

The Diverse Genetic Landscape of Neurodevelopmental Disorders

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Abstract

Advances in genetic tools and sequencing technology in the past few years have vastly expanded our understanding of the genetics of neurodevelopmental disorders. Recent high-throughput sequencing analyses of structural brain malformations, cognitive and neuropsychiatric disorders, and localized cortical dysplasias have uncovered a diverse genetic landscape beyond classic Mendelian patterns of inheritance. The underlying genetic causes of neurodevelopmental disorders implicate numerous cell biological pathways critical for normal brain development.

INTRODUCTION

The complexity of the human brain is astounding. During development, distinct cell types must proliferate, differentiate into various fates, migrate to their proper locations, and integrate into a cohesive circuitry. If all goes well, at the end of development, 85 billion neurons (8) will form the highly specified and specialized human brain, capable of complex language, cognition, and emotion. These elaborate and uniquely human processes have intrigued scientists and philosophers alike. To understand how these processes occur, physicians and scientists have often focused on disorders of brain function. By studying how brain development and function go awry, we can better understand the critical components of normal development and function.

Human genetics is a powerful tool to dissect brain development because it is increasingly clear that humans as a population show mutations in every gene—if not every codon—of the genome (21, 134, 135), providing a rich opportunity to identify disease-causing genes essential for development. Historically, geneticists have relied heavily on Mendelian principles of inheritance, based on the premise that each gene possesses a specific function that, when perturbed, causes a specific set of disease symptoms (26). However, the era of next-generation, high-throughput deep sequencing has changed the way we study human genetics (79). The ability to sequence massive amounts of DNA rapidly, cost effectively, and at greater sequence read depth has led to an explosion of computationally based gene identification, in which the tens of thousands of genetic variants in an individual's exome are filtered by multiple layers of criteria (including inheritance model, type of mutation, absence in control populations, allelic frequency, and predicted pathogenicity) to rapidly arrive at a small subset of candidate disease genes (88, 89). Compared with the years required to identify disease-causing mutations two decades ago, we can now identify variants throughout an individual's exome in a matter of weeks. These techniques have led to a surge in the identification of novel disease genes and new alleles in known disease genes.

In particular, high-throughput sequencing has allowed us to more readily capture mutations causing subtle or atypical phenotypes, including hypomorphic and less-penetrant alleles. As a result, novel mutations in known disease-causing genes are being linked to phenotypes separate from their typical Mendelian phenotype. Recent studies in neurodevelopment, reviewed here, indicate that the genetic and phenotypic diversity of known disease genes is even broader than previously anticipated and shed light on the structural and connective complexity of the brain.

MENDELIAN DISORDERS OF NEURODEVELOPMENT HIGHLIGHT PATHWAYS CRITICAL FOR CORTICOGENESIS

The normal human cortex lies at the surface of the brain and is composed of six distinct histological layers. Development begins from neuroepithelial progenitors lining the lateral ventricles that divide to expand the progenitor pool and then give rise to intermediate progenitors, which subsequently divide and give rise to neurons (see sidebar Overview of Neuronal Development along with **Figure 1**). The neurons migrate from the proliferative ventricular zones toward the pial surface of the brain to form the layered cortex, where the connections between neurons form and mature (78). The convolutions of the brain surface, known as gyri and sulci, are thought to be due to the dramatic expansion of neurons in number and surface area, relative to the volume of the brain (109, 145).

Diseases have been identified that affect each stage of neurodevelopment (**Figure 2**). For example, microcephaly (“small brain”) is caused by defects in progenitor proliferation, resulting in a decreased number of neurons and smaller brain size. The identification of autosomal recessive primary microcephaly (MCPH) genes reflects the changing dynamics of human genetics as a

OVERVIEW OF NEURONAL DEVELOPMENT

The neocortex comprises two major cell types: neurons and glia. Neurons are the signaling cells of the nervous system, and glia perform myriad functions to support the function of neurons. Neurons can be further subdivided into excitatory projection neurons and inhibitory interneurons. The development of excitatory neurons differs from that of inhibitory neurons.

Projection neurons are born from polarized neuroepithelial cells lining the lateral ventricle, which divide and differentiate into radial glial progenitors, with cell bodies along the lateral ventricle and a long basolateral fiber extending to the pial surface (78) (see **Figure 1**). Radial glia subsequently divide to give rise to intermediate progenitors and outer radial glial progenitors, forming the proliferative ventricular zones of the brain. These progenitors divide and give rise to excitatory projection neurons, which migrate along the radial processes of the radial glia toward the pial surface and form the cortical plate. Early-born neurons form the deepest layers of the developing cortex, and later-born neurons migrate to the more superficial surface to form the upper layers of the cortex, eventually forming a highly organized, laminar structure. After neurogenesis is complete, progenitors give rise to glia (48). In contrast, inhibitory interneurons are born in the ventral regions of the developing brain, in the ganglionic eminences, and first migrate tangentially into the dorsal telencephalon. Upon reaching the dorsal cortex, they turn and migrate radially into the cortical plate and integrate with the excitatory projection neurons (138).

Mutations in various genes have been shown to interfere with the proliferation and migration of neurons. Microcephaly genes, including *WDR62* and *NDE1*, discussed in this review, are implicated in ventricular zone progenitor proliferation, and lissencephaly genes, including *LIS1*, *DCX*, and *ARX*, affect neuronal migration from the ventricular zones to the cortical plate. In addition, polyalanine tract expansions in *ARX* affect interneuron migration, resulting in imbalances between excitation and inhibition, which are thought to be the cause of epilepsy. Cognitive and neuropsychiatric disorders are also believed to be due to perturbations in synapse function.

result of high-throughput sequencing. Initial linkage analyses more than a decade ago identified six loci for MCPH (107), and subsequent positional cloning revealed causative mutations in genes encoding centrosomal and pericentriolar proteins (including *MCPH1*, *CDK5RAP2*, *ASPM*, and *CENPF*) (15–17, 49, 61, 68, 90, 119, 122, 143). However, several loci, notably *MCPH2* and *MCPH4*, remained elusive (63, 108), as the linked intervals simply contained too many genes. With the advent and power of high-throughput sequencing, three groups nearly simultaneously discovered *WDR62* as the causative disease gene at the *MCPH2* locus (15, 90, 143). More than a dozen new null and hypomorphic *WDR62* alleles were identified and connected *WDR62* to a wide array of structural brain defects beyond primary microcephaly. The causative disease gene at the *MCPH4* locus also required more than a decade to identify, with studies using high-throughput techniques eventually finding two separate genes at this locus, *CASC5* and *CEP152*, that cause microcephaly (42, 49). In addition to the six classic MCPH loci, more novel MCPH genes have been identified in the past few years, including *STIL*, *CEP135*, and *CEP63* (60, 68, 122).

A common theme arising from genetic studies of microcephaly is the role of the centrosome in neuronal proliferation. The centrosome is a key microtubule-organizing center that helps maintain the cellular cytoskeleton (18, 92). During meiosis and mitosis, centrosomes nucleate the microtubule spindles to coordinate the segregation of duplicated chromosomes. Nearly all of the identified MCPH genes encode centrosomal proteins, or proteins required for proper chromosomal segregation. Given the colocalization of microcephaly proteins to the interphase centrosome and mitotic spindle poles, and the similarity of their mutant phenotypes, it is tempting to posit that they may function together in a complex or as part of a common pathway to regulate neuronal progenitor proliferation.

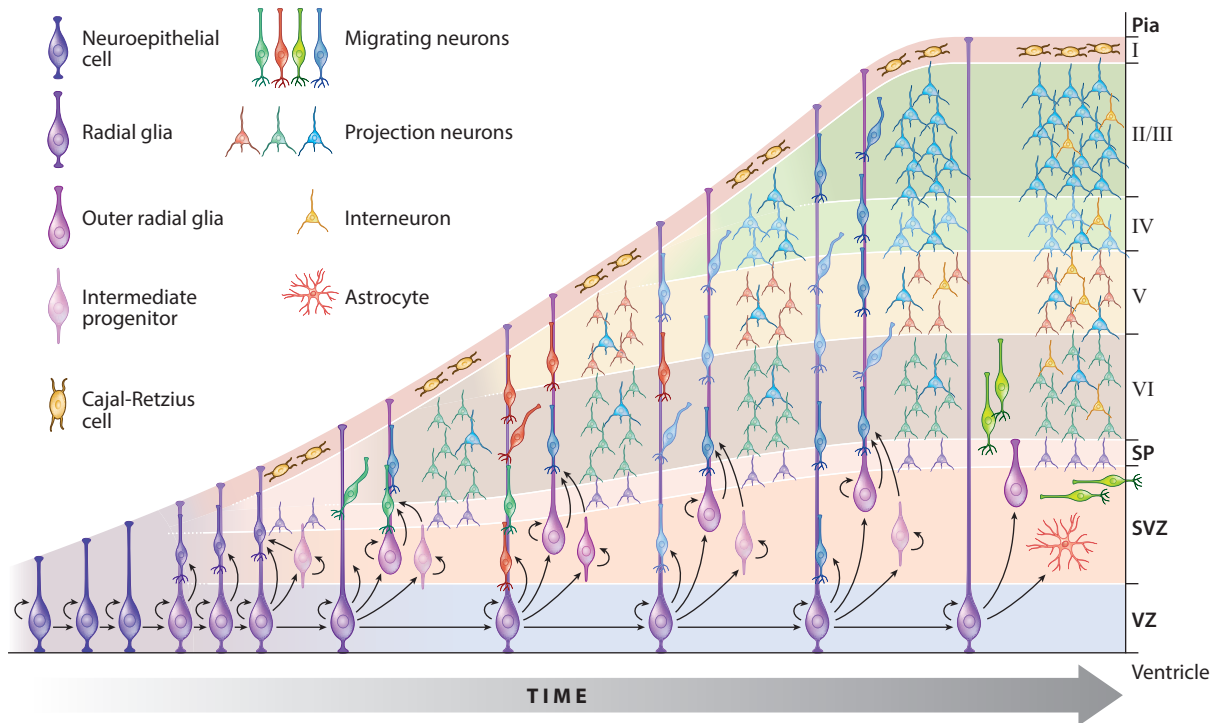


Figure 1

Schematic of neocortical development. The development of the cortex begins at the ventricular zone (VZ) for excitatory projection neurons and at the ganglionic eminences for inhibitory interneurons. Both progenitor populations undergo multiple rounds of proliferation before differentiating into neurons and migrating into the cortical plate, where they integrate into functional circuitry. (For additional details, see sidebar Overview of Neuronal Development.) Abbreviations: SP, subplate; SVZ, subventricular zone. Adapted from Greig et al. (48) with permission.

Genetic advances resulting from new sequencing techniques have also expanded our understanding of neuronal migration disorders in recent years. Lissencephaly is a striking brain malformation defined as a loss in gyral patterning on the surface of the brain, with concomitant thickening of the cortex. This thickening is caused by defective neuronal migration resulting in disorganization within the cortex, as neurons no longer form the normal laminated structure (139). The first disease gene, *LIS1* (also known as *PFAFH1B1*), was identified 20 years ago (76, 105), and the second, *DCX*, encoding the doublecortin protein, was discovered 5 years later (29, 43). The majority of lissencephaly cases are attributable to mutations in these two genes (99).

The identification of *LIS1* and *DCX* mutations utilized the traditional genetic tools of karyotyping, linkage analysis, and positional cloning, based on clues from large cohorts of patients and families. As is often the case with Mendelian diseases of severe phenotypes, mutations in *LIS1* and *DCX* represent loss-of-function alleles. Subsequent identification of additional *LIS1* alleles revealed genic deletions, missense mutations, and nonsense mutations (76). To date, all null germline mutations in *LIS1* cause classic lissencephaly, following the Mendelian “one gene, one phenotype” phenomenon. Germline mutations in *DCX*, which is X-linked, also result in stereotyped phenotypes—lissencephaly in males and double cortex in females. Loss-of-function nonsense mutations, frameshifts, genic deletions, and splicing alleles are distributed across the entire length of the protein (10, 135), whereas missense mutations cluster predominantly around two

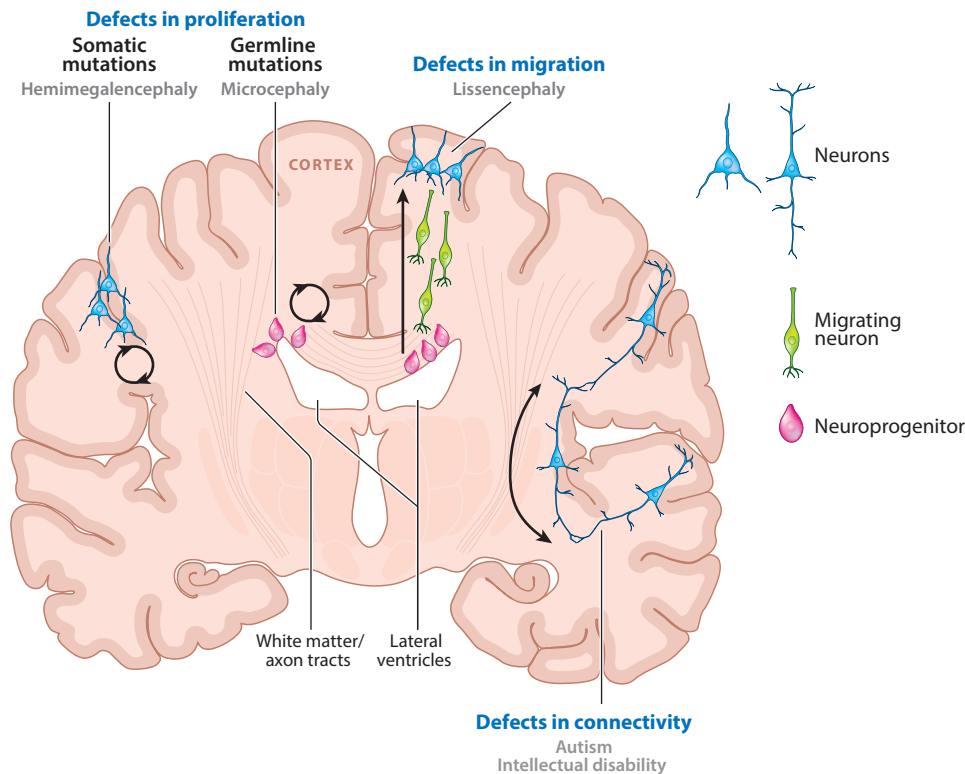


Figure 2

Schematic showing how neurodevelopmental disorder mutations affect genes that function at different stages of neurodevelopment. Defects in the proliferation of progenitors in the ventricular zone or the cortex can lead to microcephaly or hemimegalencephaly, respectively. Defects affecting the migration of neurons from the ventricular zone to the cortex give rise to lissencephaly. Connectivity defects are thought to underlie autism, intellectual disability, and neuropsychiatric disorders.

functional microtubule-binding domains and disrupt tubulin binding, also rendering the protein functionally null (112, 126). Thus, loss-of-function missense alleles can identify specific residues critical for protein structure and function.

Since the discovery of *LIS1* and *DCX*, an amazing variety of additional genetic causes of lissencephaly have been discovered at increasing rates via high-throughput sequencing, including de novo mutations in *TUBA1A*, encoding a neuronal alpha-tubulin (65). Most recently, de novo mutations in *DYNC1H1* (encoding a dynein heavy-chain isoform), *KIF2A* (encoding a kinesin heavy chain), and *TUBG1* (encoding gamma-tubulin) have also been found in association with microcephaly and pachygyria (“thick gyri”), a milder disease on the lissencephaly spectrum (102).

These discoveries add to a growing body of literature highlighting the role of cytoskeletal proteins in lissencephaly and deepening our understanding of the underlying cell biology (see sidebar The Role of the Cytoskeleton in Neurodevelopment). The complex of cytoplasmic dynein with Lis1 (36) and homologs Nde1 and Ndel1, both of which are centrosomal proteins (37, 91, 114), has long been known to be essential for neuronal migration (120, 125, 130, 141), and *Nde1*-null mice display reduced brain size and defects in cortical neuron migration (38). Recent genetic studies, nearly a decade after the initial characterization of the mouse phenotype, have identified

THE ROLE OF THE CYTOSKELETON IN NEURODEVELOPMENT

Cytoskeletal rearrangements are crucial for every aspect of neurodevelopment, from the regulation of cell division and migration, to the growth of extensive dendritic arbors and axonal branches, to the transport of cargo along those fibers. Several lines of evidence support a role for the centriolar organization of microtubules in regulating neuronal progenitor mitosis. Centrioles are microtubule-based structures that comprise the cores of centrosomes. During the G₀/G₁ phase of ciliated cells, centrioles form the basal bodies required for the proper generation of cilia and flagella. Signaling pathways transduced via primary cilia, such as the sonic hedgehog and Wnt pathways, are known to serve important functions in neuronal fate determination and regulation of cortical size. Prior to entry into the cell cycle, cilia and flagella are resorbed, and the centrioles are released from the basal body to nucleate the mitotic spindle and coordinate the segregation of chromosomes during mitosis (92).

Additionally, the genetic evidence that mutations in genes encoding microtubule-associated proteins (including *LIS1* and *DCX* as well as genes encoding dyneins, kinesins, and tubulin isoforms) cause disease points to a role for the microtubule structure itself. In particular, *Dcx* regulates microtubule stability, and perturbations cause defective migration, as rapid cytoskeletal rearrangements are necessary during migration. Additionally, motor proteins are required both for the trafficking of specific cargo and for the generation of force during structural rearrangements. For example, coupling of the nucleus to the centrosome during cell migration is necessary and depends on the Lis1-dynein-Nde1/Ndel1 complex.

mutations in *NDE1* in patients with microlissencephaly, confirming a link between the underlying cell biological pathways of microcephaly and lissencephaly (2, 12). Doublecortin, a microtubule-associated protein that stabilizes microtubules against depolymerization (39, 44), is also required for proper neuronal migration (11), and recent biochemical and cell biological studies have focused on doublecortin as an adaptor that directly binds to tubulin molecules, facilitating the interaction of kinesins (and potentially also dyneins) at the microtubule interface (75).

Finally, recent studies have identified mutations in different genes encoding tubulin subunits and kinesin proteins associated with cortical brain malformations and neurological disorders beyond microcephaly and lissencephaly, ranging from polymicrogyria to nodular heterotopia to eye movement abnormalities. These include mutations in *TUBB2B* (encoding a beta-tubulin isoform) (62), *TUBB3* and *TUBB5* (encoding neuronal beta-tubulin isoforms) (22, 128), and *KIF5C* (encoding a neuron-specific kinesin) (102). Notably, unlike the almost exclusively loss-of-function mutations in *LIS1* and *DCX*, the identified tubulin, dynein, and kinesin alleles are nearly all heterozygous de novo missense changes. To date, null homozygous or heterozygous alleles—which would suggest loss of function or haploinsufficiency as the cause of disease—have not been identified, raising the possibility that these mutations harbor a specific dominant-negative effect (22, 102) and that loss of function or haploinsufficiency may be incompatible with life. Given the rare, sporadic nature of these mutations, high-throughput sequencing was instrumental in their identification.

ALLELIC DIVERSITY BROADENS THE SPECTRUM OF NEURODEVELOPMENTAL PHENOTYPES

Since the initial identification of the classic loss-of-function *LIS1* and *DCX* mutations, missense mutations as well as somatic and germline mosaic mutations have been found to cause variable brain phenotypes. These include somatic *LIS1* mutations and somatic *DCX* mutations in males, causing double cortex (45, 121), and missense *DCX* mutations in females, causing intellectual

disability (ID) and cryptogenic epilepsy (50). Although these examples were by far the minority, they hinted that subtle perturbations in protein function or in a limited subset of cells may manifest differently from the complete loss-of-function phenotype, and they foreshadowed the broadening of genotype–phenotype correlations revealed in recent years.

An early example of phenotypic diversity occurs in the gene *ARX*, encoding Aristaless-related homeobox protein. Null alleles of *ARX* classically cause a syndrome of X-linked lissencephaly with agenesis of the corpus callosum and ambiguous genitalia with early lethality (OMIM 300215) (64, 66, 132), whereas missense changes and polyalanine tract expansions cause X-linked ID with various forms of epilepsy (115, 124) and seizures, infantile spasms, and mild to moderate ID with or without dystonia, ataxia, or autism (1, 14, 33, 123, 131). There is a general correlation between genotype and disease: Truncating and null mutations cause the most severe malformations, whereas missense and polyalanine tract repeat alleles cause ID and seizures, often without any identifiable structural involvement, although the severity of ID can vary widely (40, 64, 80).

Another outstanding example of the power of human genetics to define biochemically related proteins while showing a range of phenotypic diversity is the dystroglycanopathies, a group of disorders causing muscular dystrophy with variable central nervous system (CNS) involvement. The primary defect is in O-glycosylation of alpha-dystroglycan, which interferes with its ability to bind extracellular matrix ligands (84). Mutations of multiple genes in the glycosylation pathway have been identified as causative mutations for a wide spectrum of dystroglycanopathies (47), and recently, two missense mutations in the gene encoding alpha-dystroglycan were shown to cause disease (41, 52), punctuating the genetic links to a biochemical pathway.

The severity of dystroglycanopathies varies widely, from congenital onset with profound brain and eye malformations and early infantile death to adult-onset muscular dystrophy with no brain or eye involvement (47). Furthermore, mutations in the same gene can cause widely different diseases (25, 81). For example, *FKRP*, encoding Fukutin-related protein, was first identified as causing severe congenital skeletal muscle defects without brain or eye abnormalities (23). However, mutations in *FKRP* were soon thereafter identified as causing milder muscular dystrophies with an age of presentation as late as adulthood (24); Walker–Warburg syndrome (OMIM 613153) and muscle-eye-brain disease (OMIM 613153), both of which have severe CNS manifestations; and other dystroglycanopathies with CNS abnormalities (13, 77, 82, 129). In fact, this has prompted a reclassification of dystroglycanopathies based on the genetic underpinnings rather than the constellation of phenotypes (46, 47).

The broad phenotypic spectrum caused by mutations in the same gene is a constant theme in recent genetic studies of neurodevelopmental disorders. *WDR62* patients exhibit variable gyral simplification, cortical lamination, and abnormalities of the corpus callosum (15, 90, 143). Mutations in *DYNC1H1* can also cause Charcot–Marie–Tooth neuropathy (OMIM 614228) (136) and peripheral neuropathy in a form of spinal muscular atrophy (OMIM 158600) (53), and *DYNC1H1* patients with cortical malformations have variable neuropathy (102). Finally, *TUBB3* mutations, which cause eye movement disorders affecting the oculomotor nerves, show variable white-matter tract abnormalities (128).

In fact, the same mutation in the same gene can, less commonly, cause different diseases. Previously, it was generally believed that the range of phenotypes was due to allelic differences. However, the same alanine repeat expansion in *ARX* causing X-linked ID without structural brain abnormalities was recently reported to cause agenesis of the corpus callosum with periventricular heterotopia, frontal polymicrogyria, and interhemispheric cyst (97). Identical alleles of *POMGNT1* (140), another member of the glycosyl transfer pathway, cause different dystroglycanopathy severities (30), even within the same family (127), and a familial *DYNC1H1* allele affects children more severely than a mother who harbors the same mutation (102).

The growing diversity of disease-associated phenotypes identified in recent years highlights the complexity of neurodevelopmental disorders (**Figure 3**). This may be partially attributable to the fact that new sequencing methods capture more rare, hypomorphic, and less-penetrant alleles, whereas prior linkage studies were necessarily biased toward functionally null and highly penetrant mutations. Taken together, these data clearly indicate that other genetic and nongenetic factors that modify disease phenotypes are much more prevalent than previously thought.

A RANGE OF ALLELES IN MENDELIAN DISEASE GENES CAUSE COGNITIVE AND NEUROPSYCHIATRIC DISORDERS

The plummeting cost of sequencing (137) and concomitant introduction of high-throughput sequencing technologies paved the way for studies of neurodevelopmental diseases that do not have structural abnormalities and are of unclear inheritance. Several large-scale studies using whole-exome sequencing have identified a high rate of de novo mutations in nonsyndromic cognitive disorders, including ID. Unlike inherited mutations, de novo mutations are present in a child but not in that child's parents, and likely arose in the germline of the parent. De novo mutations in ID consist of large copy-number variants that delete one allele of many genes (27, 55, 58) and more recently were found to also include isolated point mutations (28, 104).

Another example where modern sequencing techniques have elucidated the genetic underpinnings of nonsyndromic cognitive disorders is autism spectrum disorders (ASDs). ASDs are a complex and genetically heterogeneous group of diseases characterized by impaired social interaction, communication deficits, limited interests, and stereotyped behaviors (59). Multiple genetic and nongenetic factors contribute toward the phenotype, increasing the challenge of studying ASDs. Furthermore, ASDs commonly occur in sporadic individuals without significant family history and would have been impossible to study using traditional methods of linkage analysis and positional cloning. Only with the advent of sequencing techniques in which we can survey broad regions of the genomes of many individuals have we been able to probe the genetic landscape of ASDs. Several recent studies have shown the importance of de novo copy-number variants (72, 100, 110, 117) and de novo point mutations in ASDs (7, 83, 87, 94–96, 111).

A second theme that has emerged from whole-exome sequencing studies is the contribution of recessive hypomorphic mutations to ASDs. These mutations partially inactivate genes that are known to cause more severe phenotypes when completely inactivated. Unlike de novo point mutations, which inactivate only one of two copies of a typical autosomal gene, leaving one copy of the gene still functional, both alleles are mutated in recessive, hypomorphic mutations causing ASDs. For example, ASD mutations have been identified in *AMT*, encoding aminomethyltransferase, which is widely expressed in human tissues (69). Mutations in *AMT* cause nonketotic hyperglycinemia (NKH; OMIM 605899) (5), a recessive syndrome resulting from impaired glycine cleavage. Depending on the severity of *AMT* mutations, NKH symptoms can vary from progressive lethargy, hypotonia, severe seizures, and death within the first year of life to delayed age of onset, developmental delay, and behavioral problems (51). More recently, hypomorphic mutations in *AMT* have been identified in patients with a range of neurodevelopmental symptoms, including ASDs, language and motor delays, ID, and severe epilepsy (142). The biochemical abnormalities that are typically detected in classic NKH often escape clinical detection methods in milder atypical NKH (4, 31). Functional assays to characterize the ASD *AMT* mutations demonstrated that the enzymatic activity of the mutant *AMT* was partially reduced, confirming that the affected children had a mild form of undiagnosed NKH presenting as an ASD with seizures (142).

Another example of a metabolic disease gene where hypomorphic mutations result in ASDs is *PEX7*, encoding a peroxisomal receptor, peroxisome biogenesis factor 7, that is required for protein

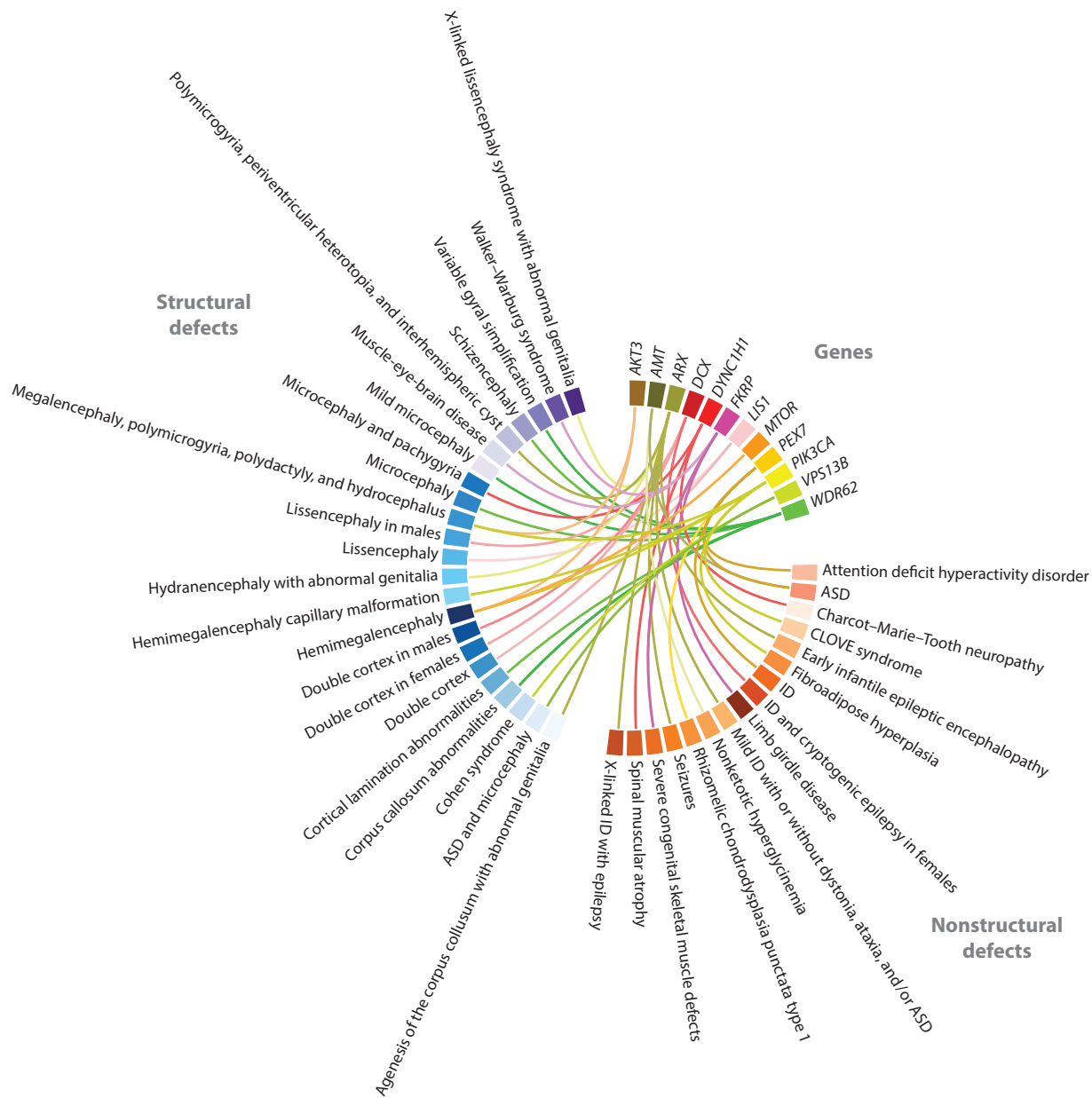


Figure 3

Circle plot illustrating the allelic and phenotypic diversity of a subset of neurodevelopmental disease genes. Mutations in the same gene cause diverse neurodevelopmental phenotypes. Advances in high-throughput sequencing techniques have expedited the identification of hypomorphic and somatic mutations, increasing the spectrum of phenotypes associated with any single gene. Phenotypes are grouped into structural brain defects and abnormalities without any structural brain defects. Abbreviations: ASD, autism spectrum disorder; CLOVE, congenital lipomatous overgrowth, vascular malformations, and epidermal nevi; ID, intellectual disability.

import into the peroxisome. *PEX7* is ubiquitously expressed, and mutations in the gene classically result in rhizomelic chondrodysplasia punctata (RCDP) type 1 (OMIM 215100), a metabolic syndrome of severe ID, growth retardation, skeletal dysplasia, and cataracts, with most cases not surviving beyond two years of age (19, 20, 86). Patients with partial loss-of-function mutations in *PEX7* often lack the skeletal abnormalities typically associated with RCDP and can have ASD, severe attention deficit hyperactivity disorder, or ID (19). Mutations in another neurometabolic disease gene, *PAH*, encoding phenylalanine hydroxylase, the cause of phenylketonuria (OMIM 261600), were one of the earliest-described causes of ASD (144).

Cohen syndrome (OMIM 216550) is a disorder of ID, microcephaly, motor abnormalities, hypotonia and joint laxity, facial dysmorphisms, retinal dystrophy, and intermittent neutropenia (56). Cohen syndrome is caused by recessive mutations in *VPS13B*, encoding vacuolar protein sorting 13 homolog B (yeast), which is widely expressed in human tissues (67). There is significant variability in the spectrum of Cohen syndrome clinical features (85, 118), but many patients with biallelic null mutations in *VPS13B* can present with typical ASD features. In addition, milder forms of Cohen syndrome, often resulting from missense mutations suggestive of partial loss of function, have been identified that are associated with ASD, microcephaly, mild dysmorphic features, and joint laxity (32).

With advances in sequencing technology and the introduction of clinical sequencing, it is anticipated that additional mild hypomorphic mutations in syndromic disease genes will be identified in ASDs and other neuropsychiatric disorders. Notably, syndromic disease genes with hypomorphic ASD mutations are ubiquitously expressed (20, 67, 69) and cause multiorgan system disease with complete loss of function. This is in contrast to more traditional Mendelian disorders of neurodevelopment, such as lissencephaly caused by mutations in *DCX* and *ARX*, where gene expression is limited mostly to the nervous system (29, 43, 66, 98, 124). The restricted expression patterns of *DCX* and *ARX* explain the tissue-specific phenotypes caused by loss-of-function alleles. For hypomorphic alleles of ubiquitously expressed genes causing ASDs, an explanation for the prevalence of neurological phenotypes remains elusive, although one explanation could be the selective sensitivity of the brain to mild perturbations in the levels of these proteins compared with that of other organs.

The significant variability in the phenotypes that can be associated with developmental syndromes makes genetic diagnosis of the milder atypical forms presenting as ASDs and other nonmalformation neurodevelopmental diseases challenging. Clinical sequencing should help overcome this challenge in at least a subset of cases, increasingly driving genetic rather than phenotypic classification. However, functional validation of candidate hypomorphic mutations will remain critical.

SOMATIC MUTATIONS CAUSE LOCALIZED BRAIN MALFORMATIONS

Some of the most remarkable findings about complex patterns of mutation have come from the realization that some de novo mutations occur after fertilization and might not be present in all tissues, but nonetheless affect the brain. Early confirmation came with the identification of somatic mutations in *LISI* and *DCX* (45, 121). However, these mutations were found in relatives of known *DCX* patients. It would take another decade to develop techniques capable of identifying somatic mutations in an unbiased manner. Key breakthroughs allowing the study of somatic mutations have included deep sequencing (to capture alleles present at low frequencies in bulk tissue) and single-cell sorting and genomic amplification techniques (to isolate and allow sequencing of individual cells).

Recently, somatic mutations in the PI3K-AKT-mTOR pathway were identified as causing hemimegalencephaly (71, 101, 106). Hemimegalencephaly is an overgrowth disorder of one hemisphere of the brain, often causing severe epilepsy that requires resection of the entire hemisphere (3, 6, 54, 113, 116). The sporadic nature of the disease and localized brain involvement is consistent with a noninherited pattern and highly suggestive of somatic mutation (9).

Studies of the resected diseased tissue identified mutations in the PI3K-AKT-mTOR pathway present in a subset of cells from the brain that were either absent from circulating lymphoblasts (101) or present at lower levels (106). Based on the sequencing of single cells, the proportion of affected alleles was estimated at 39% of neurons and 27% of nonneuronal cells (35). Surprisingly, a mutation in only a small proportion of affected cells is capable of causing disease of the entire tissue. Germline activating mutations in the PI3K-AKT-mTOR pathway have not been identified and are likely incompatible with life. In support of this hypothesis, the mutations identified to date in genes of this pathway that are associated with numerous other overgrowth syndromes, including Proteus syndrome (asymmetric overgrowth of the hands and feet, nevi, adipose tissue, and vascular malformations; OMIM 176920) and CLOVE syndrome (congenital lipomatous overgrowth, vascular malformations, and epidermal nevi; OMIM 612918), are also mosaic in nature (70, 73, 74).

The identification of somatic mutations in hemimegalencephaly raises the question of whether somatic mutations also cause focal neurological diseases, including focal cortical dysplasias. Tuberous sclerosis complex (TSC) is a neurocutaneous syndrome causing growth of nonmalignant hamartomas in multiple organ systems, including cortical tubers in the brain that are similar to focal cortical dysplasias. TSC is caused by mutations in *TSC1* and *TSC2*, which are components of the mTOR pathway (34, 133), and somatic second-hit mutations overlying a germline mutation are common in non-nervous-system hamartomas (57, 93) and can cause cortical tubers, albeit at low frequencies (103).

Whether somatic mutations also cause diseases of connectivity without structural abnormalities, including neuropsychiatric diseases and ASDs, where de novo mutations are common, remains unanswered. For focal malformations, tissue resection is often necessary to control epilepsy (3, 6, 54, 113, 116) and provides a means for postoperative diagnostic confirmation. In hemimegalencephaly patients with other nonbrain malformations, low levels of mosaicism were detected in blood and saliva samples (106), suggesting that in cases where somatic mutations occur early during embryogenesis and affect multiple organ systems, noninvasive diagnosis is possible. Additionally, given the recent evidence from recessive autism mutations in which germline hypomorphic mutations preferentially affect cognitive function, it is possible that systemic mosaicism may also preferentially affect the brain, even in the absence of gross malformations. Studies of brain-specific mosaicism, however, will require access to brain tissue and may limit our ability to detect late, organ-specific somatic mutations in disease.

CONCLUSIONS

The examples reviewed here illuminate how the landscape of human genetics is expanding, from classic Mendelian inherited diseases and mutations of high penetrance, to more complex genes causing variable phenotypes, to de novo somatic mutations detectable only in subsets of cells. The genotype-phenotype boundaries are clearly breaking down. These discoveries would not have been possible without great advances in sequencing technology that led to an exponential drop in costs and expanded sequencing coverage of the genome, as well as the ability to capture high read depth to detect low-level mutations. Many of the more complex genes discussed here were originally identified as Mendelian disorder genes, with new technology aiding in the identification of additional alleles. With whole-exome sequencing becoming the new standard for genetic studies,

we may find that the identification of hypomorphic alleles will soon precede the identification of classic null alleles.

The broad spectrum of genes that cause neurodevelopmental disorders when mutated illustrates the high sensitivity of the developing CNS compared with other organ systems. The fact that hypomorphic mutations in ubiquitously expressed Mendelian disease genes cause neurodevelopmental disorders without the typical multiorgan syndromes supports this hypothesis. Initial studies of lissencephaly syndromes revealed the structural complexity of the brain, and later reports of epilepsy and autism genetics revealed the complexity of connectivity in the brain. Given the absence of structural malformations in most cases of epilepsy, autism, and ID, it seems that connectivity is the most susceptible aspect of neurodevelopment, with diseases of connectivity manifesting before defective neuronal proliferation, differentiation, or migration can cause structural abnormalities.

In the examples presented here, mutations in many different genes often give rise to similar phenotypes. Such data suggest an overlap in cellular mechanisms and pathways. Indeed, lissencephaly genes not only are required for the same cellular process of neuronal migration, but also encode proteins that bind microtubules. Similarly, microcephaly proteins localize to the centrosome and are hypothesized to regulate mitosis, and autism genes overlap significantly with synaptic and metabolic genes. Although the precise pathways of these diseases have not yet been elucidated, recent work on hemimegalencephaly and the perturbation of the PI3K-AKT-mTOR pathway suggests that this will be true for other diseases as well.

The future of genetics is high-throughput sequencing, and clinical centers and geneticists are rapidly embracing whole-exome sequencing as a diagnostic tool. Thus, over the next few years, the rate of gene discovery will continue to accelerate, with an exponential explosion of novel genetic findings.

DISCLOSURE STATEMENT

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Errata

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