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The Ciliary Baton: Orchestrating Neural Crest Cell Development

Ching-Fang Chang[*,†], **Elizabeth N. Schock**[*,†], **Aria C. Attia**[‡], **Rolf W. Stottmann**[†,‡], **Samantha A. Brugmann**[*,†,1]

[*]Division of Plastic Surgery, Department of Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

[†]Division of Developmental Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

[‡]Division of Human Genetics, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

Abstract

Primary cilia are cell surface, microtubule-based organelles that dynamically extend from cells to receive and process molecular and mechanical signaling cues. In the last decade, this organelle has gained increasing popularity due to its ability to act as a cellular antenna, receive molecular stimuli, and respond to the cell's environment. A growing field of data suggests that various tissues utilize and interpret the loss of cilia in different ways. Thus, careful examination of the role of cilia on individual cell types and tissues is necessary. Neural crest cells (NCCs) are an excellent example of cells that survey their environment for developmental cues. In this review, we discuss how NCCs utilize primary cilia during their ontogenic development, paying special attention to the role primary cilia play in processing developmental signals required for NCC specification, migration, proliferation, and differentiation. We also discuss how the loss of functional cilia on cranial and trunk NCCs affects the development of various organ systems to which they contribute. A deeper understanding of ciliary function could contribute greatly to understanding the molecular mechanisms guiding NCC development and differentiation. Furthermore, superimposing the ciliary contribution on our current understanding of NCC development identifies new avenues for therapeutic intervention in neurocristopathies.

1. INTRODUCTION

Over 100 years ago, biologists observed a thin, singular eyelash-type extension on various cell types (Zimmermann, 1898). These scientists, without any of the molecular knowledge or tools we have today, hypothesized that this "cilium" had a sensory function within the cell (Zimmermann, 1898). Sadly, after this initial discovery the field of ciliary biology lay essentially dormant for the next 80 years. Now, in the past two decades, the field of ciliary biology has undergone a renaissance due to improved molecular and cellular tools and a better understanding of what these nearly ubiquitous organelles are doing during various cellular processes. This review will discuss what a cilium is, the process of ciliogenesis

[1]Corresponding author: samantha.brugmann@cchmc.org.

and ciliary function. We will highlight what is currently known regarding how neural crest cells (NCCs) utilize primary cilia during development and the disorders affecting the NCC derivatives that can arise when ciliary structure or function is disrupted.

2. THE PRIMARY CILIUM: DEFINING THE ORGANELLE

2.1. Structure equals function

A cilium is a microtubule-based cellular projection (Fig. 1). There are several types of cilia, which are typically classified by their microtubule arrangement, capacity for motility, and number per cell. Generally, and for the purposes of this review, a primary cilium is defined by three criteria, (1) a 9+0 microtubule arrangement, (2) lack of motility, and (3) cellular singularity. Establishing how these three criteria define the organelle is important to understand primary cilia function during various cellular processes, including NCC development. Furthermore, understanding how variations in these key characteristics affect ciliary function is also important for gaining a better understanding of how cilia, primary and otherwise, function within a cell.

The 9+0 conformation of a primary cilium, as the name implies, refers to an arrangement of axonemal microtubules in an outer ring of 9 microtubule doublets with an absence of any inner microtubule pair (Fig. 1). Other types of cilia can have a 9+2 arrangement consisting of an outer ring of 9 microtubule doublets and a central core of 2 microtubules. Even 9+4 cilia have been reported in some species (Feistel & Blum, 2006).

The second feature common to primary cilia is their nonmotile status. Microtubule arrangement broadly correlates with cilium motility: 9+0 cilia are canonically not motile and 9+2 cilia are motile. While this is a general rule to go by, it is more accurate to use the presence or absence of dynein arms to determine motility of the cilium (AfZelius, 1976; Blum, Hayes, Whisnant, & Rosen, 1977). Dyneins are large motor protein complexes that generate force, causing the movement of eukaryotic cilia and flagella. The dynein protein complexes directly connect to the outer ring of microtubules (Neesen et al., 2001). Generally, 9+0 cilia and nonmotile 9+2 cilia lack dynein arms (Bloodgood, 2009).

Finally, primary cilia are reported to be solitary cellular extensions (i.e., each cell only extends one primary cilium). Similar to motility, cilia number has also been linked to microtubule arrangement. Monociliated cells are generally 9+0 and nonmotile, whereas multiciliated cells are generally 9+2 and motile. There are, however, several exceptions to this generality, thus it should not be considered a strict rule. Nodal cilia are perhaps the best example of an exception to this general classification. Current data suggest that the node may possess a mixture of both motile and nonmotile monocilia (McGrath, Somlo, Makova, Tian, & Brueckner, 2003) as well as a mixture of 9+2 and 9+0 monocilia (Caspary, Larkins, & Anderson, 2007).

2.2. Widespread and dynamic

The last 10 years have witnessed increased interest in the primary cilia (Beales & Jackson, 2012). The major causes of this resurgence are twofold; cilia are omnipresent and highly dynamic. First, as with most organelles, they are present in almost all cells rendering their

function relevant to just about every tissue and organ system. However, there are a handful of cells and organisms that lack primary cilia. Yeast, fungi, and higher plants do not extend cilia. Of the "model organisms" classically used in biomedical research, the invertebrate *C. elegans* extend cilia only on sensory neurons, and none of the cilia in the nematode are motile. Similarly, *Drosophila* also extend cilia on sensory cells, but they are not utilized in the same way as mammalian cilia (Avidor-Reiss et al., 2004; Han, Kwok, & Kernan, 2003; Ray et al., 1999). Primary cilia are much better understood in vertebrates. Still, there are some mammalian cells that lack primary cilia including hepatocytes, acinar cells, and lymphocytes and granulocytes in the hematopoietic lineage (Wheatley, 1969; Wheatley, Wang, & Strugnell, 1996).

Second, unlike many organelles, primary cilia are highly dynamic in their extension, size, and specialization. Primary cilia are extended during times of quiescence (G_0) and begin to retract during cell cycle re-entry. This cyclical extension and retraction suggests a mutually exclusive relationship between the cell cycle and ciliogenesis. Indeed, various studies have shown that cell cycle progression requires the continued suppression of primary cilia formation (Goto, Inoko, & Inagaki, 2013). Furthermore, links have been established between ciliary extension and cancer, with some cancers being devoid of primary cilia and some depending on ciliary signaling (Basten & Giles, 2013; Molla-Herman et al., 2010; Wong et al., 2009). Although a bona fide understanding of the exact nature of the relationship between cilia and the cell cycle remains elusive, the current data open the door to the possibility of therapeutic intervention via ciliary targeting, in diseases of aberrant cellular proliferation (Basten & Giles, 2013).

In addition to their dynamic extension and retraction, primary cilia can also vary in length. Under most circumstances, primary cilia are between 1 and 10 μm long, but they can extend to over 20 μm. Various factors can influence the length of cilia including injury/hypoxia (Verghese, Weidenfeld, Bertram, Ricardo, & Deane, 2008; Verghese, Zhuang, Saiti, Ricardo, & Deane, 2011), treatment with monovalent cation chlorides (LiCl, NaCl, and KCl) (Miyoshi, Kasahara, Miyazaki, & Asanuma, 2009; Ou et al., 2009), cytoskeletal dynamics (Miyoshi, Kasahara, Miyazaki, & Asanuma, 2011), and autophagy (Tang et al., 2013; Tang, Zhu, & Zhong, 2014). It is hypothesized that with all other variables being constant, increased length of the cilium is directly related to the sensitivity of the organelle. Understanding the factors that regulate cilium length could determine how sensitive a cell is to the molecular and mechanical stimuli in the surrounding environment. Thus, a feedback system dictating primary cilium length could be an important yet underappreciated factor regulating development (Miyoshi et al., 2011).

2.3. Ciliogenesis: Building the cilium

The cilium itself is composed of various functional domains required for proper ciliary function: basal bodies (centrosomes), transition fibers, transition zone, intraflagellar transport (IFT) machinery, axoneme, and a specialized ciliary membrane (Fig. 1). In cycling cells, two centrioles (mother and daughter) together with pericentriolar material form the centrosome, which serves as microtubule organization center. Once cells enter G_1/G_0 phase, the mother centriole differentiates into a basal body before ciliogenesis initiates (Kobayashi

& Dynlacht, 2011). Similar to the mother centriole, a basal body is comprised of 9 microtubule triplets arranging in a barrel shape, a subdistal appendage (basal foot), and a distal appendage (transition fiber, Fig. 1).

Depending on cell type, ciliogenesis can occur via two distinct mechanisms: (1) extracellular ciliogenesis, where the basal body docks and fuses to the plasma membrane directly or (2) intracellular ciliogenesis, where the basal body first fuses to a ciliary vesicle in the cytoplasm before docking to the plasma membrane (Molla-Herman et al., 2010; Reiter, Blacque, & Leroux, 2012; Sorokin, 1962; Fig. 2). In both pathways, the initial docking is facilitated by the interaction between the membrane and transition fibers. The primary ciliary vesicle adds additional membrane by recruiting secondary vesicles. The enlarged vesicle soon deforms into an invaginated sac, which forms a double membrane sheath (later becoming the ciliary pocket) surrounding the apical end of basal body (Fig. 2). At this point, the transition zone begins to emerge as microtubule doublets start to assemble within the vesicle sac.

While the transition zone is growing, the basal body–ciliary vesicle migrates to the membrane through a process dependent on both the actin cytoskeleton and membrane-associated components in the transition zone (Dawe, Farr, & Gull, 2007). As the basal body–ciliary vesicle complex migrates to, and fuses with, the plasma membrane, the transition zone matures and is now able to function as a ciliary gate. The transition zone is characterized by distinctive Y-shaped fibers, which connect microtubule doublets to the ciliary membrane. Structurally, the transition zone stabilizes the basal body–ciliary vesicle after the initial docking. Functionally, all these components collectively serve as the ciliary gate, a domain required for establishing a distinct ciliary compartment separate from the rest of the cell body.

After formation of the ciliary compartment, the axoneme starts to extend via microtubule lengthening. There is a distinct set of posttranslational modifications on axonemal tubulins as the microtubules are acetylated, detyrosinated, polyglutamylated, and polyglycylated (Konno, Setou, & Ikegami, 2012). These modifications are important for axonemal stability. The microtubule extension occurs through a process called IFT. In IFT, the anterograde transport is carried out by IFT-B complexes and kinesin II motors. IFT builds the microtubule scaffold and carries essential ciliary proteins to the distal tip of the axoneme. Finally, numerous receptors and ion channels localize to the specialized membrane surrounding the fully extended axoneme. With the axoneme extended and functional, cilia are now capable of surveying the cell’s molecular environment. Various cell types utilize these cilia in different ways. In Section 3, we focus on how NCCs utilize primary cilia during their development and differentiation.

3. THE ROLE OF PRIMARY CILIA DURING NCC ONTOGENY

During development, NCCs progress through multiple phases: specification, migration, proliferation, and differentiation. While there are entire fields of study on each phase, little is known regarding primary cilia function during these phases. In this portion of the review, we summarize what is known about primary cilia with respect to each phase.

3.1. Primary cilia and NCC specification

NCC specification occurs at the boundary between the neural plate and the nonneural ectoderm. At this junction Wnt, BMP, and fibroblast growth factor (FGF) signaling initiates a transcriptional program (e.g., *Pax3, Pax7, Msx*, and *Zic1*) that will define the neural plate boarder (Garcia-Castro, Marcelle, & Bronner-Fraser, 2002; LaBonne & Bronner-Fraser, 1999; Marchant, Linker, Ruiz, Guerrero, & Mayor, 1998; Mayor, Guerrero, & Martinez, 1997; Monsoro-Burq, Fletcher, & Harland, 2003). Within this region, both NCCs and placodal cells are specified; however, NCCs express a unique combination of transcription factors including *Snail2, Sox10, Sox9*, and *FoxD3* (Bronner & LeDouarin, 2012). While no in-depth study has been conducted specifically examining if primary cilia are required for NCC specification, postmigration data can be used to infer the answer to this question. Various conditional knockouts of ciliary proteins, using NCC drivers, still exhibit the formation of NCCs (Brugmann et al., 2010; Brugmann, Stottmann, unpublished). This suggests that NCCs are specified, to some appreciable extent, in the absence of normal primary cilia function. This conclusion is further supported by data from zebrafish *bbs* morphant embryos, which exhibit normal domains of *foxd3* or *sox10* expression suggesting that NCCs are specified properly (Tobin et al., 2008).

While data from both of these model systems support the hypothesis that primary cilia are not absolutely required for proper NCC specification, data from $Fuz^{-/-}$ murine mutants suggests the cilia regulate NCC number. In these mutants, lineage tracing of NCCs indicates an expansion in the domain and number of NCCs in the midbrain, hindbrain, and maxillary process (Gray et al., 2009; Tabler et al., 2013). Taken together, these studies suggest a number of interesting possibilities regarding NCCs and primary cilia. First, perhaps only certain ciliary proteins play a role in NCC specification. Whereas, both Kif3a and the Bardet–Biedl syndrome (BBS) proteins are required for ciliogenesis and localize to the cilia and basal body (Nachury et al., 2007; Yamazaki, Nakata, Okada, & Hirokawa, 1995), respectively, Fuzzy is a cilia-associated protein that functions in the cytoplasm as an effector of the planar cell polarity (PCP) pathway (Brooks & Wallingford, 2012; Park, Haigo, & Wallingford, 2006). Thus, $Fuz^{-/-}$ mutants may have defects in NCC specification due to a cilia-independent mechanism. Second, since $Fuz^{-/-}$ is a null mutant (rather than the conditional knockout targeting ciliary function specifically in NCCs), it is possible that the loss of cilia in surrounding tissues negatively affects the molecular crosstalk required for NCC specification. Continued examination of NCC specification in various ciliary mutants is necessary to clarify this issue.

3.2. Primary cilia and NCC migration

Following specification, NCCs will undergo an epithelial to mesenchymal transition, delaminate from the neural tube and migrate in a highly regulated process. Broadly, NCC migration requires two main types of interactions: (1) between NCCs and the surrounding tissues and (2) between individual migrating NCCs. These interactions are dependent upon NCCs receiving and interpreting either long-range or short-range cellular signals (Teddy & Kulesa, 2004). For migration to efficiently occur, NCCs must be capable of responding to attractants/repellants and creating discrete, directional migration streams (Kuriyama & Mayor, 2008; Teddy & Kulesa, 2004). Chemotaxis, the movement of a cell toward a

directive substance, is also a guiding force of NCC migration (Theveneau & Mayor, 2012). Several chemotactic molecules essential for cranial NCC migration have been identified including SDF-1, PDGF-AA, and VEGFA (Theveneau & Mayor, 2012). The receptors for some of these molecules are found on primary cilia (Schneider et al., 2005).

Several ciliary mutants exhibit aberrant NCC cell migration. For example, *bbs* zebrafish morphants have a significant reduction in the migratory streams of both cranial and trunk NCCs. Through transplantation studies, it was determined that *bbs8* is required cell-autonomously for proper NCC migration (Tobin et al., 2008). *Fto* zebrafish morphants also have NCC migration defects as evidenced by diffuse and reduced *Sox10* expression in the head (Osborn et al., 2014). More caudal NCC migration is also severely disrupted in these mutants. NCCs posterior to the seventh somite fail to migrate away from the dorsal neural tube and melanocytes are mislocalized (Osborn et al., 2014). In support of these data from animal models, fibroblasts from ciliopathy patients also fail to migrate normally and exhibit disruptions in actin cytoskeletal architecture (Hernandez-Hernandez et al., 2013; Madhivanan, Mukherjee, & Aguilar, 2012; Tobin et al., 2008).

While, it is clear that aberrant ciliary function can negatively impact NCC migration, we currently lack an understanding of *how* primary cilia regulate migration. One potential mechanism is platelet-derived growth factor (PDGF)-dependent chemotaxis as PDGF is a known NCC chemoattractant, PDGFRα localizes to the axoneme, and PDGF signaling is transduced through the primary cilium (Schneider et al., 2005). Additionally, studies in both fibroblasts and mouse embryonic fibroblasts (MEFs) demonstrated primary cilia are required for directed migration toward a PDGF-AA source (Schneider et al., 2005, 2010). Interestingly, loss of PDGFRα on cranial NCCs yields aberrant cranial NCC migration (He & Soriano, 2013). Another possible mechanism to affect NCC migration is via regulation of the PCP pathway. The PCP proteins Vangl2 and Dishevelled have been shown to localize to the primary cilium and have genetic interactions with both IFT and basal body proteins (Jones et al., 2008; Ross et al., 2005; Veland et al., 2013). Additionally, ciliopathies such as Lowe, Joubert, and Bardet–Biedl syndromes have defects in RhoA or Rac1 localization or activity, and RhoA has also been show to localize to the basal body (Hernandez-Hernandez et al., 2013; Madhivanan et al., 2012; Valente et al., 2010). All of these parallel findings support the hypothesis that primary cilia play an important role during NCC migration, and we suggest this is an area ripe for continued investigation.

3.3. Primary cilia and NCC proliferation

After NCCs have finished migrating, they will continue to proliferate and greatly expand in their target tissues. Primary cilia extension and the cell cycle are tightly linked together. For example, IFT88 is associated with the centrosome throughout the cell cycle (Robert et al., 2007) and is required for spindle orientation in mitosis (Delaval, Bright, Lawson, & Doxsey, 2011). Furthermore, overexpression of IFT88 prevents the G_1–S transition and induces apoptotic cell death, whereas knockdown of IFT88 promotes cell cycle progression (Robert et al., 2007). Data from mouse ciliopathic mutants indicate that primary cilia on NCCs are essential for regulating proliferation, especially in the developing face. *Kif3a*$^{f/f}$*;Wnt1-Cre* mutants have increased NCC proliferation. This increase is most notable at the facial midline

of embryos and correlates with an expansion of *Gli1* expression. Phenotypically, the medial increase in proliferation manifests as an expanded midline in these mutants (Brugmann et al., 2010; Fig. 3).

Although the link to proliferation is clear in *Kif3a*$^{f/f}$*;Wnt1-Cre* mutants, the effect that loss of other ciliary proteins has on proliferation is highly variable. *Fuz*$^{-/-}$ mutants have hyperplastic maxillary prominences that result in a high, arched palate, yet NCC proliferation is actually decreased (Tabler et al., 2013). Furthermore, there are other ciliary mutants that do not show any indication of alterations in NCC proliferation or midline development, such as *Ttc21b*$^{f/null}$*;Wnt1-Cre* (Fig. 3, Stottmann unpublished). These data reiterate that cilia have a role in proliferation, but also suggest that the exact role the cilia play during proliferation is unclear. Currently, the most accurate conclusion to draw from these data is that individual ciliary proteins play specific roles during proliferation.

3.4. Primary cilia and NCC differentiation

NCCs give rise to a wide variety of cell types including bone and cartilage cells in the facial skeleton. Both patients with ciliopathies and ciliopathic animal models often have dysmorphic craniofacial skeletons. In zebrafish, *bbs, ift*, and *ofd1* morphants have dysmorphic craniofacial cartilage (Ferrante et al., 2009; Lunt, Haynes, & Perkins, 2009; Tobin et al., 2008). Murine ciliopathic mutants also frequently have skeletal abnormalities. *Msk*^{krc} mutants exhibit misshapen skulls and a decrease in frontal and parietal bone ossification (Weatherbee, Niswander, & Anderson, 2009). Humans with Meckel syndrome, often caused by mutations in the Msk gene, have skeletal malformation of the cranial base (Kjaer, Hansen, Keeling, Nolting, & Kjaer, 1999). Thus, taken together, these data demonstrate that cranioskeletal defects are a common feature of human ciliopathies and further suggest that primary cilia may play an important role in NCC differentiation into skeletal elements.

In addition to the importance of primary cilia for initial skeletal differentiation, they are also critical for postnatal changes in the skeleton. Mechanosensation is another cellular process that helps to shape the architecture of skeletal elements. Primary cilia are able to respond to mechanical signals (Hoey, Downs, & Jacobs, 2012). Specific subsets of ciliary proteins have been shown to be involved in these mechanical-based signaling responses. For example, PKD2 localizes to the primary cilium and is essential for fluid-flow sensation (Nauli et al., 2003). *Pkd2*$^{f/f}$*;Wnt1-Cre* postnatal mutants exhibit dome-shaped skull defects, anterior-posterior snout compression, and abnormal fusion in the face. These abnormalities are not detected in embryos, suggesting that these phenotypes are the result of an inability of NCC derivatives to properly respond to postnatal mechanical forces. These data suggest that primary cilia continue to play a role in development and remodeling of NCC-derived structures even after cell differentiation.

4. CRANIOFACIAL PHENOTYPES IN ANIMAL MODELS AND HUMAN PATIENTS SUPPORT A ROLE FOR PRIMARY CILIA IN NCC DEVELOPMENT

4.1. Insights from animal models

In addition to the ciliary mutants in which early stages of NCC development have been analyzed, there are a significant number of ciliary mouse mutants with craniofacial phenotypes suggesting aberrant NCC behavior (Table 1). For example, several mutants have cleft lip and/or palate, including the *Ift172*^{avc1} allele with cleft secondary palate (Friedland-Little et al., 2011), *Rpgrip1l* with cleft lip and hypoplastic mandible (Delous et al., 2007), *Mks1* with cleft lip/palate and pointy snout (Weatherbee et al., 2009), and *Kif7* with cleft lip/palate (direct submission by Cecilia Lo to MGI). Other mutants are reported to have phenotypes in the arches, without clefting defects, including *Dync2h1* (formerly *Dnchc2*), which exhibits micrognathia (C Lo direct submission to MGI), *ft57 (Hippi)*, which displays hypertelorism and small arches (Houde et al., 2006), *Ift52*^{hypo} with fused maxillary prominences (Liu et al., 2005), *Tmem67* with mandibular hypoplasia (Abdelhamed et al., 2013), and *ft122* mutants with enlarged branchial arches (Cortellino et al., 2009). Furthermore, an in-depth morphometric analysis was performed on BBS mouse models and both *Bbs4* and *Bbs6* homozygous mutants have larger ratios of mid-face width to height and shortened snouts (Tobin et al., 2008). These craniofacial defects strongly suggest NCC patterning and/or development is frequently affected in ciliary mutants.

Other mutations seem to reveal broader requirements for some ciliary genes. The *Ift88*^{Tg737Rpw} mice have been described with poorly differentiated branchial arches, minor clefting, supernumerary teeth, and disorganized and patent frontal sutures (Murcia et al., 2000; Zhang et al., 2003). Loss of *Ofd1* leads to both severe cleft palate and a shortened skull (Ferrante et al., 2006). *Evc* mutants have abnormal incisors (Ruiz-Perez et al., 2007). These findings would suggest that ciliary proteins execute a number of roles in craniofacial development, but that loss of particular ciliary genes leads to specific phenotypes. This latter conclusion resonates with our model that cilia in distinct regions of an embryo have possibly unique roles in specific developmental signaling events and not all cilia have the same complement of proteins. However, we must also consider that mouse experiments on different genetic backgrounds may incorporate different modifier genes, which further complicate comparisons between ciliary gene loss-of-function phenotypes. This is consistent with the known multi-allelic effects in ciliopathies (Davis et al., 2011).

Additional insight into the role of cilia in NCC development can be gleaned from a recent study of the *Fuzzy* mouse (Tabler et al., 2013). This is a unique mutant that exhibits a high arched palate, a phenotype identified in a high proportion of BBS patients (Beales, Elcioglu, Woolf, Parker, & Flinter, 1999). A detailed developmental analysis presented in this study uncovered an enlarged maxillary process and aberrant patterns of NCC migration in E9.5 embryos. Interestingly, a NCC specific ablation of *Fuzzy* with *Wnt1-Cre* resulted in a true cleft phenotype without the associated maxillary hypoplasia or hypoplastic tongue (Tabler et al., 2013). Together, these findings are consistent with our model that cilia are actually performing discrete tasks in different regions of the embryo and future studies will need to incorporate this idea to fully explain the role of cilia in craniofacial NCCs.

While mouse models allow for insights into mammalian ciliary function, both fish and chicken models have proven very valuable, especially when evaluating early NCC development. Several fish mutants and morphants exist allowing for rapid evaluation of NCC development, migration, and differentiation (Beales et al., 2007; Ferrante et al., 2009; Lunt et al., 2009; Tobin et al., 2008; Walczak-Sztulpa et al., 2010; Table 1). In addition, two separate, naturally occurring ciliopathic models have been identified in the chicken. The causal genetic elements for both the *talpid*^{2} and *talpid*^{3} mutants have recently been identified (Chang et al., 2014; Yin et al., 2009). The accessibility of the chicken embryo, and its availability for culturing and transplanting tissues, provides promise for careful analysis of how primary cilia participate in each individual step of NCC development.

4.2. Human craniofacial ciliopathies

Given the data generated in animal models, it is not surprising there is a growing class of human craniofacial ciliopathies with features indicative of aberrant NCC development. Some years ago, Baker and Beales reviewed 102 human conditions of known or possible ciliopathies. Of the 102 conditions, the craniofacial complex was primarily affected in up to 30% of cases (Zaghloul & Brugmann, 2011). Although primary cilia extend from almost all cells in the body, the clinical features observed in patients with ciliopathies highlight the importance of primary cilia in development of certain types of tissue. The phenotypes associated with human craniofacial ciliopathies were similar to those observed in animal models: midline, cephalic, oral, and dental anomalies (Zaghloul & Brugmann, 2011; Table 2). Three such examples of human craniofacial ciliopathies include Meckel–Gruber syndrome (MKS), BBS, and Oro-facial-digital syndrome (OFD). MKS presents with cleft lip in 20% of cases and cleft lip and palate in 45% of cases. Clefting is accompanied by tongue malformations including ankyloglossia, bifid tongue, and anterior marginal hamartomas (Fried, Liban, Lurie, Friedman, & Reisner, 1971; Moerman, Verbeken, Fryns, Goddeeris, & Lauweryns, 1982; Rehder & Labbe, 1981). BBS is associated with oligodontia, hypodontia, and numerous other dental abnormalities (Lofterod, Riise, Skuseth, & Storhaug, 1990). Additional studies have reported characteristic midfacial defects hypothesized to be due to improper hedgehog induced NCC migration (Tobin et al., 2008). A host of other defects related to NCC development/migration including hypopigmentation, heart defects, middle ear defects, choanal atresia, and Hirschsprung disease have also been reported in BBS patients (Gorlin, Cohen, & Levin, 1990). OFD is characterized by a wide nose with a broad, flat nasal bridge, hypertelorism, cleft lip and palate, glossal deformities, hyperplastic frenula, the growth of noncancerous tumors and hypodontia. OFD has long been linked to mutations in *OFD1* (Ferrante et al., 2001; Gurrieri, Franco, Toriello, & Neri, 2007). Recently however, a new subtype of OFD has been identified and linked to mutations in *C2CD3* (Thauvin-Robinet et al., 2014); the same gene recently found responsible for the *talpid*^{2} avian mutant (Chang et al., 2014). The rapid identification of new ciliary genes and ciliopathic models suggests that many yet to be classified syndromes may in fact be ciliopathies. Furthermore, the high frequency of dysmorphic faces suggests NCCs are indeed highly sensitive to the loss of primary cilia.

5. BEYOND THE FACE: TRUNK NCCs ARE ALSO AFFECTED BY THE LOSS OF PRIMARY CILIA

Although NCCs make substantial and vital contributions to the face, they also contribute to many other lineages and primary cilia have been implicated in proper development of many of these populations as well. The best-studied NCC lineage outside the face is arguably the cardiac NCC. Cardiac NCCs normally make a significant contribution to the outflow tract of the heart, which separates the pulmonary and systemic circulations.

An elegant example of a role for primary cilia in cardiac development is the zebrafish *cobblestone (cbs)* mutant. *Cbs* is a hypomorphic allele of *Ift88* and homozygous mutants present with a number of the most common congenital heart defects seen in newborn humans including: ventricular and atrial septal defects, and persistent truncus arteriosus (failure of outflow tract to divide). Further study of this mutation showed that the cardiac NCCs migrated normally into the heart, but the NCC-derived outflow tract exhibited a significant reduction in expression of *Bmp4* (Willaredt, Gorgas, Gardner, & Tucker, 2012). In our own studies, we have noted frequent edema in late-embryonic stage *Ttc21b*^{aln} mutants and abnormal patterns of NCC migration into the heart (Fig. 4). Thus, primary cilia are important for the molecular signaling that guides outflow tract development.

Another NCC derivative affected by loss of cilia is the enteric nervous system, the main division of the autonomic nervous system that innervates the gut. A failure of NCC migration to the gut or differentiation into enteric ganglia results in Hirschsprung disease (aganglionic megacolon; McKeown, Stamp, Hao, & Young, 2013), a disease in which variable portions of the gut lose the ability to relax. Hirschsprung disease is frequently present in both BBS patients (Amiel et al., 2001) and ciliopathic animal models. Zebrafish *bbs8* morphants have decreased vagal NCC and incomplete enteric nervous system formation leading to disordered gut motility (Tobin et al., 2008). Furthermore, reduction of *ADP ribosylation factor-like 6 interacting protein 1 (Arl6ip1)* in zebrafish produces disorganized cilia, dampened *sonic hedgehog (Shh)* signaling and subsequent reduction in the number of enteric ganglia (Tu, Yang, Huang, & Tsai, 2012).

Various other NCC derivatives extend cilia and are affected by a loss of primary cilia. Melanocytes extend cilia, both *in vitro* (Le Coz, Benmerah, & Larue, 2014) and *in vivo* (Wandel, Steigleder, & Bodeux, 1984) and zebrafish *bbs8* morphants exhibit a pigmentation phenotype (Tobin et al., 2008). Primary cilia have been identified on corneal endothelial cells in a number of mammals (Collin & Barry Collin, 2004; Doughty, 1998) and Polaris mutants have disorganized patterning of the corneal endothelium (Blitzer et al., 2011). Although no studies have reported defects in the dorsal root ganglia or sympathetic ganglia, we have noted defects in both of these lineages in the *Ttc21b*^{aln} mutants (Stottmann, unpublished).

6. NCCs UTILIZE PRIMARY CILIA FOR TISSUE-TISSUE INTERACTIONS

6.1. Structures that require reciprocal signaling are disrupted in ciliary mutants

Throughout the course of development NCCs interact with cells from all three germ layers to form epithelial specializations or discrete organs. Many structures that require reciprocal signaling with NCCs for their formation are disrupted in ciliary mutants. Prime examples of structures or tissues that require molecular input from NCCs are teeth, whiskers, and the tongue.

Odontogenesis, or tooth formation, requires reciprocal interactions between the oral ectoderm and the underlying NCC-derived mesenchyme (Thesleff 2006). Many human ciliopathies including Ellis–van Creveld syndrome, OFD syndrome, and Cranioectodermal dysplasia exhibit abnormal dentition (Gurrieri et al., 2007; Mostafa, Temtamy, el-Gammal, & Mazen, 2005; Walczak-Sztulpa et al., 2010; Table 2). Studies in mice provide the genetic advantage of tissue-specific conditional deletions of primary cilia. *Ift88* hypomorphs develop supernumerary teeth accompanied by an expansion of *Gli1* expression; however, when a keratin (K)-5-Cre driver is used to delete *Ift88* from epithelial cells, no dental abnormalities are observed (Ohazama et al., 2009). This suggests that cilia are required specifically on NCCs for proper tooth formation. This hypothesis is supported by data from *Kif3a*$^{f/f}$*;Wnt1-Cre* mutants where Shh and Wnt activity in the dental lamina is aberrant (Brugmann et al., 2010), and tooth development is abnormal (Fig. 5). Together, these data suggest that primary cilia are essential mediators of NCC-epithelial signaling during odontogenesis.

A curious example of a ciliopathy causing dental abnormalities is the avian ciliopathic mutant, *talpid*^{2}, which develops crocodilian-like teeth (Brugmann et al., 2010; Chang et al., 2014; Harris, Hasso, Ferguson, & Fallon, 2006). Modern birds lack teeth. However, some studies suggest that the avian oral ectoderm still possesses instructive signals for forming teeth. Thus, it was hypothesized that avian dentition does not form because the competent NCC-derived mesenchyme is not in a position to receive instructive signaling from the ectoderm (Kollar & Fisher, 1980; Kollar & Mina, 1991). In *talpid*^{2}, the oral/aboral boundary is shifted such that competent mesenchyme is in a position to receive instructive signal for the ectoderm, promoting specification of dentition (Harris et al., 2006). It is unclear the exact role primary cilia play in this context, as this study was performed prior to the identification of *talpid*^{2} as ciliopathic mutant, but these data further suggest that reciprocal signaling between oral ectoderm and NCC-derived mesenchyme require primary cilia.

Similar to ondontogenesis, whisker formation requires reciprocal interactions between NCC-derived mesenchyme and the overlying ectoderm (Chiang et al., 1999). When NCCs lack primary cilia, as in the *Kif3a*$^{f/f}$*;Wnt1-Cre* mutants, whiskers still form; however, they are reduced in number and disorganized (Brugmann et al., 2010). Instead of forming in a distinct spatial pattern, they are irregularly spaced (Fig. 5). While an in-depth analysis of whisker formation has not been carried out in cilia mutants, one might expect defects in primary cilia-dependent reciprocal signaling between overlying ectoderm and NCC-derived mesenchyme to at least partially explain this anomaly.

The developing tongue is comprised of three different cell types: epithelial cells encase the tongue, NCC-derived mesenchyme contribute to the vasculature and connective tissue of the tongue, and the mesodermal-derived myoblasts give rise to the muscles of the tongue (Parada, Han, & Chai, 2012). Mesoderm-derived myoblast progenitors associate with NCC-derived mesenchyme once they both populate the tongue anlage (Huang, Zhi, Izpisua-Belmonte, Christ, & Patel, 1999). In order for muscle differentiation to occur, there must be reciprocal interaction between NCCs and the mesoderm. Signaling molecules such as Fgf4, Fgf6, and BMP4 are important for myogenic cell proliferation and differentiation in the tongue and when BMP signaling is reduced intrinsically in NCCs, as is the case for *Alk5*$^{f/f}$*;Wnt1-Cre* mutants, tongue muscles are disrupted (Han, Zhao, Li, Pelikan, & Chai, 2014). Similarly, Shh signaling is also essential for tongue development and both *Gli3*$^{-/-}$ and *Smo*$^{n/c}$*;Wnt1-Cre* mice display abnormal tongue development (Huang, Goudy, Ketova, Litingtung, & Chiang, 2008; Jeong, Mao, Tenzen, Kottmann, & McMahon, 2004).

Several human ciliopathies and animal models encompass tongue malformations. Human ciliopathies that often present with glossal defects include OFD syndrome and Joubert syndrome (Table 2). Patients with OFD syndrome will frequently have a bifid tongue, sometimes accompanied by hamartomas (Gurrieri et al., 2007). With Joubert syndrome, a small percentage of patients will develop soft tissue tumors of the tongue (Pellegrino, Lensch, Muenke, & Chance, 1997). Others exhibit a groove in the midline of the tongue or frequently protrude their tongue rhythmically (Dahlstrom, Cookman, & Jain, 2000; Parisi, 2009). Numerous animal ciliopathic models have glossal defects. The avian *talpid*^{2} mutant exhibits hypoglossia/aglossia (Chang et al., 2014) and both the *Kif3a*$^{f/f}$*;Wnt1-Cre* and *Ift88*$^{f/f}$*;Wnt1-Cre* mouse mutants display aglossia (Brugmann et al., 2010; Fig. 3). It is tempting to hypothesize that primary cilia on NCCs are required to interpret the Shh signal from the epithelium or other molecular signals from the mesoderm important for specification/patterning of the tongue. It should be noted, however, that other ciliary mutants such as *Ttc21b*$^{f/f}$*;Wnt1-Cre* have normal tongues (Fig. 3). Further investigation is needed to understand why loss of certain ciliary proteins results in aglossia, while others do not.

In addition to craniofacial defects, many ciliopathic patients also have brain defects, including anencephaly, exencephaly, and hydrocephaly (Carter et al., 2012). Similar brain phenotypes are observed in several ciliary mutants including *Dynch2*^{GT}*, Hippi*$^{-/-}$*, hennin, Ift122*$^{-/-}$*, Msk*, and *Fuz* murine mutants (Caspary et al., 2007; Cortellino et al., 2009; Gray et al., 2009; Houde et al., 2006; May et al., 2005; Weatherbee et al., 2009). Interestingly, brain abnormalities can also occur when primary cilia are lost on NCCs. *Kif3a*$^{f/f}$*;Wnt1-Cre* mutants present with agenesis of the corpus callosum (Brugmann et al., 2010). In this case, the expression pattern of *Wnt1* in dorsal neural stem cells does not preclude that these phenotypes are due to loss of cilia within the neural plate. However, the possibility remains that loss of cilia affecting NCC derivatives, such as smooth muscle cells or pericytes, could contribute to the aberrant brain development observed in these mutants.

7. THE ROLE FOR PRIMARY CILIA IN MOLECULAR SIGNAL TRANSDUCTION

The data outlined above show NCCs require primary cilia for several developmental processes and intracellular interactions. As previously discussed, primary cilia are essential for the reception and processing of multiple signaling pathways. Below, we briefly summarize what is known regarding the role of primary cilia and transduction of various signaling pathways important in NCC development.

7.1. Sonic hedgehog

NCC survival, proliferation, and patterning are dependent on Shh signaling (Ahlgren & Bronner-Fraser, 1999; Ahlgren, Thakur, & Bronner-Fraser, 2002; Brito, Teillet, & Le Douarin, 2008; Marcucio & Helms, 2002; Wada et al., 2005) and defects in these processes are reflected in the tissues of the face in ciliopathy patients (Brugmann et al., 2010; Tobin et al., 2008). Identification of the primary cilium as a signaling hub for the Hedgehog pathway came from seminal experiments reporting that anterograde and retrograde IFT proteins in the cilium are required for Shh signal propagation (Huangfu & Anderson, 2005; Huangfu et al., 2003). Subsequent analyses showed that Ptch1 and Smo receptors localize to the primary cilium and that in the presence of ligand, Ptch1 is sequestered from the cilium facilitating an accumulation of Smo and activation of signaling (Corbit et al., 2005; Rohatgi, Milenkovic, & Scott, 2007). For a number of years, it was believed that there was a strictly linear relationship between Shh signaling and primary cilia (e.g., the loss of functional cilia would result in a loss of Shh function). In some mutants, however, the exact opposite is the case; loss of cilia causes a gain of Shh signaling (Brugmann et al., 2010; Cortellino et al., 2009; Tran et al., 2008). How might Shh signaling be activated as a consequence of losing ciliary proteins? To begin to hypothesize how this seemingly paradoxical event could occur, we must first understand the relationship between the cilia and components of the Shh pathway.

The most well-known links between primary cilia and the Shh pathway are (1) that the receptors of the pathway are localized to the cilium and (2) that IFT proteins are involved in trafficking and processing of Gli2 and Gli3 from full-length isoforms, into either activator or repressor forms (Haycraft et al., 2005). In the absence of Shh, Ptch1 is present in cilia and Gli3 (and Gli2) may translocate at low levels in and out of cilia, being phosphorylated by PKA. Presumably, IFT proteins are then involved in shuttling Gli3FL to the pericentrosomal proteasome where it is processed into Gli3R and subsequently translocated to the nucleus to inhibit *Gli1* and *Gli2* (Wen et al., 2010). In the presence of Shh, Smo displaces Ptch1 in cilia, thereby inhibiting PKA phosphorylation and Gli3FL (and Gli2FL) accumulates at the distal tips. Thus, one mechanism by which a ciliary defect could result in a gain of Shh signaling is via aberrant proteolytic processing of Gli3 (the predominant repressor of the pathway). Deregulation of Gli3R production would result in an increased Gli3FL:Gli3R ratio and subsequent increase in Shh activity. This scenario has been observed in numerous IFT mouse models (Haycraft et al., 2005; Huangfu & Anderson, 2005; Liu et al., 2005; May et al., 2005; Tran et al., 2008; Willaredt et al., 2008).

Whereas Gli processing is the most commonly examined aspect in ciliary mutants, the identification of novel ciliary proteins implicates additional mechanisms that could explain how the loss of cilia could cause a gain of Shh activity. For example, recent work identified G-protein-coupled receptor 161 (Gpr161) as a ciliary protein that negatively regulates Shh activity via promoting PKA-mediated generation of GliR (Mukhopadhyay et al., 2013). With respect to Gpr161 function, loss of cilia could alleviate negative regulation of the pathway and thus also cause a gain of Shh activity. Furthermore, some studies have suggested "noncanonical" and ciliary -independent mechanisms for Shh signaling that do not function through Gli activity (Robbins, Fei, & Riobo, 2012). In light of this rapidly emerging data, caution must be taken when making generalizations about the role of primary cilia and a signaling pathway because many of these mechanisms appear to be tissue-specific (Zaghloul & Brugmann, 2011).

7.2. Wnt

Wnt signaling is required throughout NCC development. Various Wnt ligands are expressed in developing neural tissue at stages that strongly suggest a role for Wnt proteins in NCC induction in several species (Rogers, Jayasena, Nie, & Bronner, 2012). Furthermore, Wnts also have reiterative roles later in NCC development during migration (Mayor & Theveneau, 2014), proliferation (Brugmann et al., 2007), and differentiation (Brault et al., 2001). Initial evidence for the involvement of the canonical *Wnt* pathway in ciliary function came from *in vitro* experiments demonstrating that *Inversin* localized to the cilia and physically interacted with *Wnt* pathway component *Dishevelled (Dvl)* (Otto et al., 2003; Simons et al., 2005; Watanabe et al., 2003). One study reported that a knockdown of Kif3a led to increased Wnt signaling, suggesting that the primary cilium restricts canonical Wnt signaling in mouse embryos, primary fibroblasts, and embryonic stem cells (Corbit et al., 2008). Other studies focused on different ciliary proteins have supported this hypothesis. MEFs isolated from retrograde protein *Ttc21b* mutant mice showed an approximately fivefold increase in reporter activity in response to *Wnt3a*-conditioned media (Tran et al., 2014). Similarly, loss of primary cilia in the mammary ducts of *Ift88* mutant mice resulted in increased Wnt signaling (McDermott, Liu, Tlsty, & Pazour, 2010).

Despite this plethora of data suggesting a role for cilia in Wnt signal transduction, there is significant evidence supporting the contrary. Zebrafish *ift88* mutants fail to make cilia and display characteristic Shh-related phenotypes, but expression of Wnt targets is unchanged (Huang & Schier, 2009). Another study found that *Axin2* expression levels and readouts from Wnt reporters were indistinguishable between wild-type and a number of ciliary mutants (Ocbina, Tuson, & Anderson, 2009). Furthermore, wild-type and ciliary mutant MEFs displayed no difference in response to exposure to both Wnt3a and Wnt5a ligands (Ocbina et al., 2009). In NCCs specifically, the loss of Kif3a did not appear to alter Wnt activity in early facial mesenchyme (Brugmann et al., 2010). Despite these opposing findings, several studies still support a role for ciliary proteins in restraining Wnt signaling. In light of these seemingly contradictory data, one possibility to consider is that the perturbations of the Wnt pathway observed in ciliary mutants may not be direct. Gli proteins can bind to and directly affect transcription of Wnt effector genes. Thus, ciliary-dependent disruptions of posttranslational Gli processing could secondarily affect Wnt activity. Clearly

the complexity of the issue requires further investigation regarding Wnt signaling and cilia involvement in NCCs and other cell types.

7.3. Fibroblast growth factor

The FGF signaling pathway has been associated with growth and development of the facial prominences, the facial skeleton, and craniofacial musculature (Creuzet, Schuler, Couly, & Le Douarin, 2004; Macatee et al., 2003; Szabo-Rogers, Geetha-Loganathan, Nimmagadda, Fu, & Richman, 2008; Trumpp, Depew, Rubenstein, Bishop, & Martin, 1999). Similar to Wnts, many FGFs are expressed in spatiotemporal patterns that suggest a role in NCC induction and patterning (Rogers et al., 2012). More recently, a novel role for FGF signaling in ciliary assembly has been hypothesized (Neugebauer, Amack, Peterson, Bisgrove, & Yost, 2009). *Fgfr1* morphant zebrafish exhibited reduced expression of the ciliary genes *foxj1* and *rfx2* as well as diminished *ift88* expression, indicating that FGF signaling may exert its effects on cilia by regulating IFT (Neugebauer et al., 2009). Furthermore, cilia length was affected in *fgf24/fgf8* double mutants, suggesting that cilia length may be regulated by functional redundancy across FGF ligands. Additional support for this hypothesis comes from studies that report decreased length and number of cilia as a result of knocking down FGF target genes *ier2* and *fibp1* (Hong & Dawid, 2009). These experiments raise the possibility that FGF-mediated craniofacial malformations could be cilia-related, and in fact, recent studies have begun to substantiate this mechanism (Tabler et al., 2013).

7.4. Platelet-derived growth factor

Aberrant *PDGF* signaling has been linked with cleft lip/palate in humans (Smith & Tallquist, 2010). *Pdgfa/Pdgfc* double mutants develop cleft face and cranial bone defects (Ding et al., 2004). Mice deficient in *Pdgfra* in the neural crest lineage display palatal fusion defects as well as nasal septation and various other anomalies of bone and cartilage (Morrison-Graham, Schatteman, Bork, Bowen-Pope, & Weston, 1992; Soriano, 1997; Tallquist & Soriano, 2003). Primary cilia are required for PDGFα signaling (Schneider et al., 2005, 2009). PDGFα receptor localizes to primary cilium in growth-arrested NIH3T3 cells. Upon signaling activation, the downstream protein Mek1/2 is phosphorylated and localizes to primary cilium. This behavior is lost in cilia mutant $Ift88^{orpk}$ fibroblasts. Moreover, the primary cilium is required for PDGF-AA induced directional cell migration during wound healing (Schneider et al., 2010).

7.5. Notch

Experiments across several species have implicated a role for Notch signaling in NCC induction (Cornell & Eisen, 2002, 2005; Endo, Osumi, & Wakamatsu, 2002, 2003; Ezratty et al., 2011; Glavic, Silva, Aybar, Bastidas, & Mayor, 2004; Leitch, Lodh, Prieto-Echague, Badano, & Zaghloul, 2014; Mead & Yutzey, 2012; Noisa et al., 2014). Notch signaling is required for the induction of NCC-specifier genes in premigratory neural crest-like cells (pNCC) derived from human pluripotent stem cells (hPSC) (Noisa et al., 2014). Activated *Notch1* intracellular domain (*NICD1*) directly binds to and induces expression of NCC-specifier genes. Interestingly, Notch activity is also required for the maintenance of the pNCC fate and the suppression of Notch signaling led to the generation of NCC-derived

neurons (Noisa et al., 2014). Furthermore, in avian models, gain and loss of Notch both result in loss of NCC markers (Endo et al., 2002, 2003).

The link between Notch signaling and primary cilia is just beginning to emerge. The Notch3 receptor is enriched in the ciliary membrane while Presenilin-2 (the enzyme responsible for Notch cleavage and activation) is localized to the ciliary basal body. *bbs1* and *bbs4* morphant zebrafish have increased expression of Notch targets (Leitch et al., 2014), and loss of *Bbs1* and *Bbs4* caused Notch receptor to accumulate in late endosomes and subsequent lysosomal degradation of Notch receptor was impaired. These experiments strongly suggest that the Notch pathway utilizes the cilium for signal transduction. Uncovering the relationship between Notch signaling, cilia, and NCC development holds great promise for identifying novel molecular mechanism of ciliopathies and possible avenues for therapeutic intervention.

8. CONCLUSIONS

The resurgence of interest in the primary cilium has been impressive and offers new perspectives on how cells sense and respond to their molecular environments. Continued study of primary cilia is vital to both basic science and biomedicine. For basic research, more insight into cilia function will be necessary for truly understanding the molecular mechanisms of signal pathway transduction. For the biomedical community, ciliary research will identify both the basis for ciliopathies as well as potential avenues for therapeutic intervention. Relative to the field of NCC biology, understanding the role primary cilia play during ontogenic development of NCCs could provide novel insights into the etiology of specific neurocristopathies.

Although the field has made significant strides in the last decade, there is still much to learn. For example, why do ciliopathies have differing phenotypes? Why do some ciliopathies have severe craniofacial phenotypes while others do not? Are primary cilia unique depending upon which type of cell they extend from or which signaling pathway they are geared to transduce? In order to address these questions, cilia must be carefully examined both at different time-points during development and on different cell types (Irigoin & Badano, 2011).

The data summarized here emphasize the shift in the current paradigm that primary cilia are homogeneous organelles and that ciliary function can be uniformly defined across multiple tissues and organ systems. Although a plethora of phenotypes have been reported in various ciliopathic animal models (Zaghloul & Brugmann, 2011; Table 1), the perception still exists that disruption of cilia equates simply to a loss of Shh function. Our work, as well as that of many others, suggests that not only will different tissues interpret the loss of cilia in a unique manner, but also the same tissue in different molecular environments will have a unique response to ciliary disruption. It will be of great interest to multiple fields to see what is in store for primary cilia research in the next decade.

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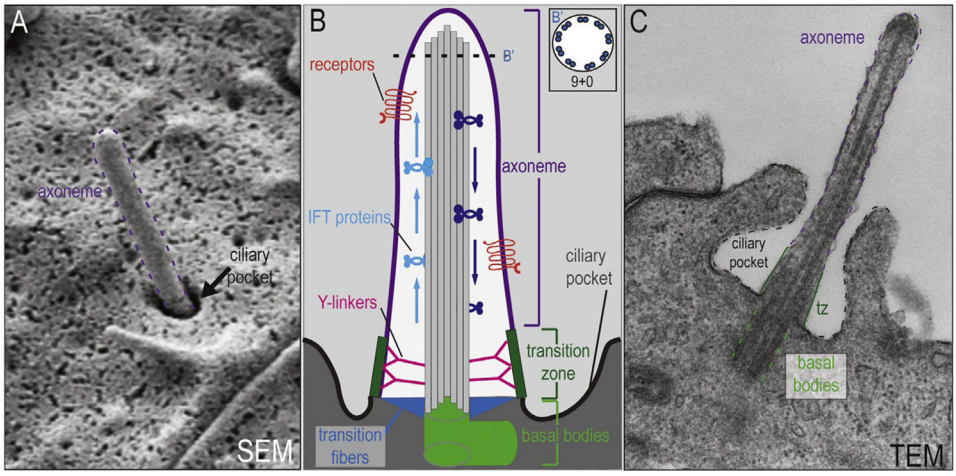

Figure 1.

Structure of the primary cilium. (A) Scanning electron microscopy (SEM) of the primary cilium. The axoneme is outlined in purple and the ciliary pocket is indicated by an arrow. (B) Schematic diagram of the primary cilium. The axoneme (purple) extends out from the basal bodies (light green) into the extracellular space (light gray). Receptors (red) for various signaling pathways localized to the membrane of the axoneme. (B′) Schematic cross-section of the axoneme reveals the microtubules in a 9+0 conformation. Intraflagellar transport proteins travel along microtubules, moving cargo up (light blue) and back down (dark blue) the axoneme. The more proximal portion of the primary cilium contains the transition fibers (blue), transition zone (dark green), and Y-linkers (pink). Collectively, these structures are important for ciliogenesis and act as a selective barrier between the intracellular space (dark gray) and the ciliary compartment (white). (C) Transmission electron microscopy (TEM) of the primary cilium. The axoneme (purple dotted lines), transition zone (tz;dark green lines), basal bodies (light green dotted lines), and ciliary pocket (black dotted lines) have all been highlighted in the image.

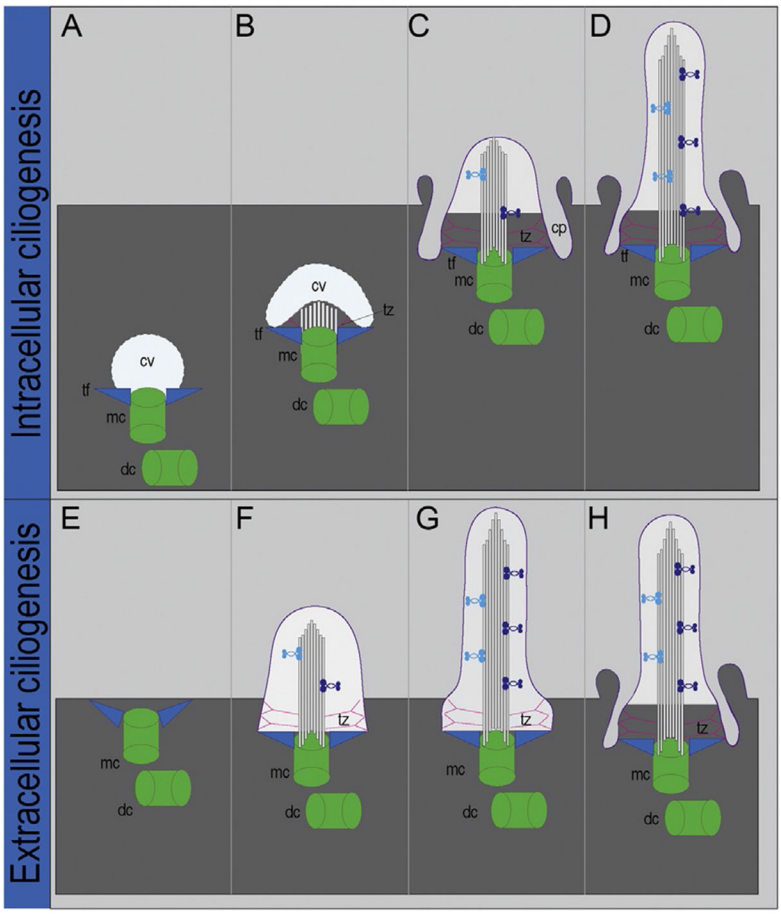

Figure 2.

Models of intracellular and extracellular ciliogenesis. *Steps of intracellular ciliogenesis*. (A) A ciliary vesicle (cv) binds to the distal end of the mother centriole (mc) via associations with transition fibers (tf). (B) Microtubule and transition zone (tz) outgrowth protrudes, causing the cv to invaginate. (C) Docking of the centriolar/cv complex to the plasma membrane. (D) Axonemal outgrowth. *Steps of extracellular ciliogenesis*. (E) The mother centriole (mc) docks directly to the plasma membrane via associations with transition fibers (tf). (F–G) Axonemal outgrowth occurs. (H) Ciliary pocket forms. dc, daughter centriole;pc, primary cilium; cp, ciliary pocket. *Figure modified from* Reiter et al. (2012) and Molla-Herman et al. (2010).

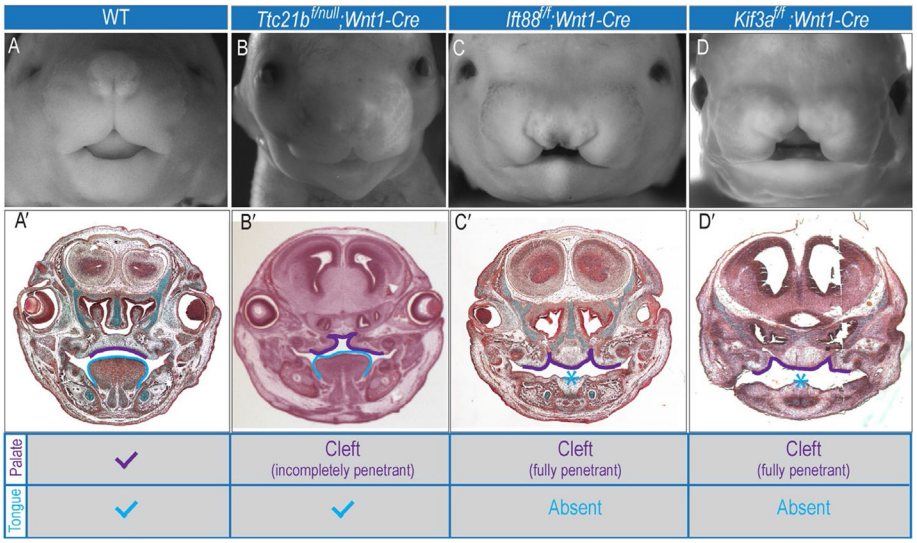

Figure 3.

Craniofacial phenotypes of ciliary mutants. Whole-mount images of e14.5 (A) Wild-type, (B) *Ttc21b*$^{f/null}$*;Wnt1-Cre*, (C) *Ift88*$^{f/f}$*;Wnt1-Cre*, and (D) *Kif3a*$^{f/f}$*;Wnt1-Cre* mice. (A′–D′) Frontal sections of (A–D). (A′) Wild-type mice have a fused palatal shelves (purple outline) and a tongue (blue outline). In contrast, all ciliary mutants have (B′–D′) some degree of cleft palate (purple outline) and *Ift88*$^{f/f}$*;Wnt1-Cre* and *Kif3a*$^{f/f}$*;Wnt1-Cre* mutants have aglossia (C′, D′). Chart indicates craniofacial phenotype for each mouse.

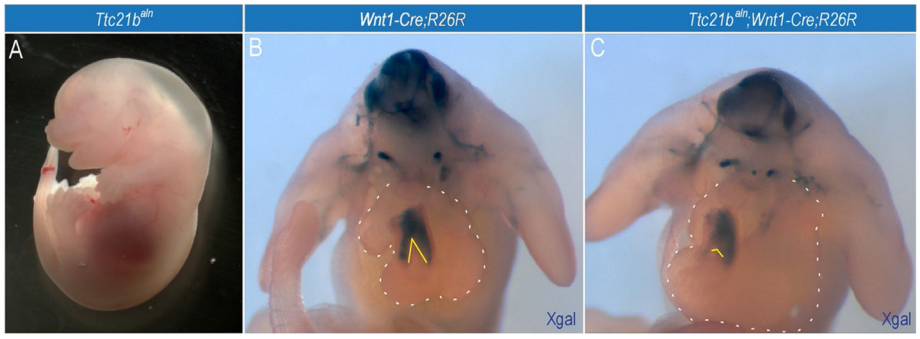

Figure 4.

Loss of primary cilia affects cardiac NCCs. *Ttc21b*^{aln} mutant has cardiac NCC defects. (A) Whole-mount image of an e14.5 *Ttc21b*^{aln} mutant with systemic edema suggestive of cardiopulmonary dysfunction. (B) Xgal staining in an e11.5 *Wnt1-Cre;R26R* embryo to trace the NCC lineage. (C) Xgal staining in an e11.5 *Ttc21b*^{aln} *Wnt1-Cre;R26R* embryo. Dotted white lines outline the heart. Solid white lines indicate the degree of cardiac NCC migration into the septating outflow tract.

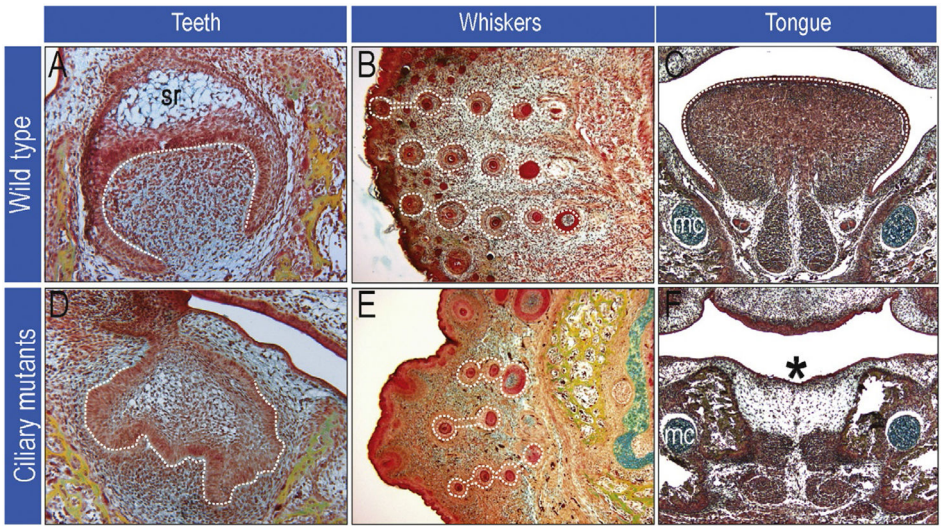

Figure 5.

Defects in craniofacial specialization. Images of wild-type (A–C) and ciliary mutant (D–F) craniofacial structures. Ciliary mutants are unable to properly form teeth (compare dotted white lines in A to D), whiskers are abnormally spaced and have aberrant morphology (compare dotted white lines in B to E) and some ciliary mutants can have aglossia (*) (compare dotted white lines in C to asterisk in F). sr, stellate reticulum; mc, Meckel's cartilage.

Table 1

Animal models for craniofacial ciliopathies

Model system	Mutant/morphant name	Gene	Craniofacial phenotype	References
Mouse	*Aln*	*Ttc21b (Ift39)*	Delayed eye and forebrain development; neural tube defects	Tran et al. (2008)
	Bbs4$^{-/-}$	*Bbs4*	Mid-facial shortening due to premaxillary and maxillary hypoplasia; shortened snouts	Tobin et al. (2008)
	Bbs6$^{-/-}$	*Bbs6*	Mid-facial shortening due to premaxillary and maxillary hypoplasia; shortened snouts	Tobin et al. (2008)
	Dync2h1^{b2b414Clo}	*Dync2h1*	Micrognathia	Cecilia Lo to MGI
	Evc$^{-/-}$	*Evc*	Chondroplasia, hypodontia	Ruiz-Perez et al. (2007)
	Fuz$^{-/-}$	*Fuz*	Exencephaly; anophthalmia; encephalocoele; mandibular defects; micrognathia; high arched palate; enlarged maxillary processes	Tabler et al. (2013) and Gray et al. (2009)
	Hippi$^{-/-}$	*Ift57*	Exencephaly; abnormal cranial flexure; hypertelorism; abnormal maxillary processes; small arches	Houde et al. (2006)
	Ift52^{hypo}	*Ift52*	Single median nostril; fused maxillary prominences; exencephaly	Liu, Wang, and Niswander (2005)
	Ift122$^{-/-}$	*Ift122*	Exencephaly; anencephaly; altered eye and branchial arch development; defects of the ventral portion of the head	Cortellino et al. (2009)
	Ift172^{avc1}	*Ift172*	Hydrocephalus; abnormal facies; shortened muzzle; cleft lip and palate	Friedland-Little et al. (2011)
	Kif3a$^{f/f}$*;Wnt1-Cre*	*Kif3a* in cranial NCCs	Anterior cranium occultum; bifid nasal septum; aglossia; severe hypertelorism; dental abnormalities	Brugmann et al. (2010)
	Kipf7$^{-/-}$	*Kif7*	Cleft lip and palate	Cecilia Lo to MGI
	Mks$^{-/-}$	*Mks1*	Occipital meningoencephalocele; hypomineralization and/or splitting of the frontal, parietal, and superaoccipital bones; hydrocephaly; cleft palate; pointy snout	Weatherbee et al. (2009)
	Ofd1$^{-/-}$	*Ofd1*	Severe cleft palate; shortened skull and facial regions	Ferrante et al. (2006)
	Orpk	*Ift88*	Dental abnormalities; micrognathia; increased gonial angle; poorly differentiated branchial arches; minor clefting; disorganized/patent frontal sutures	Zhang et al. (2003), Murcia et al., (2000), and Ohazama et al. (2009)
	Rpgrip1l$^{-/-}$	*Rpgrip1l/Ftm*	Exencephaly, microphthalmia; rounded skull; cleft upper lip; micrognathia	Delous et al. (2007)
	Tmem67$^{-/-}$	*Tmem67*	Hypoplastic mandible, semilobar HPE, occipital meningocele, midbrain and/or forebrain exencephaly	Abdelhamed et al. (2013)
Chicken	*talpid*^{2}	*c2cd3*	Short, wide upper beak; cleft lip/palate; hypo- or aglossia	Chang et al. (2014)
	talpid^{3}	*talpid3*	Ocular hypotelorism; frontonasal hypoplasia; fused maxillary prominences; micrognathia	Yin et al. (2009)
Zebrafish	*Bbs morphants*	*bbs4, bbs6, bbs8*	Mandibular dysplasia; severe craniofacial reduction; anteroneurocranium hypoplasia	Tobin et al. (2008)
	Ift mutants	*ift57, ift88, ift172*	Mild dysmorphology of craniofacial cartilages	Lunt et al. (2009)

Model system	Mutant/morphant name	Gene	Craniofacial phenotype	References
	Ift80 morphant	*ift80*	Abnormal anterior neurocranial development; midline fusion	Beales et al. (2007)
	Ift122 morphant	*ift122*	Reduced ocular development; distended cranium with hydrocephalus; otolith defects	Walczak-Sztulpa et al. (2010)
	Ofd1 morphant	*ofd1*	Abnormal blunting of the jaw; disorganization of Meckel's cartilage cells	Ferrante et al. (2009)

Ciliopathic models are grouped according to model system.

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Table 2

Human craniofacial ciliopathies and associated phenotypes

Syndrome	Gene	Craniofacial phenotype
Bardet–Biedl syndrome	*BBS1-12, MKS1, MKS3*	Mid-face shortening and flattening; nasal bridge hypoplasia; reduced length/bulbosity of the nasal tip; mild retrognathia
Cranioectodermal dysplasia/ Sensenbrenner syndrome	*IFT122, IFT144, WDR35*	Sagittal craniosynostosis; epicanthal folds; hypodontia or microdontia; everted lip; multiple oral frenula; high arched palate; skeletal and ectodermal anomalies
Ellis–van Creveld syndrome	*EVC1, EVC2*	Hypertrophy labiogingival frenulum; upper lip abnormalities; presence of teeth at birth; microdontic teeth
Joubert syndrome	*MKS3, NPHP6, RPGRIP1L, AH1, ARL13B, CSPP1*	Prominent forehead; high rounded eyebrows; epicanthal folds; ptosis; upturned nose with evident nostrils; hypertelorism; open mouth and tongue protrusion with rhythmic tongue motions
Lowe syndrome	*OCRL1*	Frontal bossing; retrognathia; high arched palate; deep set eyes; full cheeks; maxillary prognathism; malocclusion
Meckel–Gruber syndrome	*MKS1, MKS3, TMEM231, TCTN2, CC2D2A*	Encephalocele; cleft lip and palate
Oro-facial-digital syndrome	*OFD1, C2CD3*	Facial asymmetry; hypertelorism; micrognathia; broadened nasal ridge; hypoplasia of the malar bones and nasal alar cartilages; frontal bossing; pseudocleft; cleft palate; hamartomas of the tongue; bifid tongue; hyperplastic oral frenula; up-slanting palpebral fissures