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TITLE

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ABSTRACT

The cerebral cortex is an intricate structure underlying human features such as language and cognition. Cortical functions rely on specialized neurons, which emerge during development from complex molecular and cellular interactions. Neurodevelopmental disorders occur when one or several of these steps are incorrectly executed. While a number of causal genes and disease phenotypes have been identified, the sequence of events linking molecular disruption to clinical expression mostly remains obscure. Here, focusing on human malformations of cortical development, we illustrate how complex interactions at genetic, cellular and circuit levels combinatorially contribute to diversity and variability in disease phenotypes. Through select examples and an online resource, we propose that a multi-level assessment of disease processes is key to identify points of vulnerability and develop novel therapeutic strategies.

MAIN TEXT

The cerebral cortex, or neocortex, is critical to key behavioral functions in mammals, including language, sociability, and fine motor skills. This brain structure consists of several dozens of specialized types of neurons organized across layers and areas, which are generated during development by the divisions of diverse progenitor cells. Newborn neurons undergo sequential molecular programs that drive their specific local and long-range circuit connectivity and adult function (*1*). The complexity of corticogenesis is staggering and, while necessary for proper cortical function to emerge, the myriads of molecular and cellular developmental processes involved also provide multiple points of vulnerability for "malformations of cortical development" (*2*), here termed "cortical malformations". These are usually first detected through their clinical expression, including developmental delay with various combinations of intellectual and motor disabilities, often associated with seizures. Despite the toll on patients and their caregivers, only limited treatments exist and while a number of causal genes have been identified, the sequence of events linking molecular disruption with clinical expression mostly remains obscure.

Here, to interrogate cortical malformations and highlight potential points of intervention, we first present basic principles of neocortical development and highlight vulnerable cellular compartments and processes, with particular focus on neurogenesis and neuron migration. Second, we present different "levels" of developmental processes, from genes and gene products to cells, circuits, and clinical expression. Focusing on cortical malformations, we illustrate how complex interactions within and across these levels may account for variable disease patterns. Finally, we propose a framework integrating these different levels of organization, towards better understanding and treatment of the disease, as well as an online resource (http://genebrowser.unige.ch/humous/) to identify and compare genetic and cellular developmental processes in mice, human embryos, and human organoids.

Neocortical development

During embryonic neocortical development, neurons are not born at the place where they will reside in adulthood, but instead migrate relatively long distances to reach their destination. Not only are cortical neurons born at different places, but distinct types of neurons are born at different times, over several days in mice (from embryonic day (E) 11 to 17 (*3*)) and several weeks in humans (from post-conception week (pcw) 8 to 24 (*4*)) (Fig. 1A). Generation of the cortex results from billions of cells undergoing four key sequential and partially overlapping

processes: (1) progenitor division and neurogenesis, (2) migration, (3) neuritogenesis (*i.e.* extension of axon and dendrites) and (4) synaptogenesis (Fig. 1A-C). Cortical malformations occur when one or several of these developmental events are improperly executed (Fig. 1B); they typically manifest as macroscopic anatomical defects visible with brain imaging techniques such as magnetic resonance imaging (MRI) (Fig. 2). Morphological manifestations include microcephaly (decreased brain size), lissencephaly (disrupted cortical folding and lamination), polymicrogyria (numerous small cortical folds), and heterotopias (abnormally positioned cells in periventricular or subcortical regions) (*5*). In other cases, more subtle defects are visible only in pathology specimens, *e.g.* in focal cortical dysplasias (abnormal cortical lamination) (*2*).

From E8.5-E9.5 in mice and pcw4 in human (*4*), progenitors of the dorsal pallium, at the rostral end of the neural tube, which initially form a single-layered neuroepithelium, undergo self-replicating divisions to increase in numbers before generating apical radial glia (aRG, also called apical progenitors) and forming an expanded germinal zone called the ventricular zone (*3*). Around E11.5 in mice and pcw8 in human, aRGs start dividing asymmetrically to give rise to their first neuronal progeny (Fig.1A) (*4*). Different types of excitatory cortical neurons are then sequentially born between E11.5 and E17.5 in mice (pcw8-24 in human) and distribute to form six radially-organized layers in an inside-out manner: deep layer neurons, which project subcortically, are born first while superficial layer neurons, which project intracortically, are born last (Fig. 1A) (*1*). Of note, inhibitory GABAergic neurons, which are not discussed in detail in this review, are born in a distinct germinal zone, the ventral pallium, and migrate at later timepoints into the cortex to form local circuits with glutamatergic neurons (*6*).

Radial cortical organization – The laminar organization of the adult neocortex tightly relies on the radial polarity of progenitors and their daughter cells during development. Accordingly, aRGs are highly polarized cells with a radial process extending to the pial surface, necessary to guide the daughter neuron migration (Fig. 1A). Adherens junctions at the apical (*i.e.* towards the ventricle) pole of aRGs are critical to this polarity, as they maintain a cohesive ventricular zone and regulate the balance between proliferation and differentiation through cell-cell interactions (*3*). Mutations of genes coding for adherens junction proteins, such as α/β–catenins and N-cadherin, indirectly disrupt cell divisions, leading to changes in cortical size and folding, as well as neuronal heterotopias in mice and human (*7-10*). Similarly, mutations in the genes coding for cell-cell contact proteins such as DCHS1 and FAT4 disrupt aRG polarity and neuronal migration, resulting in human periventricular heterotopias (*11*).

Another notable determinant of aRG polarity is the primary cilium, a specialized organelle which transduces signals from ventricular cerebrospinal fluid (Fig. 1A) (*3*). Mutations in genes coding for cilium proteins ("ciliopathy" genes, *e.g. BBS1*, *BBS7*, *BBS10* and *TMEM216*) affect progenitor proliferation and newborn neuron migration in mice (*12*) and mutations in the microtubule-associated protein encoding gene *EML1,* which causes neuronal heterotopia in mice and humans, are likewise thought to act by destabilization of the primary cilium (*13*, *14*).

Cycling of progenitor cells – The ability to undergo cell cycle is a central feature of progenitors. A number of mutations can perturb this process, either by affecting cell divisions, or through premature differentiation into neurons. For example, mutations of genes coding for proteins controlling mitotic spindle organization (Fig. 1A) typically lead to premature differentiation and / or aRG apoptosis, resulting in reduced neurogenesis, and manifesting in humans as microcephaly with or without lissencephaly (*e.g. ASPM*, *CENPJ*, *WDR62* (*15*)). Of note, both premature differentiation into neurons as well as excessive self-replication of progenitors may in principle both result in decreased neuronal output: in one case the progenitor pool is prematurely depleted while in the other, progenitors fail to give rise to neurons. Divisions of aRGs are initially rapid and then slow down as corticogenesis unfolds (from 8 hours at early stages to 18 hours at the end of corticogenesis in mice, and from 23 to 54 hours in primates (*16*, *17*)). The slowing down of cell cycle as corticogenesis proceeds may *per se* confer new properties to daughter cells as low-affinity DNA ligands such as transcription factors are more likely to bind when target sites are available for longer times. Hence, disruption of cell cycle not only affects the number of neurons being generated (typically decreasing neuronal output) but also the relative proportions of daughter neuron types, since distinct types are sequentially produced at different stages of corticogenesis (*18*).

Diversity of progenitor cells – aRGs not only generate neurons, but also a distinct type of progenitor, termed intermediate progenitors (also called basal progenitors), which delaminate from the ventricular zone and form a new germinal layer, the subventricular zone (Fig. 1A). Intermediate progenitors are more neurogenic than aRGs, and act to boost neuronal production (*19*). Consistent with such a function, human mutations affecting intermediate progenitors, such as mutations in *EOMES* (also known as *TBR2*), perturb gyri formation and result in microcephaly, lissencephaly or polymicrogyria (*20*). In most gyrencephalic species (*i.e.* species with a folded neocortex), including primates, additional progenitor types exist, including basal radial glia (also called outer radial glia), which form a distinct proliferative sub-compartment, the outer subventricular zone (Fig. 1A). Basal radial glia express distinctive

genes (*e.g. TMEM14B* (*21*)) and morphologies (*e.g.* many have a basal process but no apical process), and are thought to underlie the disproportionate expansion in superficial layers occurring during gyrification (*22*).

Neuronal migration – Following cell division, while undergoing a complex series of morphological changes, postmitotic daughter neurons migrate radially along aRG basal processes away from the ventricular zone to the cortical plate, where they settle in their appropriate final positions. The radial migration of neurons relies on the extension of a leading process, with the leading edge responding to environmental cues. The neuron's centrosome moves within the leading process allowing somal translocation and locomotion (Fig. 1A) (*23*). The proper execution of this migratory sequence is key to building the correct cortical architecture and relies tightly on microtubule and actin cytoskeleton-associated proteins. Accordingly, mutations in the genes coding for these proteins have dramatic effects on cortical structure (*5*, *15*), and account for a disproportionate fraction of cortical malformations, as will be detailed in a later section.

Preplate splitting, which occurs at E11 in mouse (human pcw9-10) (*24*), is a critical event during corticogenesis and evolution (Fig. 1A). The glycoprotein Reelin secreted by Cajal-Retzius cells present in the marginal zone plays an important role in these processes. Preplate splitting defects consistently give rise to abnormal cortex development due to aberrant cortical neuron position. Indeed, lamination defects in *Reeler* mice, mutant for the *Reln* gene, not only reflect abnormal neuronal migration, but also abnormal splitting of the preplate into a superficial marginal zone (which becomes layer 1) and a transient deep layer subplate (sometimes called layer 6b) (Fig. 1A). In humans, mutations in *RELN* cause autosomal recessive lissencephaly (*25*). As discussed in a later section, despite the severe cortical lamination defects in *Reeler* mouse mutants, long-range cortical input/output connectivity appears largely preserved (*26*, *27*). *Pomgnt2* mouse mutants, a model of "cobblestone" lissencephaly caused by mutation in the *POMGNT2* gene in human, also show preplate splitting defects, since abnormal clusters of subplate neurons are observed in superficial layers (*28*). The subplate itself plays an important role in the radial migration of the neurons which traverse it and, later, in circuit assembly: subplate neurons make transient synaptic connections with migrating neurons, which primes their radial migration (*29*), and also act as a scaffold for thalamocortical and inhibitory circuit maturation (*30*, *31*). Although mutations specifically affecting this process have not been reported in humans, the subplate is usually visible in

human fetal MRIs; in the future, it may be possible to reveal subtle subplate defects underlying more significant circuit dysfunction.

Building circuits – Neuritogenesis and synaptogenesis are critical for circuit assembly and a bottleneck for cortical function. They have been discussed in detail in recent reviews (*32*) and will only be briefly covered here. Axon-dendrite polarization starts soon after neurons are born, when they still are migrating (*33*). Axon and dendrite growth relies on their growth cones which are highly dynamic actin-rich protrusions sensitive to environmental guidance cues (Fig. 1A). As is the case for migration, axonal guidance and extension thus heavily rely on cytoskeletal function. Cortical malformations can be accompanied by anomalies in axonal tracts, most notably in the form of atrophy or agenesis the corpus callosum, which connects both hemispheres in human (*34*). Other anatomically less prominent axonal pathway defects are likely to occur, and the use of MRI techniques such as diffusion tensor imaging (*35*) may in the future further address this possibility. Synaptic dysfunction, including inhibitory synaptic dysfunction, is thought to underlie many if not most neuropsychiatric disorders in humans (*36*), and like migration and neuritogenesis, synaptogenesis involves a highly organized cytoskeleton (Fig. 1A). Neuropsychiatric disorders are not generally associated with salient MRI defects, yet some level of anatomical dysfunction likely exists. For example, patches of cortical disorganization and white matter defects (particularly in the corpus callosum) have been reported in patients with autism spectrum disorder (*37*, *38*). Similarly, decreased neuronal density have been reported in neuropathological specimens of patients with schizophrenia (*39*). The extent to which these anatomical findings drive the clinical phenotype or are secondary to another underlying process remains unclear.

Sources of complexity in cortical malformations

Cortical malformations have a broad range of clinical expression, including seizures, intellectual disabilities, autistic features, failure to reach developmental milestones, and non-neurological co-morbidities (*e.g.* dysmorphic facial and musculoskeletal features, skin abnormalities, cardiovascular defects) (*5*). These features alone often do not allow identification of the cause of the disease, because genetically diverse defects lead to largely overlapping clinical pictures, and impairment of single genes can lead to multiple clinical defects. Thus, while an increasing number of causal genes is being identified and a diversity of anatomical and clinical phenotypes have been recognized (*15*), the sequence of events linking molecular disruption to final outcome remains mostly out of reach. In the following section, we will highlight potential sources of variability which have precluded establishment of clear

causal relationships across genes and molecules, cell types and circuits, and clinical expression (Fig. 3A).

Genetic and molecular dysfunction – Neurodevelopmental disorders can result from single point mutations in critical genes (*i.e.* monogenic mutations; Fig. 3B), or from DNA sequence variations at multiple loci that together increase disease probability. Convergent, divergent, or mixed relationships between genetic defect(s) and disease phenotype(s) may occur (Fig. 3C-F), and penetrance of the genetic mutation may be incomplete and variable (*i.e.* the mutation is not expressed at the clinical level (Fig. 3G)), together complexifying causal analyses. Understanding this cross-level organization is critical in therapeutic terms: for example, "hidden hubs" represent convergent points of pharmacological intervention across disease phenotypes, while disorders with "hidden complexity" would require distinct treatments despite similar clinical presentations (Fig. 3E, F). The contribution of monogenic causes varies across conditions; in the case of lissencephaly for example, 20% of cases could not be associated with a single genetic cause (*40*), which may reflect yet un-identified gene mutations, mutations in non-coding regions (*41*), polygenic or multifactorial inheritance, modulated by the influence of non-genetic factors (*e.g.* infectious or toxic), or somatic mosaicism (*42*). While monogenic causes appear to predominate in cortical malformations, neuropsychiatric disorders such as autism spectrum disorders and schizophrenia instead appear to mostly involve polygenic processes including through chromosomal deletions (*39*). Of note, in chromosomal deletions, disease-causing genes need not to be in the affected chromosomal region as local DNA conformation changes may in principle epigenetically alter gene accessibility in other parts of the genome. Although not discussed in detail here, some genetic mutations occur in a clonal manner, *i.e.* do not affect the whole brain but only the progeny of some cells with somatic mutations. Somatic mutations have been clearly established as a cause of focal cortical dysplasia and hemi-megalencephaly (hypertrophy of a single hemisphere) (*42*), but whether clonal processes are involved in other disorders, and in particular whether at least some psychiatric disorders may result from mosaic synaptic dysfunction, is unknown (*43*). Overlapping clinical pictures can emerge from mutations in genes which are not apparently functionally correlated. For example, over 200 *de novo* variants have been identified in a cohort of patients with periventricular heterotopia, suggesting that many genes will be associated with this condition, with a high level of convergence in the final outcome (*e.g.* mismigration in the case of heterotopias) (*44*).

Complexifying the situation, the aforementioned developmental processes (*i.e.* neurogenesis, migration, axo- and synaptogenesis) often involve redundant molecular mechanisms. For example, the cyclin-dependent kinase CDK5 (whose gene mutation is associated with lissencephaly in human (*45*)) is critical for mitosis in mouse progenitors, but also in their daughter neurons for migration and gyrification, synapse formation and even long-term plasticity in adults (Fig. 1C) (*46*, *47*). Similarly, many, if not most, axon guidance molecules also have earlier roles in neuron migration and even neuron generation, and/or in synapse and spine formation and maturation (*48-50*). For example, the Ephrin receptor EphB2 both guides corpus callosum axons and stabilizes synaptic contacts (*51*, *52*). Gene mutations may therefore affect sequential cellular processes in parallel. Such versatility in protein function likely contributes to the complexity and variability of disease processes.

Cytoskeletal perturbations – Given their involvement in multiple cellular processes, from cytokinesis to migration and neuritogenesis, mutations in genes coding for cytoskeletal proteins are particularly prevalent causes of cortical malformations. Strikingly however, quite different anatomical defects can result from mutations in genes coding for apparently related proteins. For example, in "tubulinopathies", mutations in a tubulin-encoding gene, *TUBA1A*, generally decreases gyrification in humans, while disruption of another tubulin, *TUBB3* often gives polymicrogyria (*53*). Microtubule-related proteins can behave similarly, as mutations in the gene coding for doublecortin (DCX, which stabilizes microtubules and regulates certain molecular motors during neuronal migration (*54*)), or LIS1 (which regulates dynein and is required for interkinetic migration of aRGs as well as neuronal migration (*55*)), variably result in either subcortical heterotopias or lissencephaly in humans (*5*). In the case of *DCX* , which is located on the X chromosome, mutations in women typically lead to heterotopia while in men it is associated with lissencephaly, suggesting dose-dependent effects (*56*).

Post-transcriptional and post-translational dysregulations are also a cause of cortical malformations. For example, abnormal alternative splicing can occur in the Filamin A actin-binding transcript (*Flna*), causing periventricular heterotopias in humans (*57*). Inhibition of a neuronal "poison exon" by the Polypyrimidine Tract Binding Protein PTBP1 is necessary to maintain neural progenitor identity. In the absence of PTBP1 function, de-repression of this poison exon leads to decreased expression of FLNA and precocious neuronal differentiation (Fig. 4A). Mutations of proteins modifying the conformation and activity of other proteins, like kinases (*e.g.* PI_3K/AKT pathway (*58*)), glycosylases (*59*) and tubulin chaperones /

modifiers (*e.g.* Tubulin Folding Cofactor D (*TBCD)* (*60*)) are further post-translational causes of cortical malformations.

Obviously, not all cortical malformations are the result of disrupted cytoskeletal function. Other classes of proteins, such as transcription factors, are also involved (*e.g.* ARX, PAX6, EOMES and FOXG1 whose gene mutations lead to severe cortical malformations in humans (Fig. 2) and mice (*20*, *61-63*)). Such examples are, however, comparatively rare despite their critical function in orchestrating developmental genetic programs. One explanation for this apparent paradox is that many transcription factors may be "too" critical for corticogenesis, such that loss of their function is embryonically lethal.

Disrupted spatio-temporal choreographies – Normal gene expression is tightly regulated in space and time during development. Hence, abnormal dynamic regulation of gene expression is likely a critical factor in the pathogenesis of cortical malformations. Supporting this possibility, *Flna* mutant mice do not display neuronal migration defects, while acute RNAi-mediated knockdown of *Flna* in rats reproduces the periventricular heterotopias similar to those observed in human patients (*64*). Also, constitutive *Dcx* mutant mice have a fairly normal neocortex and behavior (*65*), while acute RNAi-mediated knockdown of *Dcx* causes subcortical band heterotopia (*66*) (Fig. 4B). In principle, while dysfunction of early-onset genes cascade onto a broad array of subsequent cellular processes and may lead to more severe phenotypes, early-onset defects may also be more easily compensated for than defects affecting late differentiation stages.

Spatial regulation of gene expression is also important, particularly in the cortex where functionally specialized areas exist. In many cases, malformations are focal or regionalized rather than diffuse, although what determines their location is usually unknown. Cortical malformations often show antero-posterior gradients (*40*). For example, *FLNA* mutations typically cause frontal-predominant heterotopias (*67*), and *GPR56* mutations frontal-predominant polymicrogyria (*68*). In contrast, *CEP85L* shows higher expression in posterior cortical regions and mutations in this gene lead to posterior-predominant polymicrogyria (*69*). Some affected genes, however, are ubiquitously expressed (*e.g. GPR56* (*70*)); interactions with locally expressed binding partners or specific developmental dynamics may here account for the stereotypic location of the cortical defect. In some cases, such as *FLNA*, *GPR56* or *EOMES*, spatial restriction of gene expression in regions underlying gyri or sulci (*71*) could further contribute to impairments in cortical folding. Finally, somatic mutations may also account for

the focal nature of a defect (*e.g.* PI_3K pathway (*72*)), although what determines area location is often unknown (*42*).

Abnormal cell types – Cell types are the end-result of the dynamic spatio-temporal expression of genes during development. Cortical malformations typically affect multiple types of neurons and/or progenitors, which is in contrast with neurodegenerative diseases, where single cell-types (*e.g.* dopaminergic neurons in Parkinson disease) are predominantly affected. The apparent lack of cell type specific impairments may reflect that differentiation processes are mostly generic across neuron types, and that cell type identity mostly emerges from the combinatorial interactions of multiple genes rather than single master regulators (*73*). In some cases (mainly focal cortical dysplasia, autism spectrum disorder, schizophrenia and epilepsy), specific defects have also been reported in inhibitory neurons, astrocytes and oligodendrocytes (*43*, *74*) (Fig. 4C). Failure in astrocyte-dependent removal of extracellular debris may lead to a chronically pro-inflammatory environment, potentially increasing susceptibility to disease, but the extent to which reported changes in non-neuronal cells are reactive to neuronal loss is, however, often unclear (*75*). Of note, since astrocytes are born towards the end of neurogenesis (likely from the progeny of aRGs), mutations that affect aRG divisions are probably associated with a decreased number of astrocytes, although this has not been systematically assessed.

Depending on progenitor types and stage of corticogenesis, daughter neurons differ and the aberrant generation of one of these progenitor types could in principle affect specific progenies. For example, mutations affecting intermediate progenitor function could preferentially affect superficial layers, since such progenitors become more and more prevalent as corticogenesis unfolds and superficial layer neurons are born last. Recently, electrophysiological properties of progenitors have been shown to contribute to the sequential generation of neuronal types (*76*) and may thus contribute to cortical malformations. Accordingly, mutation in the sodium channel $Na_V1.3$ (SCN3A) gene affects outer subventricular zone progenitors and causes focal polymicrogyria in humans, potentially reflecting excess or altered superficial neuron production (*77*).

Abnormal circuits – Although neuronal heterotopia likely affects circuit assembly, neuronal identity and basic circuit properties are often conserved despite abnormal neuronal position. In mice, ectopic neurons usually still express their proper laminar markers and display conserved long-range projections (*27*, *78*, *79*) (Fig. 4D). Similarly, L5-type neurons in L4 retain their connectivity, as do L4-type neurons in L2/3 (*80*, *81*) and ectopic neurons still attract the proper subtypes of GABAergic interneurons with whom they make synaptic connections (as if they

were normally positioned in the cortex), suggesting that local connectivity might likewise be preserved (*82*). How GABAergic interneuron migration is affected in microcephaly or other cortical malformations has not been systematically examined (but see (*83*)), but disruption of this process could provide a cellular/circuit explanation for the seizures seen in some patients. In the *Reeler* mouse, although distinct layers are no longer visible, L4-type neurons still receive proper innervation from the thalamus and somatotopic innervation of the cortex is relatively preserved (*26*, *27*) (Fig. 4D). Supporting spared circuit function, perception, learning and memory in *Reeler* mutant mice are little affected by disrupted cortical lamination (*27*), although more cortex-targeted behavioral tests would likely reveal some level of dysfunction. Together, these results suggest that neuron identity emerges largely cell-autonomously, such that long-distance projections (and possibly microcircuits) may be relatively spared in neuronal heterotopias.

Behavior and clinical features – As a consequence of the number and complexity of the molecular and cellular processes involved in cortical disorders, the clinical manifestations are heterogeneous, and include intellectual disabilities, epilepsy, and a broad spectrum of neuropsychiatric manifestations. What determines this spectrum is unknown, but postnatal, environmental factors and overall genetic background may contribute (*84*). Epilepsy often occurs in the context of cortical malformations, but which factors determine seizure susceptibility is unclear. In mouse models of cortical heterotopia, the ectopic and isotopic cortices remain interconnected, which may contribute to abnormal neuronal activation and seizures seen in human patients (*85*, *86*). In some cases, abnormal electrical activity was initiated within or adjacent to the heterotopia while in others, seizures were initiated from the overlying cortex. This suggests either that aberrantly located neurons are capable of generating dysfunctional circuits at a distance, or that the overlying homotopic cortex is abnormal (*87*). Like other clinical features, intellectual functions are also variably affected, spanning from essentially normal functions to severe disability, in which case seizures are often associated (*88*). In the absence of additional biomarkers, this relatively generic array of symptoms and signs, which have historically been the main criterion for disease classification, thus offer limited opportunities for a mechanistic understanding of the disease process.

Towards a multi-level assessment of neurodevelopmental disorders

The examples above highlight several levels (*i.e.* genetic, molecular, cellular, circuit and behavioral) within and across which combinatorial interactions may occur during cortical malformations and preclude a causal understanding of the disease process (Figs. 3-4).

Understanding the processes at play at each of these levels for individuals is key to provide patients and their families with prognostic indicators (including biomarkers and, more broadly, recognized "endophenotypes") and, in the longer term, therapeutic perspectives.

Towards these two aims, the use of animal models, including mouse, ferret and non-human primates (the latter being gyrencephalic and thus closer to humans) is important, in particular, at some point, to assess the therapeutic effect of approaches on behavior, including through insertion of human mutations via gene editing technologies (*47*). *In vitro* human models are also emerging as a promising and feasible avenue: somatic cells can now be reprogrammed into induced pluripotent stem cells (iPSCs) and serve as a basis for the generation of 3D organoids and assembloids of the brain (*89*, *90*). Organoids have been used to study a variety of neurodevelopmental disorders including cortical malformations (*e.g. CDK5RAP2* mutation leading to cobblestone lissencephaly in humans) (*91*). Despite their limitations in terms of reproducibility and restriction to early stages of corticogenesis (gyrification is for example challenging to study in these models) (*92*), organoids are poised to become broadly used in personalized medicine by giving access to the neurons of the patient under study.

Recent advances in single-cell transcriptomics now allow the dynamic developmental expression of genes in emerging cell types to be assessed (*93*, *94*) (Fig. 5A), potentially linking cellular diversity and characteristics with circuits and behavioral repertoires. Comparison of gene expression across brain development in mouse, monkey, human and primate- / human-derived brain organoids is particularly important to identify perturbed processes and select the appropriate study model. As a first step in this direction, here we provide an online resource (http://genebrowser.unige.ch/humous/) compiling transcriptional maps across development and neuron differentiation for mouse embryos, human embryos and human-derived organoids (*94-96*). As an example of an application of such a database, combinatorial analysis of arrays of genes implicated in microcephaly, lissencephaly or polymicrogyria using this resource confirms that microcephaly predominantly affects progenitor function, while lissencephaly and polymicrogyria affect postmitotic neurons (Fig. 5B). In the latter two cases, human data emphasizes that progenitors may also be affected, highlighting the value of *trans*-species comparisons (see arrowheads in Fig. 5B).

Multi-omics approaches, in which transcriptomics are combined with genomics, epigenomics and proteomics will provide an opportunity to further unravel the cell type specific processes at play and distinguish core molecular / cellular disease processes from idiosyncratic or stochastic variability. Comparing the distribution of affected processes (at any

level of analysis) across patients will help to build a spectrum of abnormal states and predict vulnerable processes (Fig. 5C). Integrative approaches including electrophysiological, imaging, and clinical and biological data from patients, for example using state-of-the art artificial intelligence algorithms (*97*, *98*), may allow the bridging of DNA mutation(s) to gene expression, cellular, anatomy and circuit consequences (Fig. 5D). This will be instrumental for a physiopathogenic classification of diseases, an essential step for a more precise patient stratification and for the design of personalized diagnostic and therapeutic tools. Although this level of integration may seem futuristic, high-throughput techniques and analytical tools are increasingly available, paving the road for such strategies in a realistically close future.

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Author contributions: D.J., E.K., F.F. & S.C. conceived and designed the presented work. D.J., E.K. & S.C. wrote the manuscript, with the help of F.F. E.K. performed the data analyses and designed the figures.

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Data and materials availability: In Fig. 5, data from mouse embryos are from (*94*); data from human embryos and human-derived organoids are from (*95, 96*).

FIGURE LEGENDS

Fig. 1: Key steps of corticogenesis and their susceptibility to disease. (**A**) Summary of embryonic corticogenesis. One neuron can be followed from its birth at the ventricular zone, to its migration toward the cortical plate, and then its maturation (arrow). The role of cytoskeletal processes at each of these steps is highlighted on the right. (**B**) Literature-based summary of knowledge on the involvement of these developmental steps in specific neurodevelopmental disorders. The numbers of publications were found using the text mining EasyPubMed R package, which automatically identifies publications containing given the combinations of keywords indicated (*e.g.* "microcephaly" and "proliferation"). These numbers were then normalized for number of publications for each of the developmental steps and each of the disorders. The dendrogram represents unbiased clustering of the diseases based on this data. (**C**) Overlapping cellular processes and pleiotropic molecular mechanisms involving CDK5 across development and in the adult brain. aRG, apical radial glia; bRG, basal radial glia; CP, cortical plate; E, embryonic day; IN, interneuron; IPC, intermediate progenitor cell; N, excitatory neuron; pcw; post-conception week; SVZ, subventricular zone; VZ, ventricular zone; WM, white matter.

Fig. 2: Cellular mechanisms at play in human cortical malformations. Cells with abnormal developmental trajectories are in pink. Reduced progenitor proliferation (sometimes associated with abnormal neuron migration) leads to microcephaly. Abnormal neuron position can lead to agyria and lissencephaly (reduced migration and/or progenitor misposition), polymicrogyria (increased migration and/or excess of basal radial glia), cobblestone lissencephaly (excessive migration), periventricular heterotopia (neurons stay close to the ventricle) or subcortical band heterotopia (neurons stay below the cortex). In focal cortical dysplasia, clonal mutations affect subpopulations of mature cells, which become dysmorphic (balloon cells, dysmorphic neurons). Genes mentioned in this review are listed (bold: genes with published human-derived brain organoid models, *: gene studied in the ferret, green: genes coding for transcription factors). Pink arrowheads on MRIs highlight the malformations. MRI illustrations are from (*15*) and (*2*) for polymicrogyria and focal cortical dysplasia (Reprinted from *Semin Cell Dev Biol* vol 76, D. M. Romero, N. Bahi-Buisson & F. Francis, Genetics and mechanisms leading to human cortical malformations, pages 33-75, 2018, with permission from Elsevier; Definitions and classification of malformations of cortical development: practical guidelines; M. Severino et al., *Brain* 2020; awaa174, with permission from Oxford University Press).

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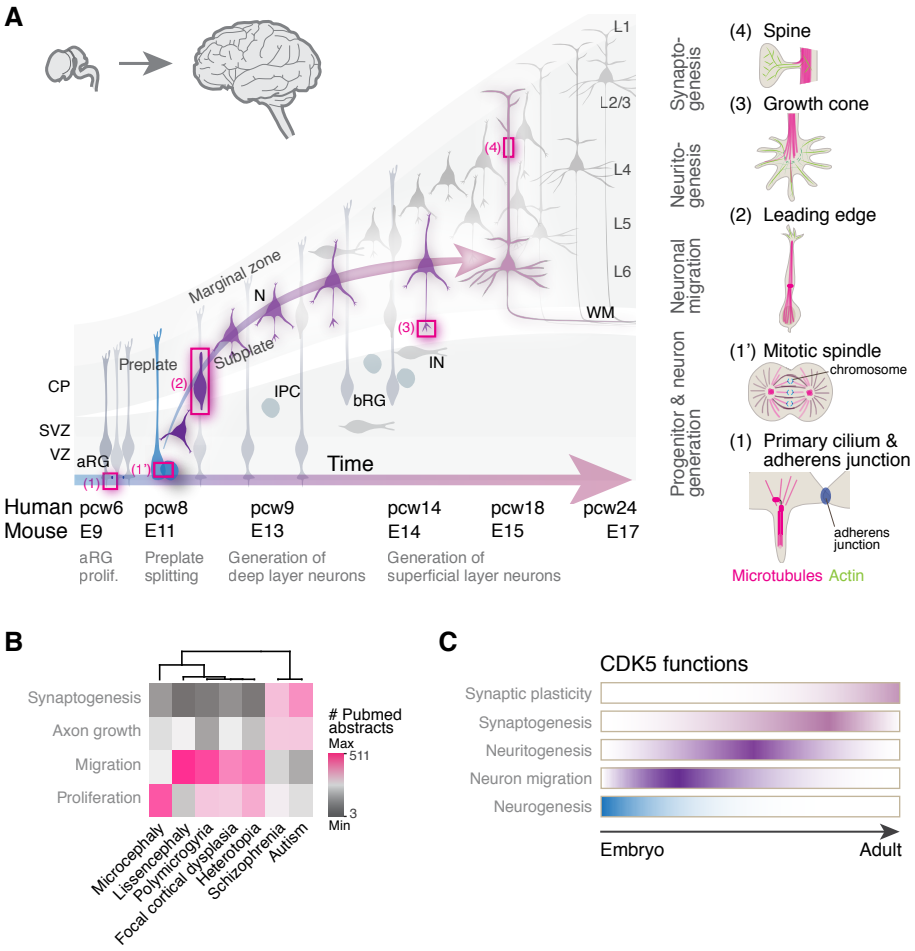

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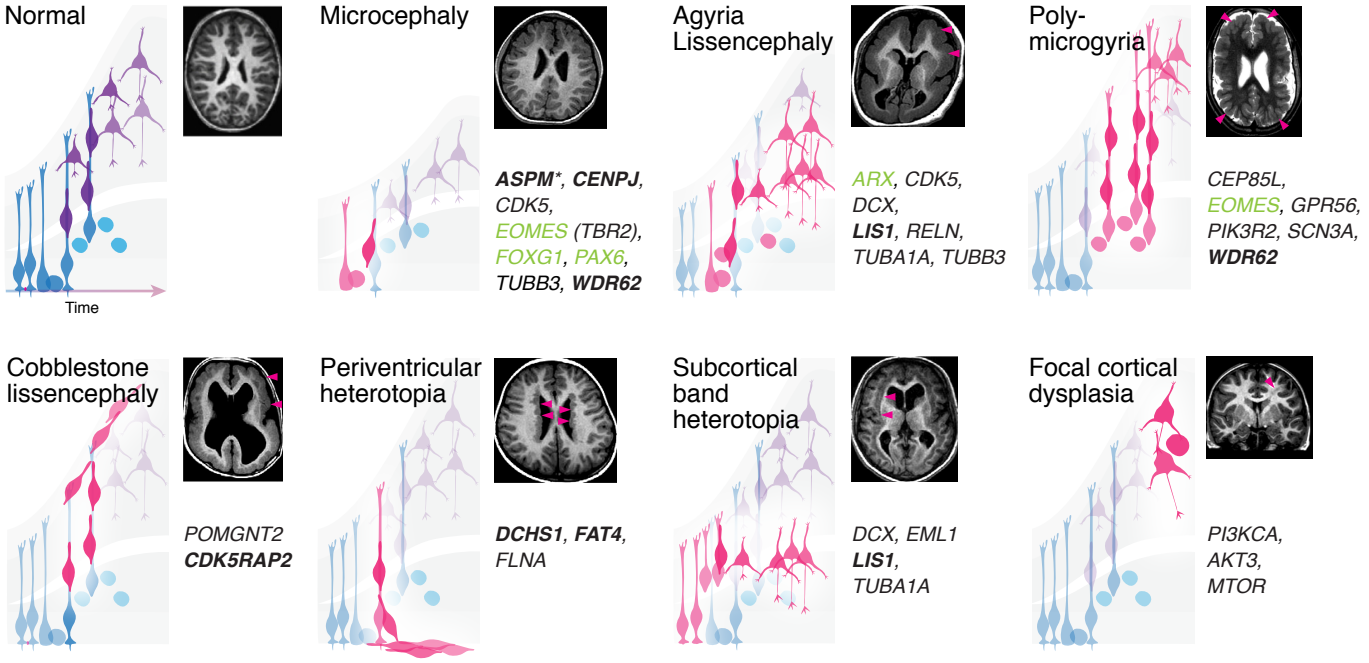

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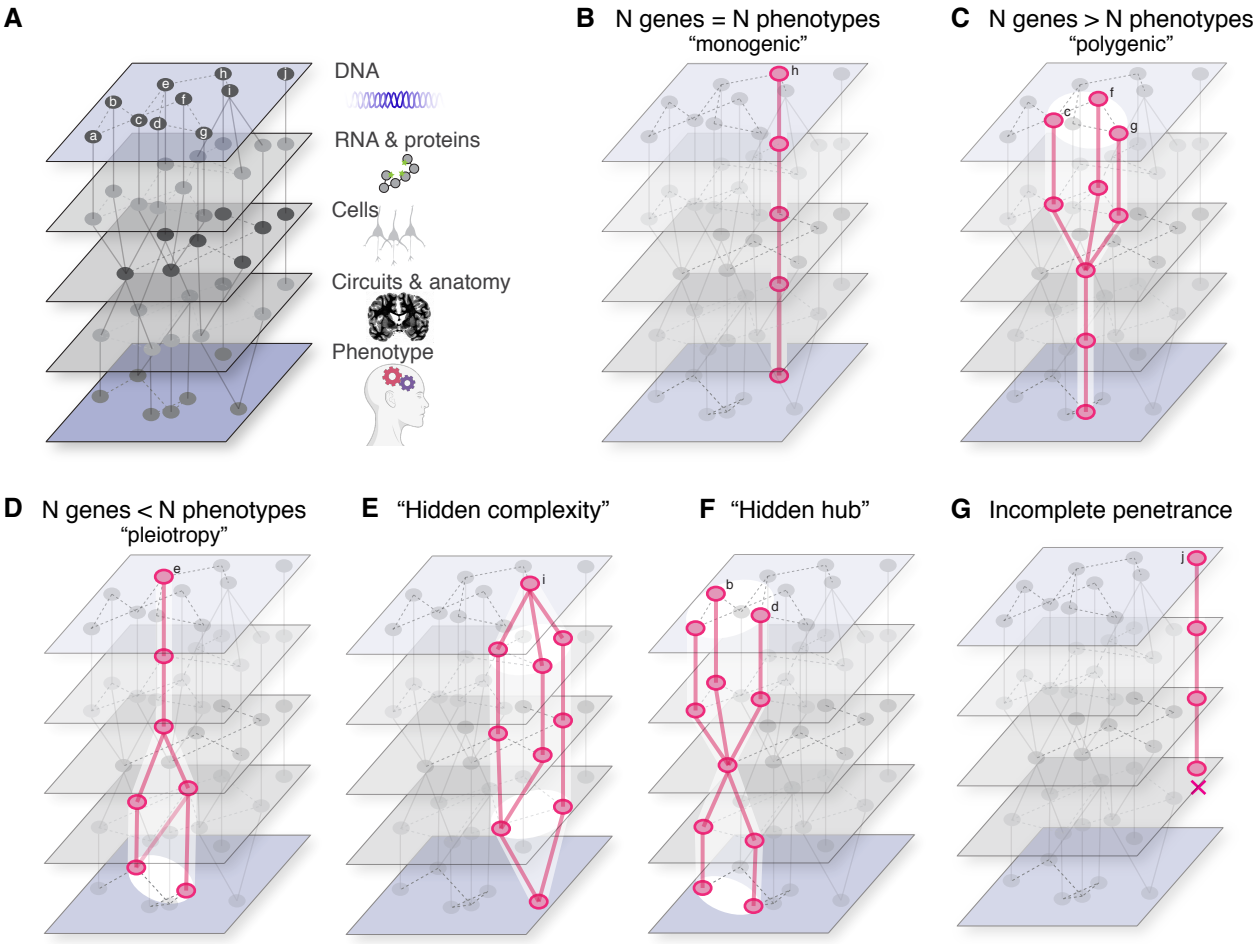

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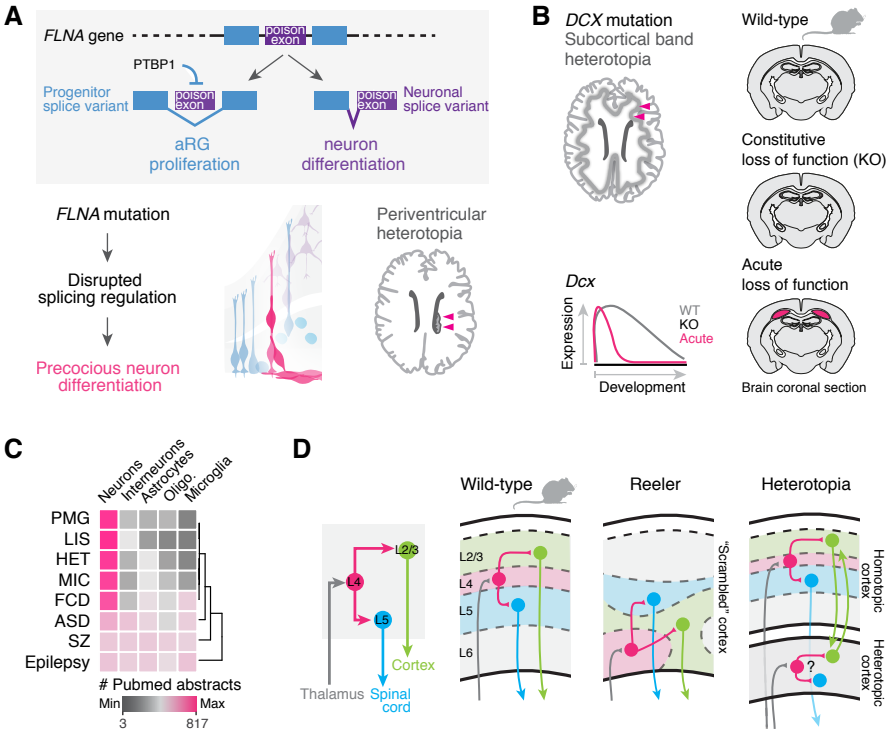

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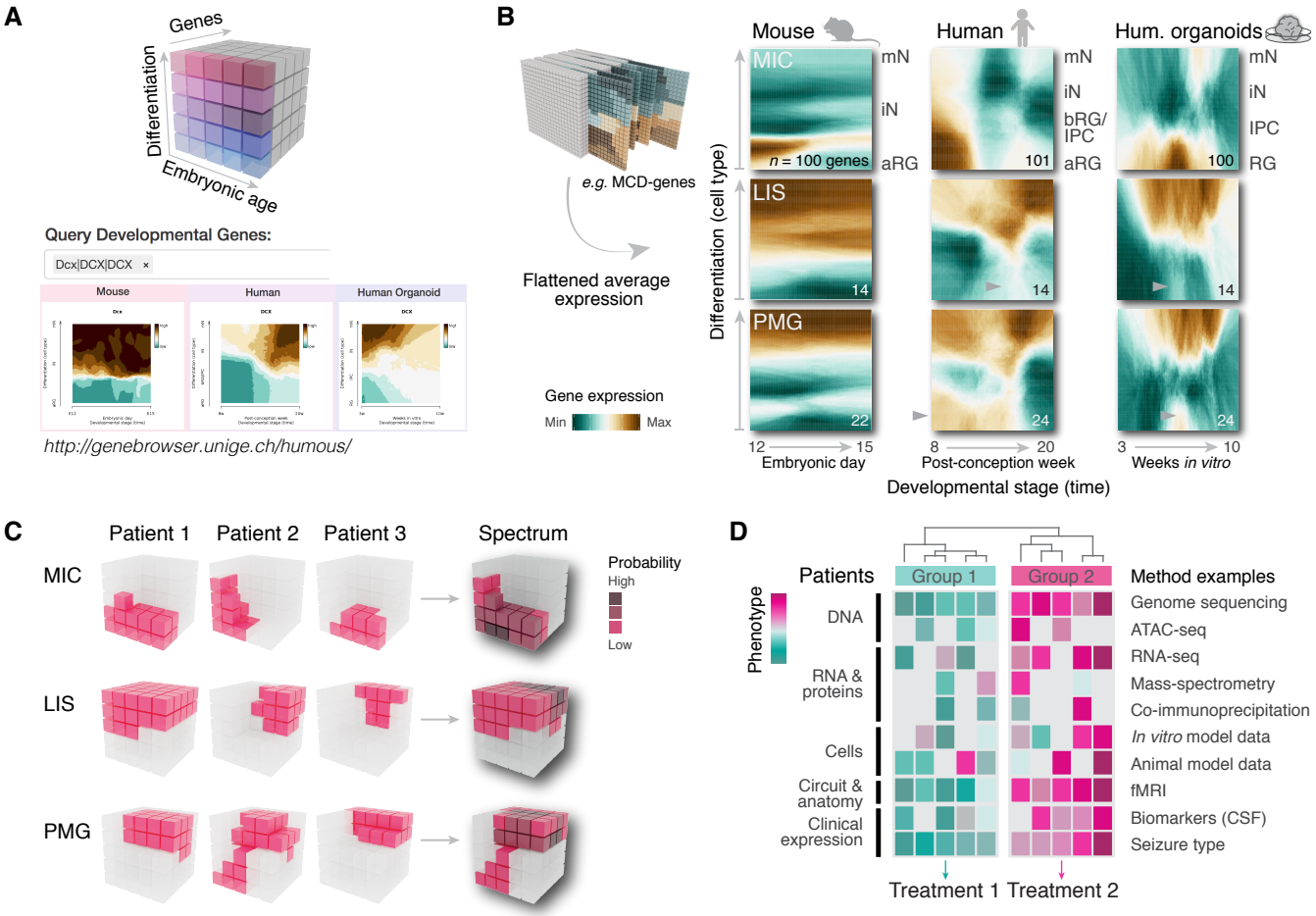

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