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Modeling Human Disease in Humans: the Ciliopathies

Gaia Novarino, **Naiara Akizu**, and **Joseph G. Gleeson**
Neurogenetics Laboratory, Institute for Genomic Medicine, Howard Hughes Medical Institute, Department of Neurosciences and Pediatrics, University of California, San Diego, La Jolla, USA

Soon the genetic basis of most human Mendelian diseases will be solved. The next challenge will be to leverage this information to uncover basic mechanisms of disease and develop new therapies. To understand how this transformation is already beginning to unfold, we focus on the ciliopathies, a class of multi-organ diseases caused by disruption of the primary cilium. Through a convergence of data involving mutant gene discovery, proteomics and cell biology, over a dozen phenotypically distinguishable conditions are now united as ciliopathies. Sitting at the interface between simple and complex genetic conditions, these diseases provide clues to the future direction of human genetics.

Until a few years ago, identifying the genetic basis of an inherited human disease was an arduous undertaking, requiring potentially a decade or more of work in ascertainment of families for linkage analysis, followed by endless fine mapping of the locus, and finally sequencing of candidate genes one-by-one until that eureka moment when the likely causative gene was identified. The newly discovered disease gene was often entirely novel, without recognizable domains or a path to understand the disease mechanism. A mouse model was then generated, in which the disease gene was inactivated. In some cases, the mouse faithfully recapitulated the human phenotype, but more often showed no phenotype or phenotypes not clearly related to the human disease. Once established, the model was studied from multiple perspectives to understand the cell biological and biochemical basis of disease, culminating in attempts to test potential therapies. Although successful in a few instances such as losartan treatment for Marfan syndrome (Habashi et al., 2006), this path has not fulfilled the promises of genomic medicine.

This strategy has begun to change over the past ten years due to increased knowledge of human genetic diseases, annotation of the human genome, and an amazing suite of tools to explore disease mechanisms. It is not uncommon now to open up a journal to find that geneticists have solved the molecular basis of a dozen or more conditions. And since we now know a lot more about the function of genes, protein domains, and networks, frequently just the discovery of the molecular cause of a disease can often partially explain its mechanism. For instance, the discovery that the Rett syndrome gene encodes a methyl-CpG-binding protein (Amir et al., 1999) immediately set the stage for a host of important discoveries in epigenetics related to brain function. The types of mutations displayed by patients, known as allelic diversity (Fig. 1), can tell us something about the effect of these disease-causing variants on protein function. By identifying patients with different phenotypes due to specific types of mutations in the same gene (i.e. genocopies) we can

Correspondence: jogleeson@ucsd.edu.

understand human disease as a network of related signs and symptoms. For example, specific types of mutations in the gene encoding p53 predispose to very different types of cancers. By comparing the genes mutated in phenotypically related human diseases, we can learn about the disturbed protein networks that underlie them. Finally, by exploring gene-gene and gene-environment interactions, we can begin to characterize genetic and epigenetic modifiers of disease. Perhaps the best example is age-related macular degeneration, in which a substantial part of the risk of disease can be quantified based on gene-environment interactions (Chen et al., 2010).

The ciliopathies: one organelle, many disorders

Although they are individually rare conditions, the ciliopathies have emerged as a dynamic new field of biology that exemplifies how genetics can be employed to drive research in basic cell biology and vice versa. The primary cilium is structured with a basal body at its base and a 9-paired microtubule axoneme, surrounded by plasma membrane, but lacking the central pair of microtubules and outer dynein arms that define its cousin, the motile cilium. Primary cilia were first observed more than a century ago and were initially thought to be evolutionary remnants. How is it that biologists missed their importance for so long?

Over a dozen disorders are now considered to be within the ciliopathy spectrum including Joubert syndrome (JBTS), nephronophthisis (NPHP), Senior-Loken syndrome (SLS), orofaciodigital (OFD), Jeune syndrome, autosomal dominant and recessive polycystic kidney disease (ADPKD and ARPKD), Leber congenital amaurosis (LCA), Meckel-Gruber syndrome (MKS), Bardet-Biedl syndrome (BBS), Usher syndrome (US) and some forms of retinal dystrophy (RD). Between them, these conditions involve nearly every major body organ including kidney, brain, limb, retina, liver, and bone (Fig. 2A), highlighting the important role of the primary cilium in development and homeostasis. These conditions were largely defined by clinical geneticists in the middle of the last century, who did their best to ascribe syndromes to unique combinations of clinical features. Individual diseases are known for the most commonly involved or diseased organ: BBS patients display the triad of obesity, polydactyly and retinopathy but can display a host of other pathologies. MKS is a lethal condition at birth with occipital encephalocele, PKD and polydactyly. JBTS is characterized by a very peculiar radiographic finding, known as the “molar tooth sign”, characterized by elongated superior cerebellar peduncles, deepened interpeduncular fossa and cerebellar vermis hypoplasia. For each of these conditions, significant phenotypic variability has been observed even between members of the same family, making clinical diagnosis a challenge.

Now that these conditions have been united by their underlying cell biology, we can begin to see commonalities between individual syndromes (Fig. 2A). For instance, low muscle tone, cystic kidney disease, agenesis of the brain’s corpus callosum, mental retardation and hyperpnea/apnea are additional clinical features often present in ciliopathy patients. Individuals affected by BBS can have several clinical features common to JBTS such as mental retardation, hypotonia and apnea. However, they also share other symptoms that are usually not present in JBTS, but common to other ciliopathies, such as polydactyly and retinal dystrophy (RD). Moreover, BBS patients are usually obese, a unique trait absent among the other ciliopathies.

The first few genes identified from positional cloning of human or mouse phenotypes for what would eventually become the ciliopathies initially did not point in an obvious way to the cilium as the site of action. It was not until evidence began to accumulate that the encoded proteins localize specifically near the cilium that the field was born (Ansley et al., 2003; Barr and Sternberg, 1999; Kim et al., 2004; Otto et al., 2003; Taulman et al., 2001).

Although the localization data is now incontrovertible, the functions of the encoded proteins at the cilium for the most part still remain a mystery, and some of the effects of these proteins do not seem to have direct relevance to ciliary function (Yen et al., 2006). The question that emerges is how a single subcellular organelle can mediate such diverse clinical features.

About 50 genes encoding predominantly ciliary-localized proteins have now been identified that are mutated in these partially overlapping syndromes (Fig. 2B). In a series of positional cloning studies, each of these ciliopathy genes was initially found as causative for a restricted phenotype. Surprisingly, in most instances, this gene identification was followed by reports of mutations in the same gene in a different ciliopathy category (Baala et al., 2007a; Baala et al., 2007b; den Hollander et al., 2006). This occurred with such regularity that the field began to wonder if any genotype-phenotype correlations would stand the test of time. How could mutations in a single gene produce such pleiotropic phenotypic effects in patients? Were these observations exceptions to the rule of strict genotype-phenotype correlation, or were they the exception that proves the rule of widespread phenotypic pleiotropy?

The primary cilium network

The primary cilium is a hair-like, immotile cellular organelle protruding from almost all eukaryotic cells, frequently described as the cell's "antenna" for transducing extracellular signals. For the purposes of this review, we focus exclusively on diseases involving non-motile cilia, and do not include diseases like primary cilia dyskinesia that involve motile cilia, since there is little phenotypic or genetic overlap. Cilia are generated during interphase from the mother centriole, by coalescence of vesicles at its distal end that fuse with the plasma membrane. After this docking of the mother centriole, the microtubule axoneme protrudes out of the cell, concurrent with recruitment of a host of ciliary-specific proteins. The basal body (i.e. the docked mother centriole) possesses several specialized accessory structures termed transition fibers, basal feet and ciliary rootlets, and is surrounded by the pericentriolar matrix, but for many years the molecular determinants of these structures were unknown.

Recent work has begun to hint at the molecular architecture of these anatomically defined structures. The 9+0 microtubule arrangement of the axoneme emerges from the basal body in a triplet configuration, and shifts to a doublet configuration at the ciliary transition zone (TZ). The Y-shaped microtubule extensions that define the TZ require the Cep290 protein for their formation in *Chlamydamonas* (Craige et al., 2010), although it is not clear if Cep290 constitutes or otherwise contributes to these structures, or if this function is conserved in vertebrates. The location of the TZ marks the ciliary diffusion barrier; a septin-2 cytoskeleton which separates the ciliary and cytoplasmic compartments (Hu et al., 2010). Attention has shifted to the TZ as the site of action of proteins mutated in several ciliopathies (Garcia-Gonzalo et al., 2011), but clear structure-function relationships are still lacking. Protein synthesis and vesicular transport do not occur inside cilia, and so the assembly of this organelle, its maintenance and function are totally dependent upon an intraflagellar transport system, by which proteins track bidirectionally along the polarized microtubules of the axoneme (Kozminski et al., 1993).

Although the involvement of primary cilia in human diseases is now well established, there are many questions about its function that remain. The current paradigm describes the primary cilium as an organelle for detecting and modulating the response to extracellular signaling molecules and as a location for organizing their cytoplasmic effectors. It is well poised to mediate both effects, as cilia typically protrude in a polarized fashion, which can

help the cell interpret the context of extracellular signals. Many cellular receptors are localized to the primary cilium, and the basal body is itself a signaling hub, probably serving as an efficient transit point to transmit signals into the nucleus. In fact, a number of transcription factors undergo processing within or near the cilium prior to nuclear entry. Numerous critical developmental signaling pathways have been directly linked to primary cilia, such as Hedgehog (Hh), canonical and non-canonical Wnt and some forms of PDGF signaling, highlighting its role as a hub (Huangfu et al., 2003; Schneider et al., 2005; Simons et al., 2005). In addition to the modulation of these signaling pathways, primary cilia are essential for mechanical, odor and photoreception. The interpretation of the ciliopathies as a unique group of disorders, associated with defects in a single organelle gave a new direction to the investigation of these human diseases.

Proteomics merges with genomics

The marriage of proteomics with genomics in the area of ciliary biology can be traced to an influential paper showing that the *RFX*-type transcription factor DAF-19 is essential for assembly of cilia in *C. elegans* sensory neurons, and regulates several genes encoding intraflagellar transport proteins (Swoboda et al., 2000). The RFX transcription factor family emerged in ciliated proto-eukaryotes but it was only later that the RFX genes were co-opted to regulate expression of cilia-specific genes based upon the presence of an X-box in their promoter (Piasecki et al., 2010). This work set the stage for a comparative genomics approach to search for X-box containing genes that were likely to encode proteins relevant to primary cilia. Two follow-up studies demonstrated the potential of this approach in identifying important components of the primary cilium (Avidor-Reiss et al., 2004; Li et al., 2004) and raised the idea of building a ciliary protein database. The ciliome (Gherman et al., 2006; Inglis et al., 2006), now consists of over 3000 genes encoding proteins either localized to cilia or essential for their assembly or function (Arnaiz et al., 2009).

The ciliary proteome has already proved to be a powerful resource, accelerating the identification of candidate human ciliopathy genes by short-listing positional candidates. For example, the cloning of *MKS1*, *BBS3*, *BBS5* and the BBS modifier *MGC1203* was achieved by sequencing a reduced set of candidate genes (Badano et al., 2006; Chiang et al., 2004; Kyttala et al., 2006; Li et al., 2004). Extending the idea of a ciliary proteome to a ciliary “interactome” was the natural extension of this work, through the identification of proteins that physically interact as part of specific complexes (Eley et al., 2008; Gorden et al., 2008). Through pair-wise testing of potential yeast-two-hybrid interactions (Otto et al., 2005) and identification of binding partners by serial mass spectrometry (Nachury et al., 2007; Sang et al., 2011), a number of discrete, functionally relevant complexes have now emerged as the likely minimal disease-causing modules (Fig. 2B).

An important observation, which has stood the test of time, is that the composition of these specific protein modules could have been predicted based upon the phenotype observed in patients. Specifically, the proteins from seven of the eight most conserved genes mutated in BBS form a core complex termed the BBSome (Nachury et al., 2007), found to play important roles in ciliary protein and vesicular transport. Importantly, this complex does not contain proteins encoded by other ciliopathy diseases genes such as NPHP, JBTS or MKS. This is perhaps surprising considering that mutations in MKS genes can cause BBS (Leitch et al., 2008). Three separate complexes containing many of the proteins mutated in NPHP, JBTS and MKS were recently identified, and although their function is still under investigation, genes for two of the co-purifying proteins, *ATXN10* and *TCTN2* were found to be mutated in patients matching the same “module” phenotype (Sang et al., 2011). In general, the complexes also display specificity in subcellular localization and function: the MKS/JBTS complex transduces hedgehog signaling and localizes to the ciliary transition

zone, whereas the BBS complex forms a coat complex to target vesicles to the cilium, and localizes to ciliary membrane (Jin et al., 2010). Proteins have non-redundant function within a given module and do not associate or function in other modules.

This work has been further corroborated by analyzing a series of *C. elegans* double mutants, which demonstrate worsened synthetic phenotypes (i.e. functional interactions) only when mutations occur in two different modules (Williams et al., 2011) but not with two different mutations in the same module. For instance, the B9 domain proteins of the MKS module functionally interact with the NPHP module (Williams et al., 2008) but not with most other genes in the MKS module. The conclusion is that each module probably mediates partially separate ciliary functions. Inactivating one component in a module is probably sufficient to fully inactivate the module, so functional interaction is only observed by inactivating a component in a different module. All together, these examples show that the availability of various disease proteomes in combination with the explosion of currently available genomic and transcriptomic datasets are driving forward biological network analysis in human disease.

Genetic complexity of human ciliopathies

Allelic diversity

What can the study of the ciliopathies teach us about the future direction of human genetic disease? Most obviously, that human genetics will be a lot more complex than many of us would have predicted. One obvious example is in the degree of multiple allelism at particular genetic loci. While some of the ciliopathy genes are associated with only a single phenotypic class to date, other gene mutations can result in phenotypes along the entire ciliopathy clinical spectrum. For instance, mutations in *CEP290* are reported in MKS, JBTS, NPHP, BBS and LCA, spanning the full breadth of severity (Coppieters et al., 2010b) whereas *ARL13B* mutations are restricted to patients with JBTS. For *ARL13B*, it is not clear if this gene is only capable of causing a restricted phenotype or if there are additional mutations to be identified in other ciliopathy class disorders. Current data would suggest the latter, since only hypomorphic mutations in *ARL13B* gene were identified in humans, whereas comparably more severe phenotypes were observed in mouse and zebrafish (Caspary et al., 2007; Sun et al., 2004), suggesting the full spectrum of disease-causing alleles has not yet been reported.

In the case of *CEP290*, despite the identification of over 100 unique disease-causing mutations, the ability to predict phenotype based upon genotype is extremely limited. The mechanism underlying the different clinical outcomes of distinct mutations is not always clear. One possibility might relate to the type of mutations and their locations as predictors of phenotypic severity. While there are some types of mutations associated with particular phenotypes, such as the c.2991+1655A-->G variant present in 21% of all LCA patients, (den Hollander et al., 2006), the exact same mutation can be seen in two different ciliopathy classes (Coppieters et al., 2010b).

There are other examples of pleiotropy in the ciliopathies. *NPHP1* mutations are found in pure NPHP and NPHP with RD (a combination known as SLS); *INPP5E* mutations are found in JBTS as well as BBS-like conditions. The case of *TMEM67* deserves special attention, in that mutations cause a broad range of phenotypic combinations that comprise renal cyst, liver fibrosis, central nervous system malformations, retinal manifestations and postaxial polydactyly. The gene was first identified as both a cause of MKS3 and the origin of the multiple phenotypes of the *Wpk* rat, which include PKD, agenesis of the corpus callosum and hydrocephalus (Smith et al., 2006). Thereafter more than 80 *TMEM67* mutations were identified, not only associated with MKS but also with a peculiar form of

JBTS involving liver fibrosis and several renal and liver ciliopathy syndromes in which missense mutations predominate (Brancati et al., 2009; Iannicelli et al., 2010). Interestingly, the presence of two truncating alleles or a missense mutation within exons 8–15 associates with the lethal MKS phenotypes, suggesting an essential function of this region of the encoded protein that is yet to be identified.

Modifiers

While allelism offers one perspective with which to view phenotypic pleiotropy, epistatic interactions and mutation load offer another important perspective that deserves consideration. Such reports are starting to emerge across the full spectrum of human disease (Gu et al., 2009; Oprea et al., 2008) but are still limited in number due to underpowered studies. Within the ciliopathies, recent studies of families with more than one affected child and larger population screenings have highlighted an important role of epistatic interactions, whereby the effect of one gene modifies the phenotypic attributes of a different gene (Leitch et al., 2008; Wiszniewski et al., 2011) (Fig. 1c). For this review, we distinguish this from mutational load, whereby the total genetic burden from accumulated deleterious variants sums to produce the phenotype. It is now clear that the precise disease manifestations and probably the timing of the appearance of symptoms are subject to modification, which could be in the form of stochastic, environmental or genetic inputs. The identification of genetically encoded modifiers offers the potential to understand gene networks, improve prognostic information and identify targetable biochemical processes for the development of therapeutic treatments.

Evidence that heritable factors in humans can alter the course of ciliopathies came initially from an elegant series of experiments involving the role of the *MGC1203* gene (Badano et al., 2006). The encoded protein, CCDC28B, contains a coiled-coil domain, and was identified in a yeast-two hybrid screen as a BBS4-interacting protein. After demonstrating CCDC28B interaction with several BBS proteins, the authors screened a BBS cohort for potential *MGC1203* mutations. While none of the patients carried a mutation known to cause BBS, a C to T transition (C430T) in *MGC1203* generated a splice defect in about 10% of gene products. To test the involvement of C430T as a potential modifier, they screened BBS and control cohorts for this variant, finding that 6.2% of patients vs. 1.4% of controls carried the variant, representing an over-transmission of the variant in transmission disequilibrium testing. The authors reported three families with affected siblings carrying a homozygous mutation on *BBS1* in which the RD severity was associated with the presence of the transition, suggesting that this variant increases the likelihood of developing more severe BBS symptoms when associated with a known BBS mutation.

Since then, other examples of epistatic effects on RD associated with ciliopathies have emerged. Mutations in either *AHI1* or *RPGRIP1L*, which both encode ciliary-localized proteins, were initially reported to cause JBTS. The *AHI1* gene product physically interacts with nephrocystin-1 (Eley et al., 2008), the product of the most commonly mutated NPHP gene, *NPHP1*. Because over 50% of patients with *AHI1* mutations display RD/LCA in addition to JBTS, investigators considered that *AHI1* might be mutated in isolated RD/LCA. However, screening a cohort of 176 mixed ancestry patients with LCA failed to demonstrate any causative mutations, indicating that *AHI1* mutations do not lead to isolated LCA in the absence diagnostic JBTS features (Louie et al., 2010). However, *Ahi1* mutant mice predominantly displayed an LCA/RD phenotype, which was more severe when synthetically combined with an *Nphp1* mutant allele, prompting investigation of epistatic interactions. Among a cohort of 153 *NPHP1*-mutant patients, there was a significant enrichment for a heterozygous c.C2488T change leading to a functional p.R830W substitution in those with RD. This translated into a 7.5-fold increase in RD in this population, which represents one of the highest known risk alterations so far described for any human disease. The c.C2488T

allele therefore significantly increases the risk of developing RD in the presence of an *NPHP1* mutation.

This association is not restricted to those with RD, as the same c.C2488T allele was more commonly found in individuals with *NPHP1* mutations displaying a more severe neurological phenotype (Tory et al., 2007). Nor are *AHI1* variants solely restricted to modifying *NPHP1* phenotypes, as three unrelated patients with the exact same *CEP290* genotype (p.R1465X) presenting with different clinical phenotypes showed variants in *AHI1* that might explain this discordance. *AHI1* heterozygous transversions (p.N811K and p.H758P) were found associated with greater severity of nephrological and neurological phenotypes (Coppieters et al., 2010a). Intriguingly the *AHI1* variants in this case had no effect on the presence of RD. Thus, these epistatic interactions are both genotype-specific and phenotype-specific.

In studies that use model systems, it is possible to manipulate gene structure or expression through a variety of techniques, but investigation of genetic modifiers in humans is limited to naturally occurring variations. This might seem like a huge drawback initially, but it has tremendous benefits in the long run because large numbers of patients can be analyzed for the more common variants. Several heterozygous variants have been reported in *RPGRIP1L* in ciliopathy cohorts, and while their individual rarity precluded further investigation, one remarkable exception, the heterozygous p.A229T, is common enough to apply statistical analysis. The RPGRIP1L protein interacts with RPGR, a ciliary protein frequently mutated in RD, and this interaction is disrupted in the presence of the p.A229T transversion (Khanna et al., 2009). Individuals with this variant alone have normal vision, suggesting that this heterozygous variant is silent in isolation. However, it is enriched in ciliopathy patients with RD compared to those without RD. There are probably many such gene-gene interactions, but defining those of highest effect size, and the mechanisms by which these epistatic effects are manifest will require new experimental strategies.

Mutational load

Is it possible that such second site mutations might modify not only the expressivity but also the penetrance of disease? BBS has long provided the classic example of oligogenicity (Katsanis et al., 2001), representing the idea that second site mutations are necessary to produce an observable phenotype. The demonstration that three mutant alleles at two different loci were required for pathogenicity was among the first example of oligogenic inheritance. In this example, investigators observed that both affected and healthy children of an outbred family carried two different nonsense mutations in *BBS2* (p.Y24X and p.Q59X). In an effort to identify the differential genetic trait that caused the disease in the affected sibling, they identified a heterozygous nonsense potentially deleterious sequence variant (PDSV) in *BBS6* (Q147X) that was absent in the healthy sibling, suggesting that the three different mutant alleles were necessary to produce this phenotype. Subsequently, additional examples were described in which BBS patients carried three mutant alleles in two different loci (Eichers et al., 2004). However, in such examples the segregation does not exclude the possibility that two mutations in the same gene are sufficient to cause disease in a different genetic background (Mykytyn et al., 2003). Moreover, the lack of recurrence of such combinations in BBS cohorts studied complicates the validation of the oligogenic hypothesis.

Although oligogenic inheritance in BBS is still hotly contested, several reports of possible oligogenic inheritance in other ciliopathies have emerged more recently (Hoefele et al., 2007; Hopp et al., 2011), where only single heterozygous PDSVs are detected, or where patients with two deleterious mutations in one gene also carry a heterozygous PDSV in a second gene. The evidence for oligogenicity is somewhat tempered by the natural limitations

of the technology used to support the findings, and whether such variants are functionally relevant and whether they are overrepresented in patients compared with similarly investigated controls is still a matter of debate. Nevertheless, the observed phenotypic and genetic variability, together with an apparent overabundance of PDSVs in ciliopathy genes have led to the general acceptance of a “mutational load” hypothesis. Defining the mutational load required for the manifestation of a given phenotypic combination or expressivity is now the main challenging issue. One potential reason for optimism is the emergence of next-generation sequencing, which will allow genome-wide unbiased exploration of the mutational load hypothesis. For instance, it would fascinating to test whether patients with ciliopathy phenotypes carry greater burdens or particular patterns of mutations in cilia-specific genes compared with housekeeping genes on a genome-wide scale.

IPSCs, cell based screens and treatments for ciliopathies

What does the future of human genetics hold and how can disorders like the ciliopathies benefit from new technological advances? Whole-genome (WGS) and whole-exome sequencing (WES) is clearly the breakthrough most likely to impact the study of Mendelian disorders like ciliopathies. The exons, accounting for ~1% of the genome, harbor most disease-causing mutations of high-effect size, and therefore WES is a reasonable approach for finding novel disease-causing genes. This is evident by the abundance of novel disease-causing genes identified in recent years. The limitations of WES can be overcome with WGS, with its ability to identify not just variants in coding regions of the genes, but also variants in introns, untranslated regions, and non-coding RNAs, such as the recent discovery of mutations in the U4atac shRNA in microcephalic osteodysplastic primordial dwarfism type I (Edery et al., 2011; He et al., 2011). Additionally, with genome-wide approaches, these datasets offer the possibility to directly test the mutational load hypothesis and identify a host of epistatic modifiers. At this point, we still require better bioinformatic tools to predict resultant human phenotypes, but this technology is already greatly benefiting the field of human genetics with an explosion in the number of newly discovered human disease genes. The ciliopathies are no exception (Gilissen et al., 2010; Hopp et al., 2011).

Combining these genetic discoveries with proteomic research is another huge area for the future, as molecular geneticists take advantage of the explosion of new human disease genes. Identifying functional complexes, modules and genetic pathways through the identification of protein binding partners and animal modeling will yield more robust platforms from which to consider therapeutics. An example is in PKD, one of the most common Mendelian diseases. Based upon the knowledge of similarities in phenotype between PKD and tuberous sclerosis complex (TSC), investigators wondered if there was a possible functional connection. The two genes mutated in TSC are involved in the mTOR pathway, and some symptoms are treatable with mTOR inhibitors like rapamycin. Investigators hypothesized that PKD1 and tuberin (one of the genes mutated in TSC) might interact. After confirming this hypothesis, they tested whether, like TSC, PKD might be abrogated by mTOR inhibitors. Strikingly, rapamycin results in a significant reversal of renal cystogenesis in two different mouse models (Shillingford et al., 2006), and these findings led to a clinical trial in humans. More recent work hints at the possibility that mTOR inhibitors may benefit other ciliopathy conditions (Tobin and Beales, 2008). Although the human trials were not successful and will need to be repeated with different endpoints, the story exemplifies the streamlining of approaches as investigators move from human disease genes to new treatments.

Because animal models can frequently be significantly different in terms of organ specificity and severity compared with their human counterparts, they have been of limited benefit in

directly exploring disease pathogenesis. For instance, both published mouse models for the most commonly mutated gene in human NPHP, *NPHP1*, shows no detectable kidney phenotype. This could be a result of functional differences in the kidney in humans versus mice, differences in the types of mutations, or in the genetic background. Although *NPHP1* mutant mice have some evidence of ciliopathy-like features, including aberrant sperm maturation and a background-dependent genetic interaction with *AHI1* in RD (Jiang et al., 2009; Jiang et al., 2008; Louie et al., 2010), in general the use of animal models of human disease often requires careful assessment of relevance.

As in many other diseases, investigators that continue to return to patients and patient-derived samples for clues to pathogenesis are more apt to make disease-relevant discoveries. Induced pluripotent stem cells (IPSCs) offer such an opportunity, especially for diseases like ciliopathies, in which the genetic background is probably critical for phenotypic expressivity. For this reason, it might be preferable to work with a patient sample carrying a known disease-causing mutation in the disease-causing genetic background than a sample from a knockout mouse where the species or the species' genetic background may not be appropriate for full expressivity. IPSCs and other cellular reprogramming strategies offer an opportunity to make discoveries in disease-relevant cells. For example, a recent application of the spheroid cellular assay to probe mechanistic insights of kidney cysts (Otto et al., 2010) might greatly benefit from the use of patient-derived cells.

Functional genomic cell-based screens also offer a complementary approach to the study of human disease, by leap-frogging past animal models in the identification of potential treatment-relevant targets. In the past year, a number of such cell-based high-throughput or high-content screening systems to identify genes required for cilium assembly and maintenance or signaling pathways known to depend upon the cilium have emerged. By combining Stable Isotope Labeling with Amino acids in Cell culture (SILAC) with BAC transgenesis in human cells, a list of 135 new centriolar components that are specific to either the mother or daughter centriole were identified (Jakobsen et al., 2011). Given the important coordination of centriole function with ciliogenesis, this list is likely to lead to many new functional discoveries in the future. Genome-wide RNAi screening in cells has identified the multitasking kinase Stk11 (a.k.a Lkb1), as a key factor in cilium stability and an integrator of Shh and Wnt signals in cells, two key pathways modulated by the cilium (Jacob et al., 2011). Finally, two recent papers established the genetic requirements of cilium assembly and length in mammalian cells, highlighting the important contribution of the actin cytoskeleton and showing it is possible to uncouple ciliary cargo transport from cilia formation in vertebrates (Kim et al., 2010; Lai et al., 2011).

Rapidly evolving sequencing methods combined with the underlying growth of informatic algorithms can provide the power to uncover both new causes as well as new potential treatments in human disease. Using the relatively simple example of the ciliopathies, we can see how the paradigm is shifting in experimental biology to one less reliant on the pure study of animal models in favor of using humans as a model to study human disease. Further integration of these new approaches has the potential to yield improved diagnosis and opens the window for new therapies across the field of genetics.

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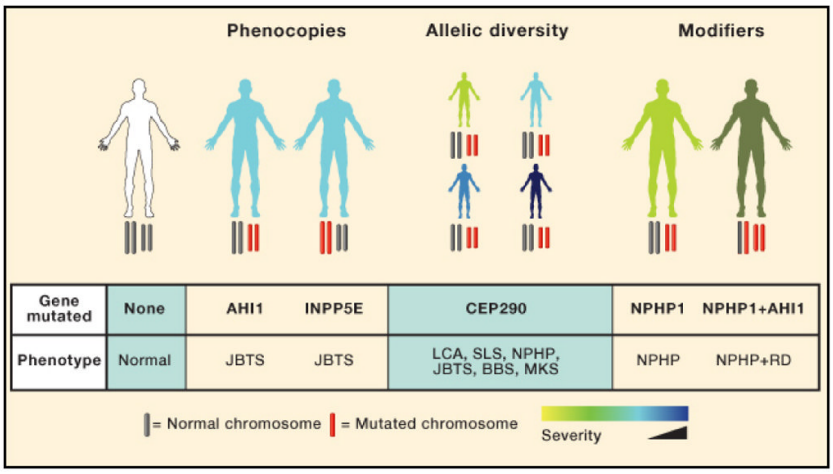

Figure 1. Human genetic interactions in the ciliopathy spectrum

Diseased individuals are in color, with severity represented by darker shading. Phenocopies refers to the finding that patients with homozygous or compound heterozygous mutations in two different genes (i.e. *AHI1* or *INPP5E*) can show indistinguishable JBTS phenotypes. Multiple allelism at the same locus indicates that mutations in a single gene (i.e. *CEP290*) can lead to various distinguishable phenotypes. Modifiers refers to evidence that potentially deleterious sequence changes in a gene like *AHI1* can modify in quantifiable ways the phenotype observed in patients with *NPHP1* mutations. Black: normal chromosome; Red: mutant chromosome. *AHI1: abelson helper integration site 1; INPP5E: inositol polyphosphate-5-phosphatase E; CEP290: centrosomal protein 290kDa; NPHP1: nephrocystin 1*. BBS: Bardet-Biedl syndrome; JBTS: Joubert syndrome; LCA: Leber congenital amaurosis; MKS: Meckel syndrome; NPHP: nephronophthisis; RD: retinal dystrophy; SLS: Senior Loken syndrome.

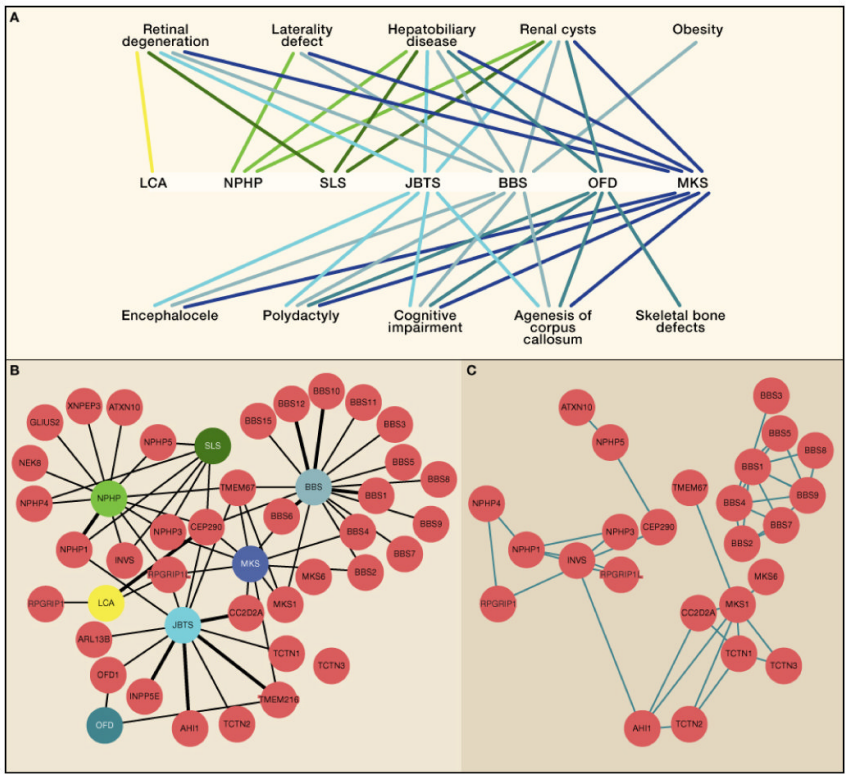

Figure 2. Phenotypic and interactome diversity of the ciliopathies based upon major organ involvement

A. Disease is represented below by abbreviation, and involvement of major organ listed above. B. Major ciliopathy diseases (color coded by severity), and gene mutated in each condition (red) linked by black bar with more common causes showing thicker lines. C. The same gene map, now indicating evidence for direct interaction between protein products. Protein interaction networks identified from published data demonstrating major clustering of interactions corresponding to disease networks. Note that genes causing a particular disorder tend to have products that interact, although there are many genes without known connections. Note that some genes like *CEP290*, which can cause several different diseases, should serve as hubs, but have few demonstrated physical interactions to date. BBS: Bardet-Biedl syndrome; JBTS: Joubert syndrome; LCA: Leber congenital amaurosis; MKS: Meckel syndrome; NPHP: nephronophthisis; OFD: Orofaciodigital syndrome; SLS: Senior Loken syndrome.

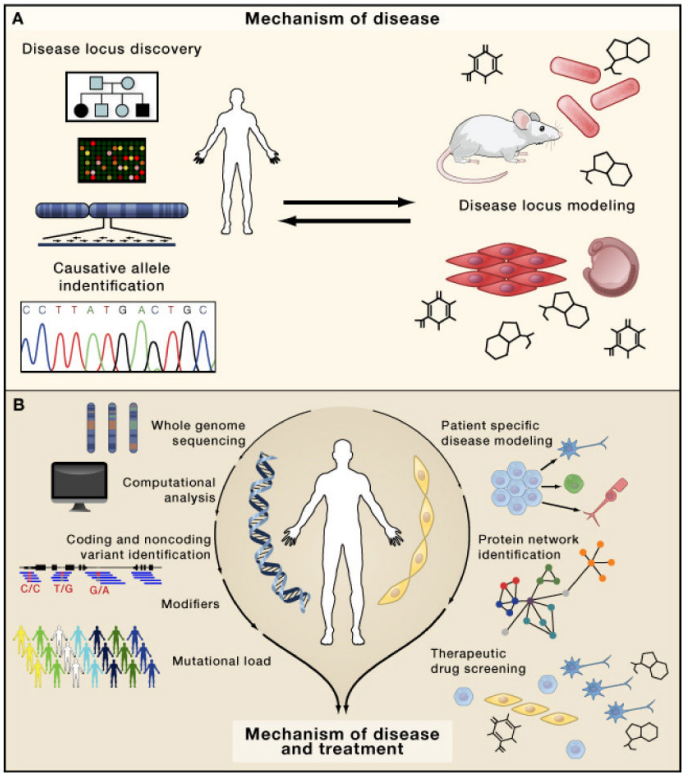

Figure 3. Discovery pipeline for mechanism of disease: past and future paradigms

A. Former strategy to identify disease mechanisms, starting with ascertainment of pedigrees for linkage, disease mapping to a particular chromosomal locus, and candidate gene Sanger sequencing. Once a mutant gene was identified, animal models were created to understand the mechanism. B. The future paradigm bypasses the need for informative pedigrees and disease mapping, instead going directly from patient ascertainment to genome sequencing, then to variant identification and expanded to identification of modifiers and mutational load. In parallel, patient-specific disease modeling using human cells coupled with protein interaction networks and therapeutic drug screening can further uncover disease mechanisms and help develop better treatments.