that was correlated with human lissencephaly (Jaglin and Chelly, 2009; Mochida, 2009; Reiner et al., 2006; Verrotti et al., 2010). LIS1 localizes to chromosome 17p13.3 (Mochida, 2009). LIS1 is a MAP that localizes primarily to the centromere in migrating neurons (Kerjan and Gleeson, 2007; Vallee and Tsai, 2006). LIS1 is highly conserved in evolution both in sequence and in multiple functional aspects (Reiner et al., 2006).

A tight relationship between LIS1, microtubule regulation, and microtubule-based motor proteins has been suggested for many organisms (Reiner et al., 2006). LIS1 controls mitotic spindle orientation in both the neuroepithelial stem cells and the radial glial progenitor cells (Verrotti et al., 2010). It has a role in preserving the normal microtubule network organization (Reiner et al., 2006; Vallee and Tsai, 2006).

In addition to a direct role for LIS1 in regulating tubulin dynamics, LIS1 interacts with a plethora of MAP (Reiner et al., 2006). This includes interactions with DCX, CLIP-170, and MAP1b (Reiner et al., 2006). LIS1 deletion causes dysfunction of the dynein, a microtubular cytoplasmic protein involved in neuronal migration processes (Jaglin and Chelly, 2009; Mochida, 2009; Verrotti et al., 2010). LIS1 interacts with several subunits of the retrograde, microtubule-based motor complex dynein/dynactin (Reiner et al., 2006; Vallee and Tsai, 2006). LIS1 regulates cytoplasmic dynein activity and participates in several dynein-mediated activities such as intracellular transport and mitosis (Reiner et al., 2006; Vallee and Tsai, 2006). In migrating cells, the presence of dynein, dynactin, and LIS1 at the leading cell cortex is essential for directed cell motility (Reiner et al., 2006).

ILS is caused by intragenic mutations or deletions of the LIS1 gene or by small deletions involving 17p13.3 (Ghai et al., 2006; Kerjan and Gleeson, 2007; Mochida, 2009; Verrotti et al., 2010). Complete deletion of both *LIS1* and 14-3-3 *αWHAE* genes on chromosome 17p13 causes MDS (Ghai et al., 2006; Kerjan and Gleeson, 2007; Mochida, 2009; Pang et al., 2008; Verrotti et al., 2010). MDS has been considered a contiguous gene deletion syndrome (Kerjan and Gleeson, 2007). αWHAE belongs to the 14-3-3 family of proteins that can have

many effects on phosphoproteins, including protection from dephosphorylation (Verrotti et al., 2010). 14-3-3 binds to CDK5/p35-phosphorylated NUDEL, and this binding maintains NUDEL phosphorylation (Verrotti et al., 2010). NUDEL is a LIS1-binding protein that, together with LIS1, regulates the cytoplasmic dynein heavy chain function through phosphorylation by CDK5/p35, a complex known to be essential for neuronal migration (Jaglin and Chelly, 2009; Mochida, 2009; Reiner et al., 2006; Verrotti et al., 2010). A smaller deletion, encompassing the region of LIS1 gene but not the 14-3-3 gene, has been associated with a milder phenotype of isolated LIS (Verrotti et al., 2010). *αWHAE* is suggested to be a dosage-dependent modifier of the severity of classical lissencephaly (Kerjan and Gleeson, 2007).

Lissencephaly due to mutations or deletions of LIS1 is a dominant trait (Mochida, 2009). Most LIS1 mutations are *de novo*, and therefore the recurrence risk is generally low (Mochida, 2009). However, in some cases, a parent harbors a balanced translocation involving the LIS1 gene, and so their risk of recurrence could be much higher (Mochida, 2009). Most cases of MDS and ILS are sporadic (Ghai et al., 2006). However, approximately 20% of patients with MDS inherited a genetic deletion from a parent (Ghai et al., 2006).

Mutations of the doublecortin (*DCX*) gene on chromosome Xq22.3 are also known to cause classical lissencephaly in males while heterozygous mutations in females are associated with subcortical band heterotopia (SBH) (Jaglin and Chelly, 2009; Mochida, 2009; Reiner et al., 2006; Verrotti et al., 2010). Males with SBH and *DCX* mutations have rarely been reported (Verrotti et al., 2010). SBH is also known as 'double cortex' syndrome as a band of heterotopic neurons is found within the cerebral white matter between a normal-appearing cortex and the ventricular surface higher (Mochida, 2009). About 20% of patients with classical ILS or SBH have mutations of the DCX gene, resulting in X-linked lissencephaly (LISX1) or double cortex syndrome (DC) (Spalice et al., 2009). Mutations in DCX may be inherited from a mother with SBH to her son, causing lissencephaly, or to her daughter, causing SBH (Mochida, 2009). Doublecortin is expressed in postmitotic neurons, but neither in proliferating cells of the ventricular zone during the development period nor in mature neurons of the adult brain (Verrotti et al., 2010). DCX is a cytoplasmic protein that appears to direct neuronal migration by regulating the organization and stability of microtubules (Jaglin and Chelly, 2009; Mochida, 2009; Verrotti et al., 2010). Nearly all mutations identified to date are premature protein truncations or missense mutations that cluster within the repeated tubulin-binding domain of DCX and inactivate its effects on microtubules (Kerjan and Gleeson, 2007).

Mutations in the alpha tubulinic complex (*TUBA1A*) gene located on chromosome 12q12–q14 have been correlated with the agyria–pachygyria-band spectrum of phenotype (Jaglin and Chelly, 2009; Kerjan and Gleeson, 2007; Mochida, 2009; Verrotti et al., 2010). This gene encodes for brain-specific alpha tubulin protein that represents one of the major component of microtubule complex required for cell movement (Mochida, 2009; Verrotti et al., 2010). Mutations in *TUBA1A* are considered to affect the folding of tubulin heterodimers and influence interactions with microtubule-binding proteins (doublecortin and kinesin KIF1A), resulting in disorders of microtubular function and deficits in the motility of neuronal progenitor cells (Verrotti et al., 2010). Congenital microcephaly, spastic diplegia or quadriplegia, and mental retardation are common clinical features seen in patients with the *TUBA1A* mutation phenotype (Mochida, 2009; Verrotti et al., 2010). In addition to microcephaly, rare occurrences of agyria and subcortical heterotopia (SBH) demonstrate that TUBA1A-related lissencephaly could encompass a large spectrum of cortical abnormalities (Jaglin and Chelly, 2009). This abnormal gyral pattern is combined with dysgenesis of the anterior limb of the internal capsule to give a dysmorphic aspect to the basal ganglia (Jaglin and Chelly, 2009). Moreover, other extracortical defects often include complete to partial ACC and mild to severe cerebellar hypoplasia (Jaglin and Chelly, 2009).

Lissencephaly due to *TUBA1A* mutations manifests as a dominant trait, and therefore, the mutations are generally *de novo*, as seen with *LIS1* (Mochida, 2009). Neuronal axonal guidance and/or growth defects, in addition to early neuronal differentiation abnormalities, are likely to be involved in the pathogeny of *TUBA1A*-related cortical dysgenesis (Jaglin and Chelly, 2009).

LIS1 and *DCX* collectively account for about three-quarters of isolated classic lissencephaly, and *TUBA1A* is estimated to account for about 4% of cases (Jaglin and Chelly, 2009; Mochida, 2009). Mutations in these three genes generally cause similar clinical phenotypes, including microcephaly, mental retardation, with or without epilepsy, and motor deficits (Mochida, 2009). Mutations of these three genes lead to this form of lissencephaly in which cortical thickness is increased fourfold and produce a recognizable gradient in which the malformation is more severe anteriorly (*DCX*) or posteriorly (*LIS1* and *TUBA1A*) (Jaglin and Chelly, 2009; Mochida, 2009).

ARX is a homeobox gene that is expressed in the ganglionic eminences and the neocortical ventricular zone (Pang et al., 2008). This gene plays an important role in the proliferation of neuronal precursors and differentiation of the forebrain (Pang et al., 2008). Mutations of ARX are a rare cause of lissencephaly, although less severe mutations result in a more common developmental

disorder, cryptogenic infantile spasms (Spalice et al., 2009). Mutations in this gene cause the X-linked lissencephaly syndrome with ambiguous genitalia (Mochida, 2009; Pang et al., 2008). These patients have neonatal-onset epilepsy, hypothalamic dysfunction causing temperature dysregulation, chronic diarrhea, and ambiguous genitalia (micropenis and cryptorchidism) (Pang et al., 2008). This gene is a transcription factor expressed in the forebrain that regulates nonradial migration of interneurons from ventral regions to the developing cortex (Spalice et al., 2009). Severe seizures are presumably related to a severe deficiency of inhibitory interneurons (Spalice et al., 2009).

Patients with *ARX* mutations have abnormalities of the basal ganglia and absence of the corpus callosum, whereas those with *RELN* and *VLDLR* mutations have less cortical thickening, absence of a cell-sparse zone, and profound cerebellar hypoplasia (Spalice et al., 2009).

Reelin (*RELN*) is a signaling glycoprotein secreted by the early neurons on the surface of the cerebral cortex known as the Cajal–Retzius cells (Pang et al., 2008). This is a large extracellular matrix protein, which, when absent, causes reversal of cortical layers with deeper layers being made up of younger rather than older born neurons (Reiner et al., 2006). Activation of the Reelin signaling pathway is thought to be essential for proper positioning of migratory neurons into the appropriate lamina of the cortex (Pang et al., 2008). Mutations in *RELN* give rise to seizures, developmental delay, and hypotonia (Pang et al., 2008). Also, the loss of cerebellar organization likely contributes to ataxia (Pang et al., 2008). Only a few patients have been described with mutations in this gene, and generalized pachygyria, severe cerebellar hypoplasia, and hippocampal abnormalities seem to be the common features (Mochida, 2009).

VLDLR mutations cause similar abnormalities to *RELN*, with severe cerebellar hypoplasia, but the simplification of gyri may be milder features (Mochida, 2009). The product of the *VLDLR* gene belongs to the same biological pathway as *RELN* (Mochida, 2009). VLDLR, along with APOER2, acts as a receptor for the RELN protein in migrating neurons and transmits the extracellular RELN signal to the intracellular signaling pathway (Mochida, 2009). There is also notable evidence of interaction between RELN signaling and LIS1 (Mochida, 2009).

In some forms of classic lissencephaly, defects in GABAergic inhibitory interneurons have been suggested (Mochida, 2009). GABAergic interneurons of the cerebral cortex are derived in the ganglionic eminence (which develops into basal ganglia) and migrate tangentially into the cerebral cortex (Mochida, 2009). The best known examples of defects in GABAergic interneurons are due to *ARX* mutations (Mochida, 2009). Also, the number of inhibitory neurons is greatly diminished in the brain of patients with a LIS1 (Mochida, 2009).

Lissencephaly is often associated with severe, intractable epilepsy, and defects in interneurons, in addition to abnormal cortical lamination, may be in part responsible for this (Mochida, 2009). Mutations in *TUBA1A* seem to be associated with a lower incidence of epilepsy compared with *LIS1* (Mochida, 2009).

31.5.1.1.3 NEURORADIOLOGICAL FINDINGS

MRI of the brain in classic lissencephaly demonstrates an hour-glass configuration with areas of pachygyria and agyria, poorly developed sylvian and rolandic fissures, and failure of opercularization of the insular areas (Pang et al., 2008). It shows some degree of abnormality in the spacing of the gyri and sulci (Kerjan and Gleeson, 2007). The cortex is moderately thickened (5–10 mm) with white matter signal abnormalities (Kerjan and Gleeson, 2007; Verrotti et al., 2010). Associated findings may include dilatation of lateral ventricles, mild hypoplasia of the corpus callosum, and persistent cavum septum pellucidum (Verrotti et al., 2010).

Mutations in *LIS1* are often associated with abnormalities prevalent in the parietal and occipital cortex, whereas DCX lissencephaly is more pronounced in the frontal and temporal cortex (Pang et al., 2008; Verrotti et al., 2010). Mutations in *TUBA1A* have led to gyral malformations that are more severe in posterior than in anterior regions of the brain, often combined with dysgenesis of the corpus callosum, cerebellar and brainstem hypoplasia, and variable cortical malformation, including subtle SBH, ventricular dilatation, and absence or hypoplasia of the anterior limb of the internal capsule (Pang et al., 2008; Verrotti et al., 2010).

In individuals harboring *ARX* mutations, the lissencephaly is worse posteriorly than anteriorly, and there is absence of the corpus callosum (Pang et al., 2008). The cortex is moderately thickened (5–10 mm) with white matter signal abnormalities as well as cystic or fragmented basal ganglia (Pang et al., 2008). In *RELN* mutations, the lissencephaly is associated with cerebellar hypoplasia and hippocampal and brainstem abnormalities (Pang et al., 2008).

31.5.1.2 Cobblestone Lissencephaly

Cobblestone lissencephaly, previously type II, is a complex brain malformation characterized by global disorganization of cerebral organogenesis (Verrotti et al., 2010). It is characterized by a defective basement membrane in which breaches are formed (Jaglin and Chelly, 2009). It refers to the nodular appearance of the cerebral cortex caused by disorganization of the cortical layers and overmigration of neurons through the pial surface of the brain into the leptomeninges (Jaglin and Chelly, 2009). The cortex displays irregular grooves

imparting a cobblestone pattern and consists of cluster and circular arrays of neurons, with no recognizable layers, separated by glial and vascular septa (Verrotti et al., 2010). The brain phenotype includes multiple anomalies such as hydrocephalus and neuronal overmigration, causing a cobblestone cortex, lissencephaly, and ACC (Reiner et al., 2006). Brainstem abnormalities also may be present (Ghai et al., 2006).

All patients with cobblestone lissencephaly show defects in the O-linked glycosylation of the glycoprotein α-dystroglycan, a protein that bridges the actin cytoskeleton of cells and the extracellular matrix component, laminin callosum (Reiner et al., 2006).

31.5.1.2.1 CLINICAL CHARACTERISTICS

It is associated with various eye abnormalities and congenital muscular dystrophies (Reiner et al., 2006). Cobblestone lissencephaly has been described in three syndromes: the Walker–Warburg syndrome (WWS), muscle–eye–brain disease (MEB), and Fukuyama-type congenital muscular dystrophy (FCMD) (Ghai et al., 2006). WWS is the most severe of these small groups of syndromes (Verrotti et al., 2010). It has a worldwide distribution, while FCMD has been found in Japan and MEB primarily in Finland (Verrotti et al., 2010).

The major clinical features of WWS are macrocephaly, cerebellar malformation, ventricular dilation/hydrocephalus, retinal malformation, anterior chamber abnormality, and congenital muscular dystrophy (Verrotti et al., 2010).

MEB disease results in a severe form of congenital muscular dystrophy with mental retardation and myoclonic jerks (Verrotti et al., 2010). Ocular disorders include progressive myopia, retinal dystrophy, glaucoma, and optic atrophy (Verrotti et al., 2010).

FCMD is the mild form of cobblestone lissencephaly, and it is characterized by severe hypotonia, progressive weakness, and developmental delay (Pang et al., 2008; Verrotti et al., 2010). The majority of patients are unable to walk unsupported (Pang et al., 2008). Mental retardation is a universal finding (Pang et al., 2008). The association of epilepsy and seizure-related disorders in FCMD is widely accepted: Febrile seizures and epilepsy with generalized tonic convulsions appear in about 50% of children, but they are usually not severe (Pang et al., 2008).

31.5.1.2.2 GENETICS

Cobblestone lissencephaly follows an autosomal recessive inheritance pattern (Pang et al., 2008). Several genes have been implicated in the etiology of WWS (Verrotti et al., 2010). Different mutations have been found in the proteins *O*-mannosyltransferase 1 and 2 (*POMT1* at 9q34 and *POMT2* genes) and also in each of the fukutin (*FKTN* at 9q31–33) and fukutin-related 9 protein (*FKRP* at 19q13–32) genes (Pang et al., 2008; Verrotti et al., 2010).

The MEB gene has been localized on the chromosome 1p32–34 (*POMGnT1* gene for protein O mannose β-1,2-*N*-acetylglucosaminyltransferase) (Pang et al., 2008; Verrotti et al., 2010). FCMD is associated with mutations of the gene *FKTN* on chromosome 9q31, which encodes a novel 461-amino acid protein termed 'fukutin' (Pang et al., 2008; Verrotti et al., 2010).

All these genes are involved in the glycoylation of α-dystroglycan, an extracellular protein capable of binding to components of extracellular matrix such as laminin, agrin, neurexin, and perlecan (Pang et al., 2008; Verrotti et al., 2010). Mutations in these genes compromise the integrity of the superficial marginal zone of the cortex, so that neurons overmigrate beyond this structure into the pial surface, forming the cobblestone (Pang et al., 2008; Verrotti et al., 2010).

31.5.1.2.3 NEURORADIOLOGICAL FINDINGS

Brain MRI demonstrates the typical cobblestone lissencephaly with varying degrees of severity (Pang et al., 2008; Verrotti et al., 2010). MRI in WWS and MEB reveals pontine hypogenesis with a distinct dorsal 'kink' at the mesencephalic-pontine junction; a 'Z-shaped' hypoplastic brainstem is considered a key feature (Pang et al., 2008; Verrotti et al., 2010).

31.5.1.3 Lissencephaly X-linked with ACC

Lissencephaly X-linked with ACC (XLAG) includes a thickened cortex and three-layered cortex with gyral malformations that are more severe in posterior than anterior brain regions, atrophic striatal and thalamic nuclei, poorly myelinated white matter, ACC, and ambiguous genitalia (Jagla et al., 2008; Miyata et al., 2009; Okazaki et al., 2008).

31.5.1.3.1 CLINICAL CHARACTERISTICS

XLGA is associated with intractable neonatal onset epilepsy, temperature instability, probably due to a hypothalamic dysfunction, severe diarrhea, postnatal microcephaly, abnormal genitalia with micropenis and cryptorchidism, and early death (Jagla et al., 2008; Miyata et al., 2009; Okazaki et al., 2008).

31.5.1.3.2 GENETICS

XLAG results from defects in the *ARX* (aristaless-related homeobox) gene located at Xp22.13 (Okazaki et al., 2008). The *ARX* gene product has two functional domains, *prd*-like homeodomain, and *aristaless* domain (Okazaki et al., 2008). Disruption of the *prd*-like homeodomain leads to XLAG (Okazaki et al., 2008). The functional domain, *prd*-like homeodomain, has very important functions in the formation of the normal brain in early development (Okazaki et al., 2008).

The major function of ARX protein is thought to be not only the regulation of proliferation and tangential migration of GABAergic interneurons but also the radial migration of pyramidal neurons death (Okazaki et al., 2008).

31.5.1.3.3 NEURORADIOLOGICAL FINDINGS

Imaging studies show a thick cerebral cortex (5–6 mm) with anterior pachygyria and posterior agyria (Okazaki et al., 2008). Other findings include abnormal signal of white matter, absence of corpus callosum, and cystic or fragmental basal ganglia (Okazaki et al., 2008).

31.5.1.4 Lissencephaly with Cerebellar Hypoplasia

LCH has been recently defined as a different group of lissencephaly, which is neither classical nor cobblestone type (Verrotti et al., 2010). It is associated with severe abnormalities of the cerebellum, ranging from vermian hypoplasia to total aplasia with either classical or cobblestone lissencephaly, and abnormalities in the hippocampus and brainstem (Hong et al., 2000; Verrotti et al., 2010).

31.5.1.4.1 CLINICAL CHARACTERISTICS

Affected children show a motor, language, and cognitive delay; they neither sit and stand unsupported nor develop linguistic skills (Verrotti et al., 2010). Hypotonia and severe ataxia are frequent; in addition, generalized epilepsy begins at an early age (Verrotti et al., 2010).

31.5.1.4.2 GENETICS

It is an autosomal recessive disorder (Hong et al., 2000) that maps to chromosome 7q22 and is associated with mutations in the gene encoding reelin (*RELN*) (Hong et al., 2000; Verrotti et al., 2010). The mutations disrupt splicing of RELN cDNA, resulting in low or undetectable amounts of reelin protein (Hong et al., 2000). RELN encodes a large secreted protein that acts on migrating cortical neurons by binding to the VLDLR, the APOER2, α3β1 integrin, and protocadherins (Hong et al., 2000). LCH is also correlated with mutations of the gene, which encodes for VLDLR (Verrotti et al., 2010).

31.5.1.4.3 NEURORADIOLOGICAL FINDINGS

MRI demonstrates diffuse pachygyria, hippocampal dysplasia, and hypoplastic brainstem (Verrotti et al., 2010). Cerebellar manifestations range from midline hypoplasia to diffuse volume reduction and disturbed foliation (Verrotti et al., 2010).

31.5.1.5 Microlissencephaly

Microlissencephaly differs from other variants of LIS1 by the presence of a severe microcephaly (Verrotti et al., 2010). It is caused by an abnormal neuronal proliferation or survival combined with neuronal migration disorders (Verrotti et al., 2010). Two main types of microlissencephaly are recognizable: type A (Norman-Roberts syndrome) with no infratentorial anomalies and type B (or Barth syndrome), which is associated with a severe hypoplasia of the cerebellum and corpus callosum (Verrotti et al., 2010). A recent form has been reported in which primordial osteodysplastic dwarfism is associated with severe microcephaly (Verrotti et al., 2010).

31.5.1.5.1 CLINICAL CHARACTERISTICS

Norman-Roberts syndrome presents with a wide phenotypic heterogeneity (Natacci et al., 2007). Clinical characteristics include microcephaly, bitemporal hollowing, a low sloping forehead, slightly prominent occiput, widely set eyes, a broad and prominent nasal bridge, and severe postnatal growth deficiency (Verrotti et al., 2010). Neurological features include hypertonia, hyperreflexia, seizures, and severe mental retardation (Verrotti et al., 2010).

31.5.1.5.2 GENETICS

Norman-Roberts syndrome is an autosomal recessive disorder (Natacci et al., 2007).

31.5.1.5.3 NEURORADIOLOGICAL FINDINGS

The brain MRI has shown changes consistent with lissencephaly type I (Verrotti et al., 2010).

31.5.2 Heterotopia

Heterotopia is a neuronal migration disorder characterized by a cluster of disorganized neurons in abnormal locations, and it includes three main groups: periventricular nodular heterotopia (PNH), SBH, and marginal glioneural heterotopia (Spalice et al., 2009; Verrotti et al., 2010).

PNH is a rare malformation in which primary neuronal cells never begin migration and remain adjacent to the lateral ventricles (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). PNH may involve both sides of the brain or, less frequently, be restricted to a single hemisphere (Spalice et al., 2009; Verrotti et al., 2010). Apparently, the cerebral cortex is normal (Pang et al., 2008).

SBH or 'double cortex' syndrome is characterized by a diffuse laminar band of gray matter located below the cerebral cortex and separated from it by a thin band of white matter (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010).

Marginal zone heterotopias or leptomeningeal glioneuronal heterotopias are one form of dysplasia in which ectopic nests of glial and neuronal cells are observed in the cortical MZ or overlying leptomeninges, respectively (Verrotti et al., 2010).

31.5.2.1 Clinical Characteristics

The spectrum of clinical presentation of PNH is wide (Spalice et al., 2009). Epilepsy is the main aspect (Pang et al., 2008; Spalice et al., 2009). About 90% of patients with PNH have epilepsy that can begin at any age, and it is usually intractable (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). There is no clear relationship between the epilepsy severity and extent of nodular heterotopia (Pang et al., 2008). Surgical removal of the heterotopia cortex is generally successful (Spalice et al., 2009; Verrotti et al., 2010).

Other symptoms of PNH include severe developmental delay, microcephaly, and infantile spasms (Verrotti et al., 2010). However, in general, these patients have normal intelligence. Some patients may have learning problems such as impaired reading fluency (Pang et al., 2008). Some patients with PNH have also been described with Chiari I and amniotic band syndrome (Spalice et al., 2009; Verrotti et al., 2010).

The main clinical manifestation of SBH is epilepsy (Spalice et al., 2009). Individuals with SBH have variable degrees of mental retardation and intractable epilepsy, which seem to correlate with the thickness of the band and the overlying cortex (Spalice et al., 2009; Verrotti et al., 2010).

The cortex may be normal or associated with pachygyria (Verrotti et al., 2010). Lennox–Gastaut syndrome is another potential presentation (Spalice et al., 2009). Epilepsy surgery for focal seizures yields poor results, while callosotomy has been associated with improvement in drop attacks (Spalice et al., 2009). The clinical spectrum of SBH in male subjects overlaps with that in females in terms of seizure type representation, epilepsy syndromes, and response to antiepileptic therapy (Verrotti et al., 2010). However, there is increased heterogeneity with respect to cognitive function, neuroimaging, and molecular genetic data in males compared with females (Verrotti et al., 2010).

31.5.2.2 Genetics

PNH can be caused by genetic mutations or extrinsic factors, such as infections or prenatal injuries (Pang et al., 2008; Verrotti et al., 2010). Mutations of the *FLNA* gene (Xq28) cause bilateral PNH in the majority of patients; this form is often fatal for males, therefore explaining the female preponderance (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). This gene encodes for Filamin A, an actin-binding protein that stabilizes the cytoskeleton to generate the forces necessary for cell mobility and mediates focal adhesions along the ventricular epithelium (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). There is enrichment of FLNa (filamin A, alpha) in postmitotic migrating neurons, an expression pattern that might be maintained in part by FILIP (filamin-A-interacting protein), a potent degrader of FLNa (Spalice et al., 2009). FLNa mutations resulting in PH often involve truncation or disruption of the actin-binding domain, indicating that FLNa's ability to cross-link actin may be necessary for migration (Spalice et al., 2009).

The autosomal recessive form of PNH is caused by mutations in the *ARFGEF2* (ADP-ribosylation factor guanine exchange factor 2) gene localized at 20q13.13, which encodes for the protein brefeldin-inhibited GEF2 (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). Mutations in ARF-GEF may impair the targeted transport of FLNA to the cell surface within neural progenitors along the neuroependyma (Pang et al., 2008; Verrotti et al., 2010). The disruption of these cells could contribute to PNH formation with microcephaly (Verrotti et al., 2010).

PNH has also been associated with copy number variations, including duplication 5p15.1 or 5p15.33 and deletion 6q26–q27 or 7q11.33 (Spalice et al., 2009; Verrotti et al., 2010). At least 15 distinct PNH syndromes have been described (Spalice et al., 2009).

SBH is caused by alterations in two genes: *LIS1* at 17p13.32 and *DCX* at Xq22.3–q23.3 (Pang et al., 2008; Verrotti et al., 2010). Mutations of the *DCX* gene have been found in all familial cases and in 53–84% of patients with sporadic, diffuse, or anteriorly predominant band heterotopia, which represent the most common forms of SBH (Verrotti et al., 2010). Other genetic causes of SBH remain unexplained (Verrotti et al., 2010). The DCX protein is thought to direct neuronal migration by regulating the organization and stability of microtubules necessary for neuronal motility (Pang et al., 2008).

31.5.2.3 Neuroradiological Findings

MRI patients with X-linked dominant mutation show bilateral symmetric nodules lying adjacent to the lateral ventricular walls; additional findings include hypoplasia of the corpus callosum and posterior fossa abnormalities such as cerebellar hypoplasia and enlarged cisterna magna (Pang et al., 2008; Verrotti et al., 2010). Unilateral PNH is commonly located in the posterior paratrigonal zone of the lateral ventricles and may involve the adjacent white matter (Verrotti et al., 2010).

Patients with autosomal recessive mutations of the *ARFGEF2* gene present with microcephaly, slightly enlarged ventricles, and delayed myelination (Pang et al., 2008; Verrotti et al., 2010). Symmetrical nodular heterotopia lining the ventricles is also seen, and the overlying cortex may be thinned with abnormal gyri (Pang et al., 2008).

MRI of the brain in SBH demonstrates two parallel layers of gray matter, a thin outer ribbon and a thick inner band, separated by a very thin layer of white matter (Pang et al., 2008; Verrotti et al., 2010).

31.5.3 Polymicrogyria

PMG is a cortical malformation characterized by an irregular brain surface with an excessive number of small and partly fused gyri separated by shallow sulci, giving the surface of the cortex its characteristic lumpy appearance (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010).

PMG can be focal or diffused, unilateral or bilateral (Pang et al., 2008; Verrotti et al., 2010). It is a very common cortical malformation and can be an isolated lesion associated with other brain malformations such as heterotopia, white matter lesions, or a part of several multiple congenital anomaly/mental retardation syndromes (Spalice et al., 2009; Verrotti et al., 2010). Bilateral involvement of the cortex is frequently seen, with a symmetric or asymmetric distribution, affecting the frontal, frontoparietal, parieto-occipital, perisylvian, and mesial occipital regions (Pang et al., 2008; Spalice et al., 2009).

PMG pathogenesis is not understood; brain pathology demonstrates abnormal development or loss of neurons in middle and deep cortical layers, variably associated with an unlayered cortical structure (Spalice et al., 2009). Two types of PMG can be identified histopathologically: a simplified four-layered form (in which there is a layer of intracortical laminar necrosis with subsequent alterations of late migration and postmigratory disruption of cortical organization) and an unlayered form (in which the molecular layers are continuous and do not follow the borders of convolutions and the neurons below have radial distribution while laminar organization is absent) (Verrotti et al., 2010). The incidence of PMG is unknown because of its clinical and etiological heterogeneity (Verrotti et al., 2010).

31.5.3.1 Clinical Characteristics

The wide spectrum of clinical manifestations is related to the extension of PMG, which varies greatly (Spalice et al., 2009). Almost all children with PMG have a high risk of developing epilepsy (Verrotti et al., 2010). Seizures usually begin between 4 and 12 years of age, and they are drug resistant in approximately 65% of patients (Spalice et al., 2009; Verrotti et al., 2010). A small number of children present with focal epilepsy, while the most frequent seizure types are atypical absences, tonic or atonic drop attacks, or tonic–clonic convulsions (Spalice et al., 2009; Verrotti et al., 2010).

In the bilateral frontal type, the most common symptoms include delayed motor and language milestones, spastic hemiparesis or quadriparesis, and mild to moderate mental retardation (Spalice et al., 2009; Verrotti et al., 2010). In the bilateral frontoparietal form, the clinical presentation is characterized by global developmental delay, esotropia, and pyramidal and cerebellar signs and seizures, which occur in 94% of patients and are mostly generalized (Verrotti et al., 2010).

Bilateral perisylvian PMG-affected patients can present pseudobulbar palsy with diplegia of the facial, pharyngeal, and masticatory muscles and pyramidal signs and seizures (Verrotti et al., 2010). Infantile spasms may be the presenting seizure type even if seizures develop only before the end or after the first decade (Verrotti et al., 2010). Most patients develop multiple seizure types, and seizure control is poor in more than half of the cases (Verrotti et al., 2010).

Other forms of PMG, such as bilateral parasagittal parietooccipital, bilateral generalized, and unilateral ones, can produce various kinds of seizures and EEG abnormalities (including status epilepticus during sleep), cognitive slowing, motor delay, and cerebral palsy (Verrotti et al., 2010).

31.5.3.2 Genetics

PMG has been associated with mutations of a few genes, including *SRPX2* (sushi-repeat-containing protein, X-linked 2), *PAX6* (paired box 6), *TBR2* (T-box-brain2), *GPR56* (G-protein-coupled receptor 56), *KIAA1279*, *RAB3GAP1* (RAB3 GTPase-activating protein subunit 1), and *COL18A1* (collagen, type XVIII, alpha 1), with all but *SRPX2* found in rare syndromes (Spalice et al., 2009).

The genetic role in the etiopathogenesis of PMG is also supported by its association with Aicardi syndrome, Zellweger syndrome, and WWS or with chromosomal abnormalities such as 22q11 deletion, 1p36 monosomy, and trisomy of chromosome 13 (Pang et al., 2008; Verrotti et al., 2010).

The familial transmission of PMG has been identified in bilateral frontoparietal, bilateral perysylvian, and bilateral generalized forms (Pang et al., 2008; Verrotti et al., 2010). Bilateral frontoparietal PMG seems to be related to the mutation of the gene *GPR56* on chromosome 16q12.2–21 with an autosomic recessive inheritance (Pang et al., 2008; Verrotti et al., 2010). This gene encodes for a G-protein-coupled receptor, a regulator of cell cycle signaling in neuronal progenitor cells at all ages, and plays an essential role in the regional patterning of the human cerebral cortex (Spalice et al., 2009; Verrotti et al., 2010).

Bilateral perysylvian PMG is mainly attributed to different patterns of inheritance, including X-linked dominant, X-linked recessive, autosomal recessive, autosomal dominant with reduced penetrance, autosomal recessive with pseudodominance, and autosomal dominant (Verrotti et al., 2010). A locus for X-linked bilateral perisylvian PMG maps on the distal long arm of the X chromosome (Xq28), but the linkage has not been confirmed and no gene has been identified (Verrotti et al., 2010).

TBR2 is a gene involved in the genesis of PMG, associated with microcephaly and corpus callosum agenesis (Verrotti et al., 2010). It maps to chromosome 3p (Verrotti et al., 2010). This gene encodes a transcription factor, a member of the T-box family, critical in invertebrate embryonic development of the central nervous system and mesoderm (Verrotti et al., 2010). It may also be involved in neuronal division and migration (Verrotti et al., 2010).

31.5.3.3 *Neuroradiological findings*

MRI in PMG demonstrates small irregular gyri and an indistinct gray and white matter junction (Verrotti et al., 2010). The polymicrogyric cortex often appears mildly thickened (6–10 mm) because of cortical overfolding (Verrotti et al., 2010). T2 signals within the cortex are usually normal, although there may be delayed myelination (Verrotti et al., 2010).

31.5.4 Schizencephaly

SCZ is a structural abnormality of the brain, characterized by congenital clefts spanning the cerebral hemisphere from the pial surface to the lateral ventricle and lined by cortical gray matter with communication between the ventricles and extra-axial subarachnoid space (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). Cleft localization varies widely, but the perisylvian region is more frequently involved (Spalice et al., 2009; Verrotti et al., 2010). The cortex overlying the cleft is often polymicrogyric (Verrotti et al., 2010). Actually, SCZ is classified within the same group as PMG (Pang et al., 2008; Verrotti et al., 2010).

This malformation can be unilateral or bilateral, symmetric or asymmetric, and can be divided into two subtypes: 'closed or fused lips' or type I (if the cleft walls are in apposition) and 'open lips' or type II (if the cleft walls are separated) (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). Type II SCZ is more common than type I (Barth et al., 2007). SCZ tends to involve the insular, precentral, and postcentral regions (Pang et al., 2008). In addition, SCZ is often associated with septo-optic dysplasia (Spalice et al., 2009). The etiology of this disorder has not been clearly established, and several causes, including genetic, vascular, toxic, metabolic, and infectious factors, may be involved (Spalice et al., 2009; Verrotti et al., 2010).

31.5.4.1 *Clinical characteristics*

Patients with unilateral closed-lipped SCZ generally have mild hemiparesis and seizures but no impairment of normal developmental milestones (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). When the cleft is open, patients have mild to moderate developmental delay, microcephaly, seizures, spasticity, and hemiparesis (Pang et al., 2008; Verrotti et al., 2010). Patients with bilateral clefts show severe mental deficits and severe motor abnormalities, including spastic quadriparesis (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). Blindness due to optic nerve hypoplasia can be common (Verrotti et al., 2010). Language development is more likely to be normal in patients with unilateral SCZ compared to patients with bilateral clefts (Spalice et al., 2009; Verrotti et al., 2010). Noncentral nervous system manifestations have also been reported, such as gastroschisis and bowel atresias (Pang et al., 2008).

Several types of seizure have been reported, including generalized tonic–clonic, partial motor, and sensorial (Spalice et al., 2009; Verrotti et al., 2010). Infantile spasms have been described in a few children (Verrotti et al., 2010). Seizures are usually resistant to medical therapy, and stabilization may be achieved through surgery (Verrotti et al., 2010).

31.5.4.2 *Genetics*

EMX2 (empty spiracles homeobox 2) gene mutations may be correlated with type II SCZ (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). *EMX2* is a homeotic gene expressed in proliferating neuroblasts and is probably involved in controlling cortical migration and structural patterning of the developing rostral brain (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010). However, recent studies criticize the true role of *EMX2* in SCZ (Pang et al., 2008; Spalice et al., 2009; Verrotti et al., 2010).

31.5.4.3 *Neuroradiological findings*

In addition to PMG, MRI may demonstrate agenesis of the septum pellucidum and agenesis or thinning of the corpus callosum, hippocampal malformations, posterior fossa abnormalities, ventricular diverticula and arachnoid cysts, and multiple calcifications (Pang et al., 2008; Verrotti et al., 2010). Periventricular heterotopic nodules have also been found in a minority of cases (Verrotti et al., 2010). The malformations associated with SCZ may also involve extracerebral structures (Verrotti et al., 2010).

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