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Review article

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Genetics of human brain development

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Abstract

Brain development in humans is achieved through precise spatiotemporal genetic control, the mechanisms of which remain largely elusive. Recently, integration of technological advances in human stem cell-based modelling with genome editing has emerged as a powerful platform to establish causative links between genotypes and phenotypes directly in the human system. Here, we review our current knowledge of complex genetic regulation of each key step of human brain development through the lens of evolutionary specialization and neurodevelopmental disorders and highlight the use of human stem cell-derived 2D cultures and 3D brain organoids to investigate human-enriched features and disease mechanisms. We also discuss opportunities and challenges of integrating new technologies to reveal the genetic architecture of human brain development and disorders.

Sections

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Introduction

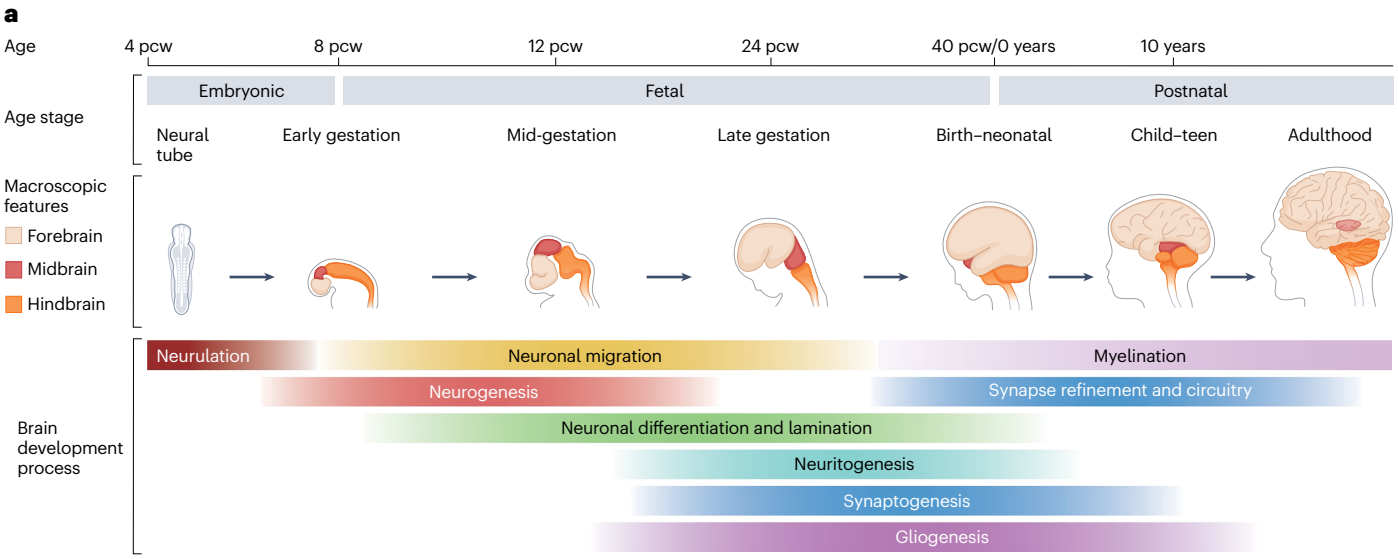
Human brain development follows sequential, orchestrated cellular and molecular steps that are driven by genetic blueprints to build an organ for sophisticated higher-level computational tasks, such as cognition, memory, emotion, language and behaviour[1]. Many brain developmental processes are conserved across mammals, and tremendous advances in understanding their shared genetic basis have been achieved through functional studies using classic animal models. However, evolution has also led to numerous species-specific features that potentially enable higher-order brain functions unique to humans. For example, although mouse models have been instrumental in establishing the genetic causality of many microcephaly-associated genes[2], a number of mouse mutants do not fully recapitulate the sharply reduced brain size observed in human patients[3–6]. These species-related differences pose substantial challenges to relying on animal models to deconvolute molecular, cellular, anatomical or circuit complexity or to predict treatment responses in humans; human cell-based approaches that capture divergent, human-specific features are therefore required[1,7–9]. Cross-species comparative genomics offers genetic clues about human-specific brain developmental features that may contribute to the remarkable expansion in brain size and complexity[10]. In addition, neurological and psychiatric disorders with disrupted critical developmental steps provide another perspective to study genetics of human brain development by linking disease traits to their genetic aetiology. Enabled by the increased availability of high-quality neurotypical and pathological human brain specimens, rapid advances in molecular detection technologies and genome-wide association studies (GWAS) have led to numerous 'omics' datasets, providing molecular characterization of the (patho)physiology of human brain development[11–13]. Despite increasing statistical power, however, these analyses provide only correlative evidence for the role of specific genes in human brain development, which requires further functional validation in human cell-based models. In conjunction with advances in genome editing, recently established human pluripotent stem cell (hPS cell)-based models have emerged as an unprecedented platform to investigate genotype–phenotype causation during human brain developmental processes, pinpointing cell type-specific genetic regulation at the molecular, cellular and anatomical levels[14,15].

Here, we first summarize our current understanding of the key steps of human brain development, highlighting human-enriched features and focusing on the cortex as an example. We next describe how studying alterations in the overall neurodevelopmental landscapes due to evolution, risk genes associated with neurodevelopmental and neuropsychiatric disorders, or the impact of environmental exposures facilitates our understanding of the genetic control of human brain developmental processes. We highlight recent discoveries of causative genetic mechanisms regulating cortical development and human-enriched cellular and molecular features from emerging hPS cell-based platforms, listing examples to illustrate how these studies can advance our knowledge of the genetics of human brain development. We articulate opportunities and challenges of implementing hPS cell-based models to identify causative variants responsible for human-specific traits in brain evolution, function and dysfunction. Finally, we discuss how integration of hPS cell-based models with in vivo animal models and other technologies can revolutionize our research paradigm to identify causative genetic links between human brain development and diseases in a cell type-specific, brain region-specific, developmental stage-specific and species-specific manner to aid in the development of therapeutic strategies for various developmental brain disorders.

Key steps of human brain development

Human brain development follows conserved spatiotemporal patterns across mammals. It starts with neurulation, a process in which the neural plate, specified from the embryonic ectoderm, folds and fuses to form a closed neural tube. This tube then segments into lineage-restricted vesicles that become the forebrain, midbrain and hindbrain (Fig. 1a). The interior wall of the neural tube contains neuroepithelial stem cells (NECs) that populate the proliferative ventricular zone (VZ)[16]. NECs then transform into radial glial cells (RGCs) that produce postmitotic excitatory and inhibitory neurons and subsequently glia (Fig. 1a,b). Mammalian neurogenesis relies on intermediate progenitor cells (IPCs), the immediate descendants of RGCs that divide in the subventricular zone (SVZ), to amplify neuronal production[1,17] (Fig. 1b). In the developing cortex, precursors of excitatory neurons migrate radially along the radial glial scaffold of RGCs to populate the cortical plate in a stereotypical 'inside-out' manner, with late-born upper-layer neurons migrating past early-born deep-layer neurons[18]. Inhibitory interneurons arise mostly from progenitors in the ganglionic eminence and migrate tangentially into the cortex[19–22] (Fig. 1a,b). Neuronal differentiation and maturation occur along with migration in overlapping temporal waves to achieve radial cell type diversification and tangential arealization to propagate the dorsal forebrain[8]. While approaching their regional destinations, neurons send out axons and dendrites through highly motile growth cones that integrate environmental cues[23]. Upon establishment of neuronal networks, dendritic spines form to enable neuronal communications via synapses. Synaptic assembly and later dendritic and synaptic pruning are highly plastic and neuronal activity-dependent, and their continuous refinement persists into young adulthood[1,24,25] (Fig. 1a). Among non-neuronal cells, astrocytes and oligodendrocytes are generated by RGCs post-neurogenesis and mature throughout early postnatal periods to modulate neuronal functions, such as regulating synaptic transmission and myelinating neuronal axons[1,25,26] (Fig. 1a,b). Microglia derived from the haematopoietic lineage and endothelial cells lining blood vessels migrate into the neuroectoderm-derived nervous system to support embryonic brain development[27].

Evolutionary genetic changes result in neoteny in humans, with temporally protracted developmental processes compared to other species in almost every brain development step[1,10], especially progenitor proliferation and neuronal development[8,28] (Fig. 1b). For example, human NECs display protracted differentiation, resulting in greater forebrain expansion than in apes[29]. RGC-mediated neurogenesis in humans occurs at a substantially slower pace (over 3 months) and involves different genetic programmes than in mice (~1 week) and macaques (1–2 months)[8,25,30,31], which probably contributes to the disproportionate enlargement of the human forebrain. Prolonged differentiation and maturation are also observed in human neurons in the cortex[25,32] and other brain regions[21,33–35], leading to an increase in the volume of neurons with more elaborate neurites and synapses[24,30,36,37]. These extended periods may allow for greater size, complexity and plasticity of the human brain, as well as increased vulnerability to genetic or environmental factors that may disrupt normal development[1]. Aside from differential timing in development, there are many special cellular features in humans. The outer SVZ layer of the cortex, one of the distinctive features in humans and species with a larger gyrified (folded) cortex that is virtually absent in rodents[38], arises from outer radial glial cells (oRGCs)[39] (Fig. 1b). These oRGCs exhibit an enhanced proliferative capacity and generate most of the upper-layer cortical neurons, coinciding with expansion of the primate cortex in size and surface area[7]. There are other cell types unique to gyrencephalic mammals



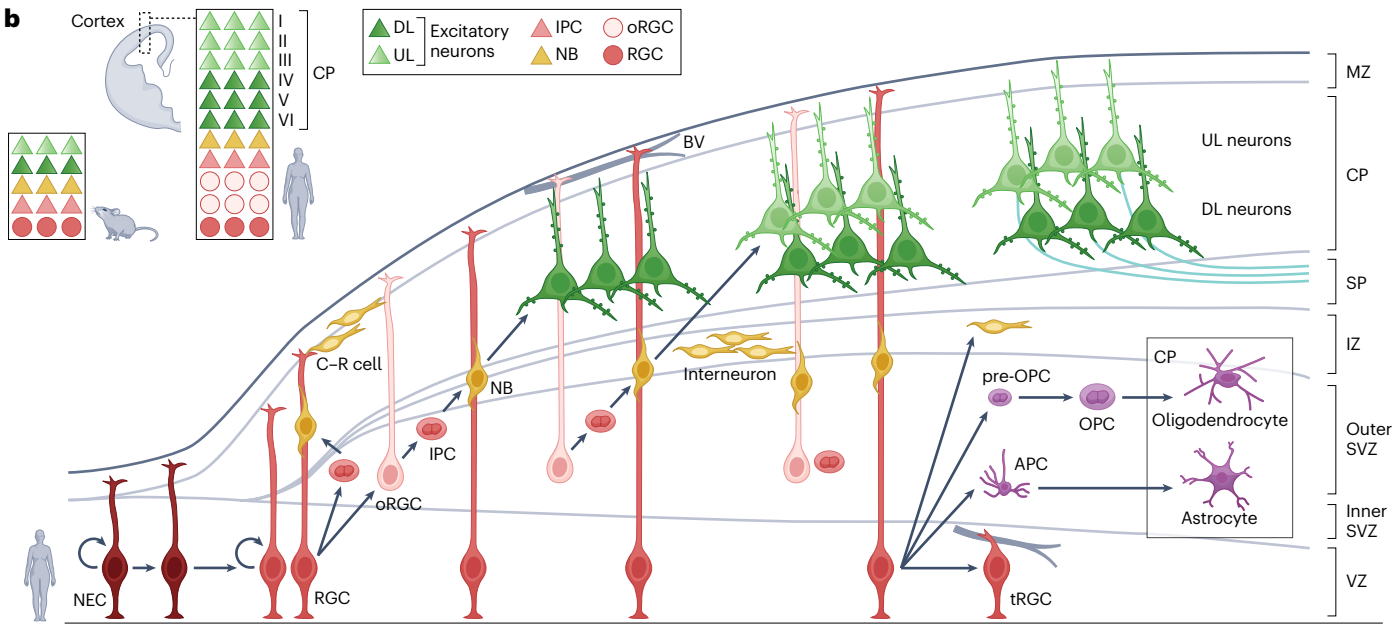


Fig. 1 | Key processes of human brain development. a, A summary of the major processes and their timing during human brain development, including neurulation and neural epithelial cell proliferation[16], neural stem cell-mediated neurogenesis[16], radial[18] and tangential[19] migration of neural precursor cells to their destination, neuronal differentiation and maturation to achieve lamination and establish areal identity[8], generation of neurites and synapses[12,23], and gliogenesis[25], as well as some processes that largely occur during human postnatal periods, including continuous synaptic pruning[24] and oligodendrocyte myelination[25,26]. Age timeline and brain size are not depicted to scale. **b**, Stepwise processes during human cortical development. Cellular features enriched in primates are included, such as outer radial glial cells (oRGCs, comprising the outer subventricular zone (SVZ)), an enlarged subplate (SP), an enlarged and more complex cortical plate (CP), pre-oligodendrocyte precursor cells (pre-OPCs) and truncated RGCs (tRGCs). APC, astrocyte precursor cell; BV, blood vessel; C–R, Cajal–Retzius; DL, deep layer; IPC, intermediate progenitor cell; IZ, intermediate zone; MZ, marginal zone; NB, neuroblast; NEC, neuroepithelial cell; pcw, post-conception week; UL, upper layer; VZ, ventricular zone.

(such as primates), including truncated RGCs[40,41], oRGC-derived EGFR$^+$ progenitors that are primed to become oligodendrocyte precursors[42], and outer-SVZ-derived white-matter astrocytes[43] (Fig. 1b). Human interneurons display unique origins and distinct cell types compared to rodents, such as possible local production of interneurons by cortical RGCs[44], subtype switching[45] and a primate-specific subtype of TAC3$^+$ striatal interneurons[46,47]. In addition, the primate subplate, a transient layer during development that is critical for establishing

cortical–thalamic connections, is five times thicker than that of other mammals, suggesting more extensive and potentially divergent circuit formation and refinement in primates[48,49]. Overall, evolutionarily distinct genetic regulation of cellular features, ranging from an extended duration of development to specialized cell types and anatomical structures, provides the architectural bases for brain expansion, connectivity and higher-order functions in humans.

Much of our knowledge about genetic contributions to molecular and cellular landscapes of brain development was procured from gain-of-function or loss-of-function studies using animal models[1,8,14,50]. For example, diffusible signals from the mesoderm during neural induction and early patterning were identified in classic studies using mouse models[8]. The 'radial unit hypothesis' during cortical neurogenesis was established by histological, molecular and physiological examination in primates[18]. Mechanisms underlying axon and dendrite pathfinding and outgrowth were revealed by *Drosophila melanogaster* models[51]. Primates remain the major model to study higher-level circuit functions for disease modelling[52–54]. Notably, emerging hPS cell-derived models, including 2D cultures and region-specific, integrated and xenografted 3D brain organoids[14], allow for functional testing of candidate genetic variants to establish their causality in cellular phenotypes in human models, fuelling current research to investigate genetics of human-specific brain development processes through the lens of evolution and brain disorders (Fig. 2).

Insight from an evolutionary perspective

Evolution offers a natural perspective for studying genetic mechanisms underlying different features across species[7,10,28,55]. Genetic variation underlying human-specific traits is predominantly identified in neurogenesis and neuronal development processes and is located in either protein-coding genes or non-coding regulatory regions, such as the human-accelerated regions (HARs)[56–58]. The resulting phenotypic alterations occur at the microscopic (cellular) level but ultimately influence an entire brain region through macroscopic changes in cell volume, complexity, connectivity and architecture, which may confer unique advantages to the human brain, such as enhanced brain volume, plasticity and cognition. A recent flurry of discoveries based on cross-species phylogenomic and comparative genomic studies followed by functional testing using humanized animal models and hPS cell-based models has revealed critical genes, pathways and regulatory mechanisms for cell type-specific and developmental stage-specific modulation of human brain development[7,59] (Table 1 and Fig. 3).

Neurogenesis variations unique to humans

Neurogenesis is mediated by neural stem and progenitor cells and is regulated by many human-specific molecular features through various mechanisms, including cell cycle regulation, transcription modulation, signalling pathways, mitochondrial dynamics and metabolism, which were traditionally revealed by ectopic expression of human genes in animal models[10,14] (Fig. 3a). A growing number of discoveries have been made using hPS cell-derived brain organoids as they robustly model the microscopic and macroscopic characteristics of early human brain development that are dominated by neural progenitor cells (Fig. 3a). For example, comparative functional studies using human and ape forebrain organoids identified *ZEB2*, which encodes a transcriptional co-repressor, as an evolutionary driver that contributes to greater cortical expansion in humans by delaying the NEC-to-RGC transition with faster cell cycles[29] (Table 1 and Figs. 3a,4a). Similarly, altering of RGC cell cycles by two HAR-regulated mechanisms involving *PPP1R17* (which encodes a protein phosphatase inhibitor and cell cycle regulator)[60] and *FZD8* (which encodes a WNT receptor)[61] leads to prolonged development and increased size of the neocortex (Table 1 and Fig. 3a). Functional cross-species comparisons revealed differential genetic control of RGC proliferation in humans through modulation of key signalling pathways, including PDGF[62], Robo[17], Notch (for example, by human-specific gene duplication of *NOTCH2NL*)[63,64], mTOR (for example, via *INSR* (which encodes the insulin receptor, an mTOR activator), *ITGB8* (which encodes a fibronectin receptor, an mTOR activator)[40,65] and hominid-specific gene *CROCCP2* (which encodes a cilia protein and regulates mTOR signalling in oRGCs)[66]) and regulatory networks, including via the *DUF1220* protein domain (human-specific increase)[67] and *ZNF558* (which encodes a human-specific mitophagy regulator)[68] (Table 1 and Fig. 3a). There are also variations that specifically affect oRGCs, a subset of RGCs enriched in primates. oRGC amplification and neocortical expansion is promoted by overexpression of the human-specific mitochondrial gene *ARHGAP11B* in mice[69,70], ferrets[71], marmosets[72] or chimpanzee organoids[73]; the hominoid-specific histone methyltransferase suppressor *TBC1D3* in mice[74] and hPS cell organoids[75]; the primate-specific cell cycle-related gene *TMEM14B* in mice[76]; or the hominid-specific fatty acid synthesis-related gene *TKTL1* in mice or genome-edited human organoids[77] (Table 1 and Fig. 3a). Taken together, these studies identified not only canonical signalling pathways regulating human-specific features in neurogenesis, such as Notch and mTOR, but also novel genetic mechanisms modulating various cellular properties of human neural stem and progenitor cells (Table 1 and Fig. 3a).

Neuron and circuit development variations specific to humans

Rates and levels of neuritogenesis and synaptogenesis related to circuit formation and connectivity are also differentially regulated in humans, the underlying mechanisms for which were mostly revealed using humanized mouse models[28,78] (Fig. 3b). For example, *FOXP2*, the earliest reported gene with evidence of human-specific evolutionary adaptation in its coding sequence[45,79], has critical roles in speech and vocalization development. It encodes a transcription factor that modulates dendritic length and synaptic plasticity[80] (Table 1 and Fig. 3b). Ectopic expression of the human-specific gene *SRGAP2C* in embryonic mice interferes with activity of its ancestral copy of *SRGAP2* (which encodes a Slit–Robo RHO GTPase activator), leading to prolonged maturation and increased density of synapses and connectivity of pyramidal neurons[81–84] (Table 1 and Fig. 3b). Primate-specific modifications in regulatory regions also result in dendritic or synaptic remodelling. For example, osteocrin (encoded by *OSTN*) is normally secreted in bones and muscles, but is evolutionarily re-purposed to be expressed in the primate brain where it interacts with the transcription factor MEF2 and regulates activity-dependent dendritic growth specifically in primate neurons[85]. Changes in the expression levels of *CBLN2* (which encodes cerebellin 2, a regulator of synaptogenesis)[86] or *PLXNA1* (which encodes semaphorin receptor plexin A1 and is involved in axon guidance)[87] owing to loss of regulatory region binding sites for their respective transcription factors, SOX5 and FEZF2, alter dendritic growth and spine density in primate neurons. Human-specific chromatin loop-regulated *EPHA7*, which encodes an axon guidance protein, exhibits elevated expression in human subplate neurons and impacts dendrite growth and circuit development[88] (Table 1 and Fig. 3b). A human-specific deletion in a putative regulatory element of *LOXL2* (a neuronal differentiation-related gene), which is a region highly conserved in other vertebrates, leads to transcriptomic changes related

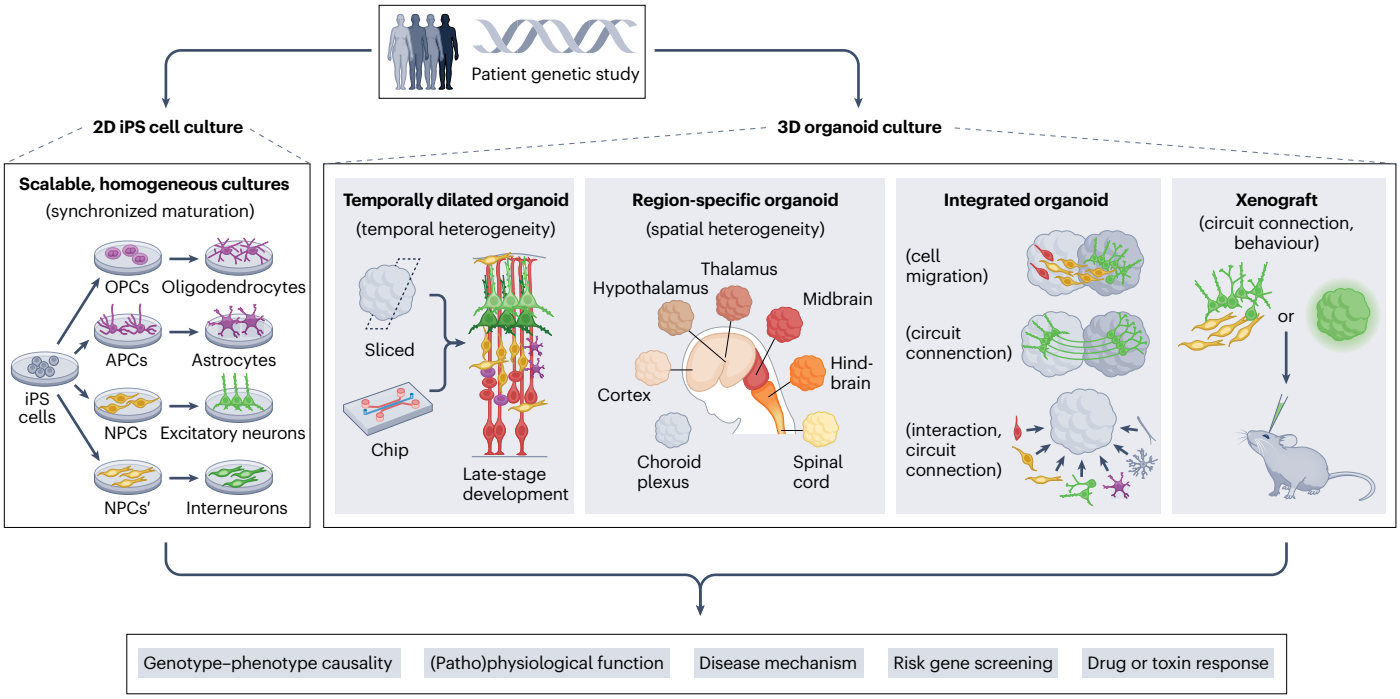


Fig. 2 | Applications of human pluripotent stem cell-based systems for studying human brain development. Genetic studies of individuals with disrupted brain development and genome-wide association studies can be used to identify causative genetic mutations and risk genes, respectively. Human pluripotent stem cell-based models derived from patients or through genetic engineering — including 2D cell culture and 3D organoids — allow establishment of causality between genotypes and phenotypes and facilitate investigations of neuropathological examination, disease mechanisms, and drug or toxin responses. They can also be used to screen risk genes to study causal genetic (mal)functions. Although 2D induced pluripotent stem (iPS) cell culture provides a homogenous, reproducible, scalable platform, advanced 3D organoid culture has the unique advantage of modelling spatiotemporal features and functions of the developing human brain and can start to recapitulate cell interactions and circuit formation events despite room for improvement, such as reducing organoid-to-organoid variability. APC, astrocyte precursor cell; NPC, neuronal precursor cell to excitatory neuron; NPC′: neuronal precursor cell to inhibitory interneuron; OPC, oligodendrocyte precursor cell.

to calcium signalling and myelination of neurons, as shown by reintroducing the conserved chimpanzee regulatory sequence for *LOXL2* into human cells[89]. In addition, a recent study revealed human-specific mapping of HARs to late-stage excitatory neurons in the developing telencephalon that are missing in chimpanzee-accelerated-regions, suggesting evolutionary rewiring of interactions between neurodevelopmental genes and HARs[57]. Moreover, genes mediating protracted neuronal differentiation and maturation in humans have been identified using mouse models (for example, *MEF2A* and *NPAS3*, which encode transcription factors involved in brain development[7], and *GADD45G*, which encodes a cell cycle repressor, apoptosis activator and epigenetic regulator[90]) and hPS cell models (for example, *GATA3*, which encodes a transcription factor that regulates action potential speeds and is uniquely upregulated in human maturing neurons compared with non-human primates to contribute to human neoteny)[91]. These findings suggest that many neuronal and circuit developmental processes are conserved across mammals, with increased levels of synaptic connections and protracted duration in humans that provide a substrate for enhanced neuronal networks (Table 1 and Fig. 3b).

Despite rapid advances, the limited number of genes studied so far suggests that we have only scratched the surface of the human-specific regulatory mechanisms that ultimately make us humans. Nevertheless, it is important to exercise caution when interpreting the results of evolution-based studies such as those described above. In particular, the recent controversies over *TKTL1* (refs. 92,93) and *FOXP2* (refs. 94,95) highlight that variants that seem to be absent from the genomes of modern humans can in fact be detected when a larger number of genomes representing greater ancestral diversity are examined. A recent preprint showed that disease risk and gene expression during human development are affected by different genetic ancestries and environmental backgrounds[96], and yet most recent human studies have focused on populations with European ancestry. It is critical that future genetic studies represent the broad genetic heterogeneity and ancestral diversity of human populations, especially those that are currently genetically under-represented[97,98], by developing resources such as the recently published draft of the more-inclusive human pangenome reference[99]. It is also important to note that animal models have limitations for evolutionary studies as ectopic expression of human proteins in cells of other species may result in nonspecific artefacts[100]. hPS cell models with determined genetic variants therefore provide a useful human-based platform to study causative links between genotypes and functional traits (Figs. 2 and 4). However, it must be remembered that single genetic variants that alter physical brain traits may not translate to effects on more complex traits, such as human-specific behaviours, that cannot be measured in these systems and are likely to be polygenic. Traits identified and modelled in both animal and hPS cell models should,

Table 1 | Genetic features that modulate human brain development as revealed by modelling evolution or diseases in animal or hPS cell models

Genetic feature	Genotypic mechanism	Phenotypic trait in human brain development	Human brain development process involved	Refs.
Human-specific evolutionary feature				
ZEB2	Delayed NEC-to-RGC transition in humans compared with apes; regulation of NSPC cell cycle and differentiation, interneuron fate specification and electrophysiological properties	Enlarged human forebrain organoids compared with apes; haploinsufficiency leads to Mowat–Wilson syndrome	Neurulation, neurogenesis, neuron development, synapse and circuitry	29,231
ARHGAP11B	Human-specific gene duplication regulating Rho GAP and calcium signalling in mitochondria in oRGCs	Neocortex expansion with more oRGCs and cortical neurons	Neurogenesis	69–73
CROCCP2	Hominid-specific gene duplication regulating cilia dynamics and mTOR signalling in oRGCs	Increased oRGC proliferation and cortical neuron production	Neurogenesis	66
DUF1220	Human-specific, dosage-dependent regulation of the *NBPF* gene family in NSPC proliferation	Increased expression and neocortex volume; copy number variation linked to 1q21.1 syndrome	Neurogenesis	67
FZD8	Regulated by HAR enhancer to modulate RGC cell cycle length	Increased neocortical size with more RGCs	Neurogenesis	61
PDGF	Human-specific PDGFD–PDGFRB signalling expression and regulation of RGC cell cycle absent in mice	Expanded germinal region; higher RGC heterogeneity	Neurogenesis	62
PPP1R17	Regulated by HAR enhancer; may increase RGC cell cycle length	Increased expression in oSVZ	Neurogenesis	60
TBC1D3	Hominoid-specific gene duplication for NSPC epigenetic regulation	Increased cortex size and folding with more oRGCs	Neurogenesis	4,75
TKTL1[a]	Hominoid-specific metabolic regulation of oRGC proliferation	Neocortex expansion with more oRGCs and upper-layer neurons	Neurogenesis	77,92,93
TMEM14B	Primate-specific gene regulating oRGC cell cycle and proliferation	Increased neocortex size and folding with more oRGCs	Neurogenesis	76
ZNF558	Human-specific expression in forebrain NSPCs absent in chimpanzees; regulation of mitophagy gene *SPATA18*	Differential regulation in cortex size and developmental timing in humans versus chimpanzees	Neurogenesis	68
NOTCH2NL	Human-specific gene duplication enhancing Notch signalling in NSPC proliferation and differentiation	Enlarged cortex, prolonged neurogenesis with more NSPCs and neurons; deletion and duplication lead to microcephaly and macrocephaly, respectively (1q21.1 syndrome)	Neurogenesis, neuron development	63,64
CBLN2	Hominoid-specific regulation of *SOX5* in neurons and synapses	Higher expression, spine density, synaptic connectivity in human prefrontal cortical neurons	Neuron development, neuritogenesis, synapse and circuitry	86
EPHA7	Human-specific loop regulation of neuron and circuit development	Elevated expression, dendrite growth, connectivity in human subplate neurons	Neuron development, neuritogenesis, synapse and circuitry	88
FOXP2[a]	Human-specific regulation of cortico-striatal circuit	Increased dendrite length and synaptic plasticity in human striatal neurons; expression in cortex: primate-specific in layer 4 granular neurons, human-specific in microglia	Neuron development, neuritogenesis, synapse and circuitry	45,79,80, 94,95
OSTN	Primate-specific regulation of *MEF2* in dendrites and neuronal activities	Activity-induced expression and regulation of primate neurons	Neuron development, neuritogenesis, synapse and circuitry	85
PLXNA1	Primate-specific regulation of *FEZF2* in neurons and axons	Lower expression level and weaker axon pruning of corticospinal connections in human cortical neurons	Neuron development, neuritogenesis, synapse and circuitry	87
SRGAP2C	Human-specific gene duplication for Rho GAP regulation in cortical neurons	Neuron neoteny, increased spine density and synaptic connectivity	Neuron development, neuritogenesis, synapse and circuitry	81–84
Human brain developmental disease-causing genetic feature (modelled in hPS cell system)				
ANKLE2	Mitosis, endoplasmic reticulum regulation in NSPCs	Microcephaly (induced by Zika virus infection)	Neurogenesis	167,168
ASPM	Centrosome function in NSPCs	Microcephaly; may be associated with primate brain expansion	Neurogenesis	106,112

Table 1 (continued) | Genetic features that modulate human brain development as revealed by modelling evolution or diseases in animal or hPS cell models

Genetic feature	Genotypic mechanism	Phenotypic trait in human brain development	Human brain development process involved	Refs.
Human brain developmental disease-causing genetic feature (modelled in hPS cell system) (continued)				
CENPJ	Centrosome, cilia function in NSPCs	Microcephaly	Neurogenesis	107
CDK5RAP2	Centrosome function in NSPCs	Microcephaly; may be associated with primate brain expansion	Neurogenesis	9,112
IER3IP1	Endoplasmic reticulum secretion	Microcephaly	Neurogenesis	111
PTEN	mTOR signalling regulation in NSPCs	Macrocephaly	Neurogenesis	113
TLR3	Activation of innate immune response and apoptosis	Microcephaly (induced by Zika virus infection)	Neurogenesis	166
TSC2	mTOR signalling in caudal late interneuron progenitors	Tuberous sclerosis complex	Neurogenesis	114
WDR62	Centrosome function, mitosis regulation in NSPCs	Microcephaly, polymicrogyria	Neurogenesis	108
FGFR1	Nuclear signalling regulating NSPC proliferation, neuron migration and differentiation, and reelin	Schizophrenia	Neurogenesis, neuron migration, neuron development	153
NF1, CRLF3	RAS signalling for NSPCs; RHOA signalling for neuronal differentiation, survival and maturation	Neurofibromatosis type 1	Neurogenesis, neuron development, neuritogenesis	15,116
DISC1	Regulate NSPC proliferation, neuron differentiation and maturation, synapse formation, etc. Downstream targets include *NDEL1*, phosphodiesterases and WNT signalling	Multiple psychiatric disorders, including schizophrenia	Neurogenesis, neuron development, synapse and circuitry	133,151,152, 203,212
FMR1	Differential human–mouse and cell-type-specific regulation; human downstream targets include *CHD2*, the PI3K pathway and retinoic acid	Fragile X syndrome	Neurogenesis, neuron development, synapse and circuitry	144,145
MECP2	A multifunctional epigenetic regulator: microRNA regulator for ERK and AKT signalling in neurogenesis and NSPC differentiation; transcriptional regulator for synaptic excitability and synchrony	Rett syndrome	Neurogenesis, neuron development, synapse and circuitry	146–148, 232
RHOA	Small GTPase RHOA signalling	Focal cortical dysplasia	Neurogenesis, neuron development, synapse and circuitry	134
CNTNAP2	Cell cycle regulation in NSPCs; known function in neurites	ASD; cortical dysplasia focal epilepsy syndrome	Neurogenesis, neuritogenesis	118
TCF4	WNT signalling regulation	Pitt–Hopkins syndrome	Neurogenesis, neuritogenesis, gliogenesis	117
ACTB, ACTG1	Mitosis in NPSCs and neuroblasts	Subcortical band heterotopia, Baraitser–Winter syndrome	Neuron migration	129
DCHS1, FAT4	Cadherin regulation for cell adhesion in RGC architecture where disruption leads to migratory defects	Periventricular heterotopia	Neuron migration	130
LIS1, YWHAE	Microtubule, mitosis in RGC architecture, where disruption leads to migratory defects	Classical lissencephaly (Miller–Dieker syndrome)	Neuron migration	120,124,125
TUBA1A	Microtubule function in migrating neuroblasts	Classical lissencephaly	Neuron migration	126
CACNA1C	Calcium channel ($Ca_v1.2$) for interneuron saltatory migration and GABA receptor activity	Timothy syndrome	Neuron migration, synapse and circuitry	131,132
OLIG2	Transcriptional regulator for interneuron production	Down syndrome (trisomy 21)	Neuron development	135
ARID1B, CHD8, SUV420H1	Epigenetic regulators of cortical interneuron and deep-layer neuron development and circuit formation; *ARID1B* regulates callosal projection neuron maturation, axon projection, synapse formation	ASD; *ARID1B*$^{+/-}$ leads to agenesis of the corpus callosum	Neuron development, neuritogenesis, synapse and circuitry	137,140

Table 1 (continued) | Genetic features that modulate human brain development as revealed by modelling evolution or diseases in animal or hPS cell models

Genetic feature	Genotypic mechanism	Phenotypic trait in human brain development	Human brain development process involved	Refs.
Human brain developmental disease-causing genetic feature (modelled in hPS cell system) (continued)				
DMPK	MECP2-related pathway in glutamatergic neurons	Myotonic dystrophy type 1	Neuron development, synapse and circuitry, gliogenesis	136
WWOX	WNT signalling and DNA damage response; maintain interneuron, astrocyte cell number and activity	Epileptic encephalopathy; HAR6 lies in the gene transcribed region	Neuron development, synapse and circuitry, gliogenesis	58,154
DGCR8	MicroRNA regulator for calcium signalling in synaptic excitability	DiGeorge syndrome / 22q11.2 deletion syndrome	Synapse and circuitry	143
SHANK3	Calcium activity, synapse scaffolding regulation	Phelan–McDermid syndrome	Synapse and circuitry	149
UBE3A	E3 ubiquitin ligase for big potassium channel	Angelman syndrome	Synapse and circuitry	150
PLP1	Myelin production in oligodendrocytes	Pelizaeus–Merzbacher disease	Myelination	156

ASD, autism spectrum disorder; HAR, human-accelerated region; hPS cell, human pluripotent stem cell; NEC, neural epithelial stem cell; NSPC, neural stem and progenitor cell; oRGC, outer RGC; oSVZ, outer subventricular zone; RGC, radial glial cell. [a]Whether the gene has an evolutionary advantage in modern humans has been under debate.

therefore, be further validated in human populations carrying the corresponding mutations. Thus, moving forward, more key causal genetic mechanisms can be discovered through larger-scale gene mapping (for example, using massively parallel reporter assays[57,89] or genome-wide genetic screens)[13,101], followed by functional testing on advanced human cellular models and validation of traits in specific human populations.

Insight from human brain disorders

Dysregulation along human brain developmental trajectories arising from mutations in critical genetic loci can lead to neurological and psychiatric disorders. Here we provide examples — far from an exhaustive list — to illustrate how identifying genetic causes of brain disorders, ranging from anatomical deficits to neuropsychiatric disorders to interactions with environmental factors, together with functional characterization in mouse and hPS cell-based models, can advance our understanding of genetic control at each step of human brain development (Table 1 and Figs. 4b,c,5).

Neural tube defects

Failures in neural tube closure expose the neuroepithelium to the outside environment, resulting in neuroepithelial degeneration and subsequent nervous system deficits[102]. Neurulation events are highly conserved across mammals as they involve an ancient mechanism that evolved before vertebrates[102], making the mouse model an invaluable system to recapitulate core genetic control mechanisms identified in clinical studies. Dysregulation in key signalling pathways (for example, bone morphogenetic protein, Notch, planar cell polarity, non-canonical WNT, Sonic hedgehog), cell cycle and survival machinery (for example, *CASP3*, which encodes caspase 3 for cell apoptosis), cytoskeleton systems (for example, *MARCKS*, which encodes an actin filament crosslinking protein), and cell adhesion and interaction functions (for example, Eph–ephrin) disrupts neuroepithelium bending or neural tube closure steps — many of which depend on NEC behaviours, such as movement, proliferation, survival and cell–cell interactions — and leads to severe disorders, such as craniorachischisis and exencephaly[102] (Table 1 and Fig. 5). Despite the identification of many key mutations, genetic causes of neural tube defects remain elusive, largely due to a polygenic aetiology attributed to complex gene–gene or gene–environment interactions[103]. New brain organoid platforms for modelling neural tube formation can systematically test risk genes in human models to investigate the underlying mechanisms[104,105].

Malformation from disrupted neurogenesis

Defects in progenitor amplification or apoptosis result in alterations in brain size and are often associated with primary microcephaly, developmental delay and epilepsy[2]. Despite diverse aetiologies, genetic causes of brain structural deficits, such as 'small head' (microcephaly) or 'smooth brain' (lissencephaly), are often attributable to genes controlling cytoskeleton architecture or cell growth machinery in various cell types[12]. In mice and hPS cell-derived cortical organoids modelling microcephaly, causative mutations in genes encoding proteins involved in centrosome function and mitosis (for example, *ASPM*[106], *CDK5RAP2* (ref. 9), *CENPJ*[107] and *WDR62* (refs. 108,109), DNA damage response (for example, *MCPH1*)[110] and endoplasmic reticulum secretion (for example, *IER3IP1*)[111] target RGC-mediated neurogenesis, resulting in RGC premature differentiation, depletion and consequently a significant reduction in cortical size[15] (Table 1 and Fig. 5). Among microcephaly-causing genes, *ASPM* and *CDK5RAP2* contribute to an increased brain size in primates[112], and identifying more such genes could reveal convergent genetic mechanisms underlying both evolution and diseases. Moreover, disruption in critical cell growth-related signalling pathways in neural progenitors can lead to abnormal proliferation and anatomical malformations. hPS cell models in which mTOR signalling was altered by deletion of *PTEN* (which encodes an mTOR suppressor)[113] phenocopied macrocephaly by affecting cortical RGC amplification, and those in which mTOR signalling was altered by deficiency of *TSC2* (which encodes a cell growth regulator)[114] phenocopied tuberous sclerosis complex owing to interneuron progenitor overproliferation (Table 1 and Fig. 5). Organoid models derived from individuals with neurofibromatosis type 1 (mutation in *NF1*, which encodes neurofibromin, a RAS GTPase activator)[115,116] and Pitt–Hopkins syndrome (mutation in *TCF4*, which encodes a transcription factor initiating neural differentiation)[117] showed alterations in both progenitor proliferation and neuron differentiation, although the *NF1* mutation acts on RAS signalling and the *TCF4*

mutation impairs WNT signalling. Organoids derived from individuals with cortical dysplasia focal epilepsy syndrome owing to a homozygous mutation in *CNTNAP2*, a neurexin-encoding gene, recapitulate forebrain overgrowth phenotypes due to faster neural progenitor cell cycle and production[118]. Homozygous silencing of the gene encoding the transcription factor EOMES affects IPC propagation and migration, causing microcephaly with polymicrogyria and agenesis of the corpus callosum (ACC)[119]. Many microcephaly pathogenic variants cause overlapping phenotypes of lissencephaly (for example, *NDE1* (ref. 2) and *LIS1* (ref. 120)), polymicrogyria (for example, *EOMES*[119] and *WDR62* (ref. 12)) and focal cortical dysplasia (for example, mTOR-related genes)[121,122], highlighting the staggering complexity of genotype–phenotype manifestations even in monogenic diseases[12] (Table 1 and Fig. 5). Notably, several mouse models of mutations that cause microcephaly in humans exhibit a mild reduction in brain size (for example, *ASPM*[4], *CDK5RAP2* (refs. 3,6) and *IER3IP1* (ref. 111)) in contrast to robust phenotypes in their corresponding hPS cell models[9,106,111], highlighting the unique role for hPS cell-based models not only to identify disease-causing variants de novo, but also to revisit risk genes previously studied only in animal models. Furthermore, hPS cell-based models can serve as a platform for large-scale screening to reveal new genetic modifiers of human neurogenesis and brain size[111] (Fig. 2).

Abnormal neuronal migration, differentiation and maturation

Neuronal precursor-mediated migration allows for brain expansion and self-organization of the architecture, which can be disrupted by pathogenic mutations in key cytoskeletal genes that regulate centrosomes at the leading edge of migrating neuroblasts or microtubules that are necessary for somal translocation[12]. The resulting abnormal precursor migration and neuronal positioning largely leads to lissencephaly, which is characterized by subcortical band heterotopia with a wide range of clinical severity in cortical abnormalities, anterior-to-posterior severity gradients, gyrification reduction, band thickness and ventricle enlargement[123] (Fig. 5). Classical (type I) lissencephaly patients display a thicker, four-layer cortical structure with a heterotopic band, whereas hPS cell-derived forebrain organoids bearing key causative mutations, including *LIS1* (which encodes a regulator of motor protein dynein), *YWHAE* (which encodes a signal transduction-mediating protein that has diverse roles, such as cell division and regulation of insulin sensitivity)[120,124,125] and *TUBA1A*[126], modelled defects in anatomical architecture and under-migration of newborn neurons (Table 1 and Fig. 5). Mutations in X chromosome-linked *DCX*, which encodes a microtubule-associated protein, cause lissencephaly in males and are associated with sex differences and dose-dependency in disease severity, as female patients are affected with milder subcortical band

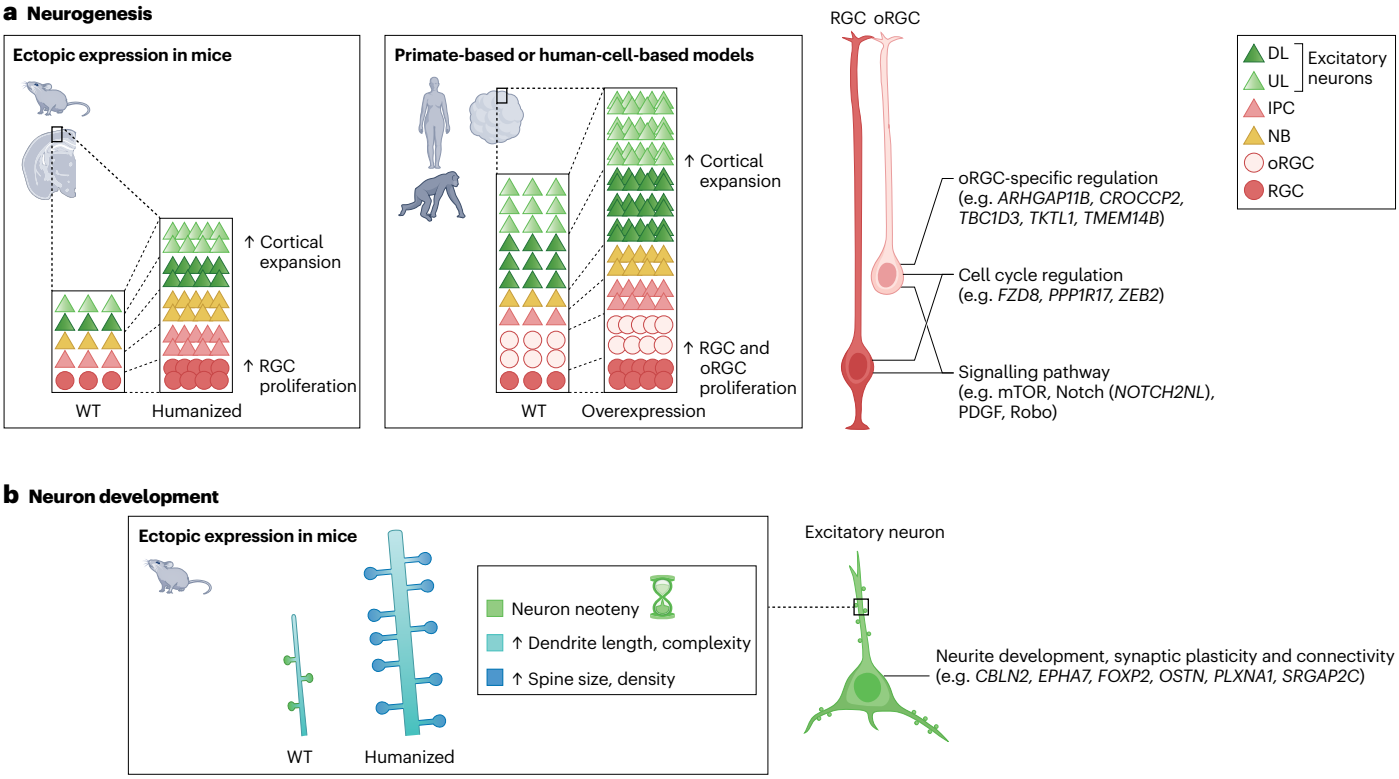


Fig. 3 | Human-specific genetic modulation of brain development. a, Examples of human-enriched genetic features that modulate neural stem cell-mediated neurogenesis, resulting in cortical expansion, that were modelled in mice and pluripotent stem cell-based systems. These genetic features can regulate the neural progenitor cell cycle (*FZD8* (ref. 61), *PPP1R17* (ref. 60) and *ZEB2* (ref. 29)), regulate signalling pathways (mTOR[40,65], Notch (for example, *NOTCH2NL*)[63,64], PDGF[62] and Robo[17]) or specifically regulate outer radial glial cell (oRGC) behaviours (*ARHGAP11B*[69–73], *CROCCP2* (ref. 66), *TBC1D3* (refs. 74,75), *TKTL1* (ref. 77) and *TMEM14B*[76]). **b**, Examples of human-enriched genetic features that modulate neuron development processes, resulting in protracted neuron maturation (which is indicated by the 'hourglass' icon) and changes in neurite development, synapse plasticity and connectivity, that were mostly studied using humanized mouse models. These genetic features include *CBLN2* (ref. 86), *EPHA7* (ref. 88), *FOXP2* (refs. 45,79,80), *OSTN*[85], *PLXNA1* (ref. 87) and *SRGAP2C*[81–84]. DL, deep layer; IPC, intermediate progenitor cell; NB, neuroblast; UL, upper layer; WT, wild type.

heterotopia[123,127,128]. Many lissencephaly-causing mutations lead to disease traits in various brain regions aside from the cortex. For example, 'tubulinopathies' and 'actinopathies' caused by mutations in genes encoding proteins involved in microtubule (for example, *TUBA1A*)[126] and actin (for example, *ACTB*, *ACTG1* based on data discussed in a preprint article)[129] regulation, respectively, lead to cortical lissencephaly and a broad range of maldevelopment in the cerebellum, hippocampus and corpus callosum, although neuropathology has only been modelled in human cortical organoids[123] (Table 1 and Fig. 5). Mutations preferentially targeting interneuron migration and differentiation, such as *ARX* (which encodes a highly conserved homeobox transcription factor involved in vertebrate cerebral development), lead to agyria lissencephaly phenotypes paired with severe neuropsychiatric disorders, such as epilepsy or ACC[123]. Mutations in *RELN* (which encodes reelin, a secreted extracellular matrix glycoprotein involved in neuronal migration and cell interactions) impair regulation of neuronal precursor migration by Cajal–Retzius cells, causing pachygyria or lissencephaly with cerebellar hypoplasia[12]. The importance of accurate spatial positioning of neurons for normal brain development is emphasized by these disease cellular phenotypes and many others, including over-migration of newborn cortical neurons in cobblestone (type II) lissencephaly (for example, owing to mutations in *POMT*, which encodes a glycosyltransferase)[123], aberrant migration and lining along ventricles of newborn cortical neurons in periventricular heterotopia that is often paired with recurrent seizures (for example, owing to mutations in genes encoding cadherin (*DCHS1*, *FAT4*)[130] or an actin-binding protein filamin A (*FLNA*)[12]), and interneuron migration and synapse deficits in Timothy syndrome (for example, owing to mutations in *CACNA1C*, which encodes a subunit of the calcium channel $Ca_v1.2$)[131,132] (Table 1 and Fig. 5). Notably, many disease-causing mutations that have a clear impact on patients, such as *DCX* and *FLNA*, do not show corresponding phenotypes in mice[12]. Recent development of brain organoid models that exhibit clear segregation of distinct cortical layers[133] and some structural convolutions[125,133] provide promising human models to examine the impact of these risk genes on neuron diversification and distribution and to investigate underlying mechanisms. More advanced organoid platforms remain to be developed to reveal the molecular and cellular basis that leads to key cytoarchitectural features, including gyrification (Fig. 2).

Aside from abnormal migration (which determines spatial positioning), dysregulation in neuronal differentiation and maturation processes can also lead to diseases, some of which have been effectively modelled with hPS cell-derived organoids[8,14]. For example, cortical organoids derived from focal cortical dysplasia patient-induced PSCs (iPS cells) recapitulated phenotypes of dysmorphic neurons and neuronal network hyperexcitability through downregulation of *RHOA*, a small GTPase RHOA signalling gene[134], which revealed a focal cortical dysplasia-causing mechanism independent of mTOR regulation of progenitor proliferation[121]. Impaired RHOA signalling caused by reduced *CRLF3* expression was also attributed to defects in neuronal differentiation, survival and maturation in forebrain organoids derived from NF1 patients[115,116] (Table 1 and Fig. 5). Moreover, patient iPS cell organoids modelling disorders with less severe structural abnormalities but more pronounced psychiatric symptoms have been used to reveal underlying cellular defects caused by mutations, which are often associated with neuron differentiation. For example, *OLIG2* was shown in Down syndrome patient iPS cell-derived organoids with trisomy 21 to be a transcriptional factor that drives interneuron fate choice, resulting in excessive $OLIG2^+$ progenitors and interneurons[135] (Table 1 and Fig. 5). A cortical organoid model of myotonic dystrophy type 1 bearing a mutation of *DMPK* (which encodes the myotonin-protein kinase that interacts with RHO family small GTPases) displays loss of glutamatergic neurons, dysregulation of glutamate synaptic signalling and a marked elevation in the number of glial cells. Glutamate-induced excitotoxicity is manifested through *MECP2*-related pathways, and the *DMPK*-mutated patient organoid model phenocopied that of *MECP2* deficiency[136] (Table 1 and Fig. 5). Analysis of three cortical organoid models with isogenic mutations of *SUV420H1*, *ARID1B* and *CHD8*, three autism spectrum disorder (ASD) risk genes that encode epigenetic regulators, revealed common phenotypes of excessive interneuron differentiation and premature differentiation of deep-layer cortical neurons[137] (Table 1 and Fig. 4b). Overall, these findings emphasize the importance of accurately controlling the positioning of neurons in both space and time to achieve the complex structural organization of the human brain.

Aberrant circuit formation and wiring

Disruption of neural circuit assembly and connectivity involving key genes regulating axon guidance and synaptic function, in addition to cytoskeleton organization, can lead to brain disorders in humans[8,12,48] (Fig. 5). Most functional studies of disease-causing mutations in axon guidance gene families found in human patients, which are often associated with epilepsy and ASD, have been performed in animal models[138]. For example, mutations in several Slit–Robo ligand-receptor gene families in mice disrupt axon midline crossing between two hemispheres and are associated with disorders such as ACC or dyslexia in humans[138,139]. Gonadotropin-releasing hormone-expressing neurons migrate from the nose through the forebrain into the hypothalamus along neuronal axon tracts, and mouse models show that mutations in semaphorin genes that cause Kallmann syndrome in humans (such as *SEMA3A*, a semaphorin-encoding gene regulated by *CHD7*) disrupt migration substrates for these neurons[138]. A recent preprint using organoids derived from individuals with ACC revealed that *ARID1B*-haploinsufficiency causes abnormalities in callosal projection neuron maturation, long-range projection formation and transcription of corpus callosum development[140] (Fig. 5). This finding demonstrates that rapid advancement of hPS cell organoids that model different brain regions and later developmental stages enables the study of neuronal projection development in humans (Fig. 2).

Genetic malfunctions in synapse formation and plasticity are major contributing factors to the pathogenesis of neurodevelopmental and neuropsychiatric disorders, including ASD, epilepsy, intellectual disabilities (ID) and schizophrenia[50,141]. Besides robust modelling of disorders with structural defects caused by deficits in progenitor proliferation, neuron migration, or differentiation, organoid systems, such as assembloids, are rapidly advancing to provide platforms for examining the pathogenesis and mechanisms underlying less anatomically distinct but more complex psychiatric disorders involving circuitry abnormalities[14,15,142] (Fig. 2). In recent years, many of the most common single-gene mutations for ASD or ID were shown to be sufficient to cause cellular deficits and to recapitulate relevant 'synaptopathies' in patient-derived organoids: examples include *CACNA1C* in Timothy syndrome[131,132], *DGCR8* (which encodes a microRNA regulator for calcium signalling) in DiGeorge syndrome[143], *FMR1* (which encodes the fragile X mental retardation protein, a brain-enriched RNA-binding protein essential for brain development) in fragile X syndrome[144,145], *MECP2* (which encodes a multifunctional epigenetic regulator) in Rett syndrome[146–148], *SHANK3* (which encodes a synapse scaffolding protein) in Phelan–McDermid syndrome[149], *UBE3A* (which encodes an E3 ubiquitin ligase for voltage-dependent big potassium channel) in

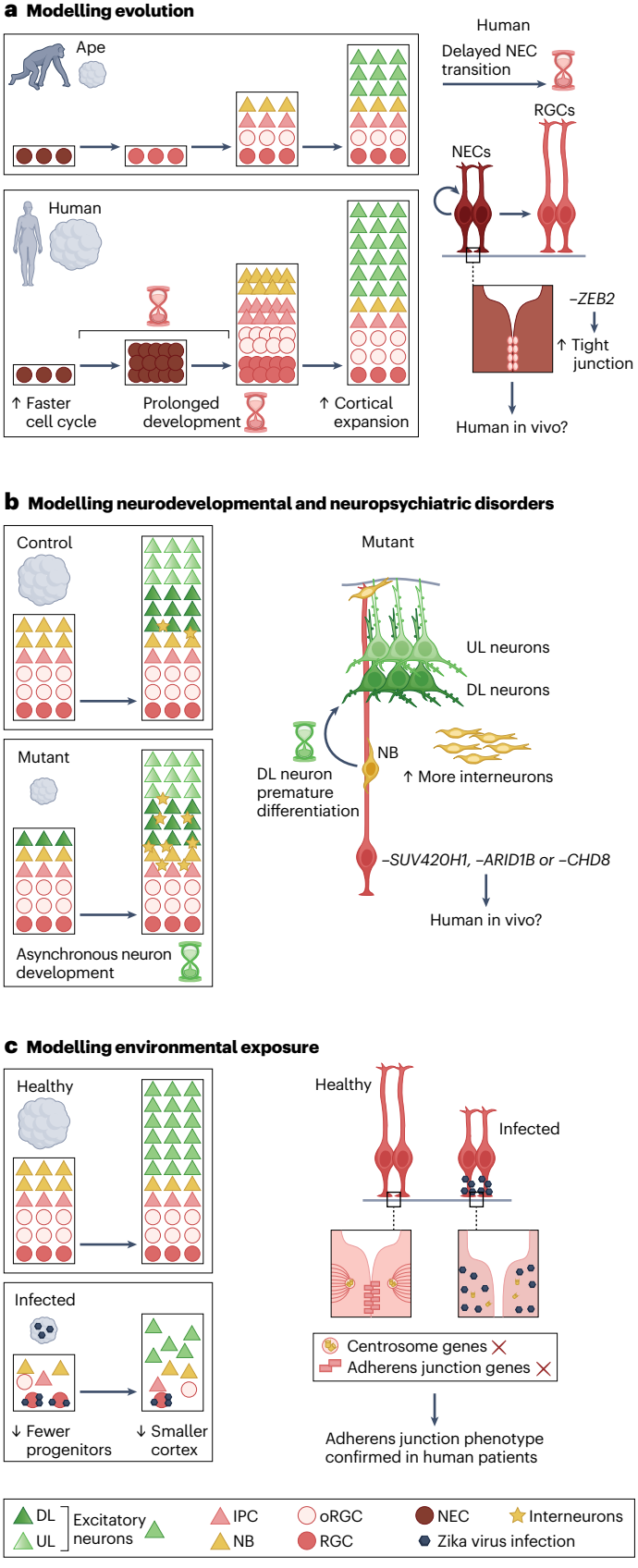


Fig. 4 | Genetic basis of human brain development uncovered using hPS cell models by studying traits led by evolution, diseases and environmental exposure. a, Evolutionary features modelled in human pluripotent stem cell (hPS cell)-derived organoid system. An illustrative study[29] is depicted that compares ape and human cortical organoids and identified differential cellular and molecular features, including *ZEB2* as a genetic driver that modulates the transition of neuroepithelial cells (NECs) to radial glial cells (RGCs) in humans. **b**, Neurodevelopmental and neuropsychiatric disorders modelled in hPS cell-derived systems derived from patients or genetically engineered. Depicted is a study[137] that examined the consequences of mutations in three autism spectrum disorder risk genes, *SUV420H1*, *ARID1B* and *CHD8*, in their respective cortical organoids and found cell type-specific developmental abnormalities, including excessive interneuron differentiation and premature differentiation of deep-layer cortical neurons. The 'hourglass' icons in parts **a** and **b** indicate alterations of developmental tempo. **c**, Environmental exposure modelled in hPS cell-derived systems. Represented are studies[161–163,166–171] that infected brain organoids with Zika virus and demonstrated substantial disruption in cellular architecture and further identified its genetic causes, including centrosomal and adherens junction genes. The microcephaly phenotypes identified and modelled in the dish were observed and validated in human patients, including aberrant adherens junctions[169]. DL, deep layer; IPC, intermediate progenitor cell; NB, neuroblast; oRGC, outer RGC; UL, upper layer.

Angelman syndrome[150], *DISC1* (which encodes a multifunctional protein, Disrupted in schizophrenia 1, involved in cell proliferation, differentiation, neurite growth, cell adhesion and synapse formation, among others)[133,151,152] and *FGFR1* (which encodes a receptor tyrosine kinase for the growth factor FGF to regulate cell signalling)[153] in schizophrenia, and *WWOX* (which encodes an oxidoreductase enzyme) in epileptic encephalopathy[154] (Table 1 and Fig. 5). In addition, mutations causing glia-associated pathology can lead to developmental delay and other disease traits owing to critical roles of glia in synapse modulation[155]. For example, organoids derived from patients with Pelizaeus–Merzbacher disease, a rare X-linked disease caused by mutation of the *PLP1* gene (which encodes myelin protein) and associated with delayed development in motor skills and hypotonia during early childhood, recapitulate patient cellular phenotypes of myelination defects[156] (Table 1 and Fig. 5). Besides molecular, cellular and anatomical phenotypes, alterations in neuronal activity are another readout identifying pathophysiology of circuit assembly and function[157]. Emerging studies reveal the roles and mechanisms of risk genes in regulating synapse formation, function and plasticity using human neurons, such as *FMR1* in fragile X syndrome[144], and we expect that more will be learned in the future, especially through comparisons between human and mouse models.

Dysregulation via environmental interactions

Human brain developmental processes can be shaped negatively or positively by genetic alterations induced by environmental factors. Biological or chemical insults, such as viral infections, chemical agents or radiation[158], can either directly induce genetic lesions or disrupt molecular cascades during one or more steps of brain development, including precursor generation, migration, positioning, or establishing neuronal connections. Affected genes have been identified using animal[159] or hPS cell-based models[160]. A notable example is congenital microcephaly induced by Zika virus. Initial studies showed reduced neural progenitor proliferation and premature differentiation upon viral infection of human brain organoids[161–163] or mice[164,165]. Mechanistic investigations then linked viral protein interactions with key molecules in neurogenesis (such as *TLR3* (ref. 166) and *ANKLE2* (refs. 167,168)) and

cytoskeleton processes (such as adherens junctions[169] and centrosome genes[170,171]) (Table 1 and Figs. 4c,5). Moreover, exposure to physiological factors (such as nutrition or the microbiome) or activity-dependent mechanisms (such as an enriched environment or experience) affects genetic elements regulating not only brain patterning and cytoarchitecture, but also neurotrophic factors and neurotransmitter systems that are altered in many neurodevelopmental and neuropsychiatric disorders, including ID, ASD and schizophrenia[172]. For example, vitamin A is an essential supplement during neural tube formation[103]. Its derivative retinoic acid — an evolutionarily differentially regulated signalling molecule that presents an anterior-to-posterior, prefrontal cortex-enriched gradient in the developing brain — has a critical role in prefrontal cortex patterning and cortical–thalamic connectivity, as loss of function of its receptor genes (*RXRG*, *RARB*) or *CYP26B1*-dependent catabolism in mouse models showed deficits[173]. Many environmentally induced alterations influence neuronal and circuit development through epigenetic regulatory mechanisms[1,50]. Notably, the severity of both structural changes (for example, via Zika virus infection)[174] and complex neurodevelopmental and neuropsychiatric disorders (such as ASD and schizophrenia)[175] largely depends on the genetic background of the patient, which can be modelled with hPS cells[176].

Emerging views on genetic multiplicity, interactions and regulation

Recent advances in studying neurodevelopmental and neuropsychiatric disorders have revealed emerging principles of genetic multiplicity, differential regulation, gene–gene interactions and interactions of non-coding genomic regions with disease-associated genes[11]. Integrating such information offers a new perspective, while posing challenges in investigating the diverse genetic architecture of human brain development among complex genotype–phenotype relationships and overlapping aetiologies.

Genetic multiplicity — both risk gene pleiotropy and disease polygenicity — has been revealed through the expanded scale of next-generation and single-cell multi-omic sequencing and GWAS analyses of ASD[141], schizophrenia[177,178] and other complex neurodevelopmental and neuropsychiatric disorders. These efforts have not only identified a growing list of risk genes and variants with high statistical confidence, ranging from common small-effect variants to rare but deleterious ones, but have also revealed their highly polygenic nature through polygenic risk scoring and through analysing variant interactions and convergence[11,12,101]. Moreover, recent studies with higher throughput and sensitivity have begun to deconvolute how ubiquitous disease-causative or disease-associated gene variants affect the brain in a region-specific, developmental stage-specific and cell type-specific manner. For example, periventricular heterotopia-causing mutations (such as *FLNA*[12] and *PRPF6* (ref. 179)) disrupt RGC proliferation and neuronal differentiation through cell type-specific mechanisms. Novel roles for several synaptic genes, including *SCN2A* and *CACNA1C* in ion channels and *SHANK3* and *SYNGAP1* in synaptic modulation, were identified in immature neurons; these roles are distinct from their known function in mature neurons and are due to differential spatiotemporal regulation[180]. Disruption in *FMR1* in a fragile X syndrome patient-derived organoid model showed abnormalities in multiple developmental steps, including neurogenesis, neuronal maturation and synapse formation[145] (Table 1 and Fig. 5). These findings highlight the level of complexity among disease traits, their causative variants and the affected developmental processes[12].

Diseases with anatomical phenotypes can involve genetic interactions. For example, genetic interactions during neural tube closure

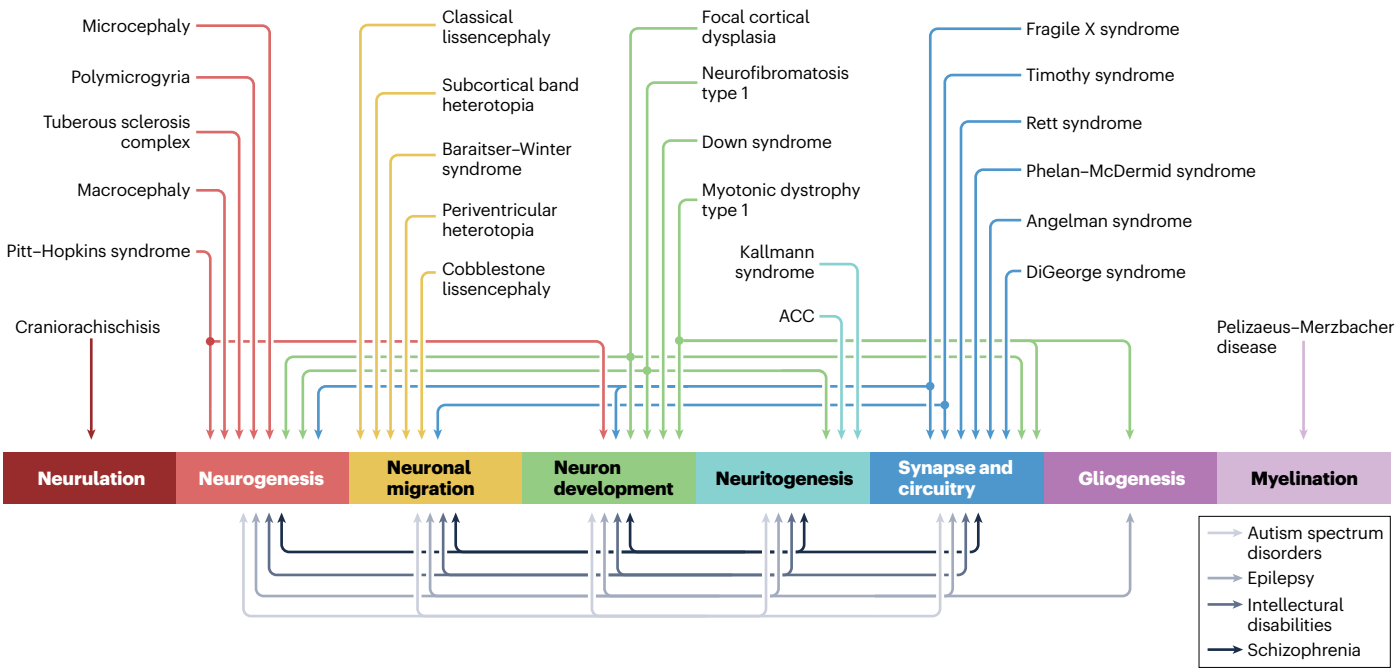


Fig. 5 | Genetic basis of human brain development revealed by brain disorders. An illustration showing examples of neurodevelopmental and neuropsychiatric disorders that have helped to determine how causal genetic variants (Table 1) impact specific neurodevelopmental processes. Some diseases commonly considered to be monogenic have overlapping phenotypes, highlighting genotype–phenotype disease manifestations, such as models for microcephaly and polymicrogyria[12], and focal cortical dysplasia displaying deficits in radial glial cell proliferation[121] and neuron differentiation[134]. ACC, agenesis of the corpus callosum.

Glossary

Gene editing
A type of genetic engineering technology in molecular biology by which a DNA sequence is inserted, deleted, modified or replaced in the genome of a living organism.

Genome-wide association studies
(GWAS). A research approach to identify genetic variants at the genome-wide level that are statistically associated with a risk for a disease or a particular trait.

Gyrified
Characterized by convolutions made of alternating gyri and sulci on the surface of cerebral cortex in certain species. Some disease conditions can alter gyrification, such as lissencephaly, where the cortical surface is smooth.

Human-accelerated regions
(HARs). Sets of segments of the human genome that are conserved throughout vertebrate evolution, but contain many substitutions in the human lineage.

Humanized animal models
Experimental animal models that have been xenografted with human cells and/or engineered to express human gene products, to obtain relevant insights in the in vivo context for understanding of human-specific physiology and pathologies.

Monogenic diseases
Genetic disorders that are caused by variation in a single gene.

Neural stem and progenitor cells
A collective term for neuroepithelial cells, radial glial cells, progenitor cells and other multipotent cells in the brain that give rise to various differentiated, postmitotic neuronal and glial cell types, often through intermediate progenitor cell stages.

Neurotypical
Description of individuals with intellectual and cognitive development typical of the larger population, as opposed to, for example, those impacted by neurodevelopmental or neuropsychiatric disorders (known as neuroatypical).

Organoids
Multicellular 3D structures — derived from primary tissue, embryonic stem cells or induced pluripotent stem cells — that self-organize in vitro and recapitulate developmental, anatomical and/or functional aspects of the primary tissue or organ counterpart.

Outer radial glia cells
(oRGCs). Radial glial neural stem cells that contain basal processes but lose their apical attachment to the ventricular surface and undergo distinct migratory and division behaviours; also known as basal radial glial cells.

Pleiotropy
The phenomenon that one gene or regulatory element affects multiple phenotypic traits (for example, biological processes, diseases).

Pluripotent stem cell
A cell that can be maintained in an undifferentiated state and can differentiate into most, if not all, cells of the body.

Polygenicity
A genetic disorder that is caused by the combined action of more than one gene.

Primary microcephaly
A brain disorder — known as 'small head' — characterized by significant reduction in head circumference at birth (more than three standard deviations below the mean for age and gender) usually coincident with intellectual disabilities.

Prime editing
A gene editing method by which new genetic information is written into a targeted DNA site in a precise 'search-and-replace' manner, involving a prime editing guide RNA capable of identifying the target site.

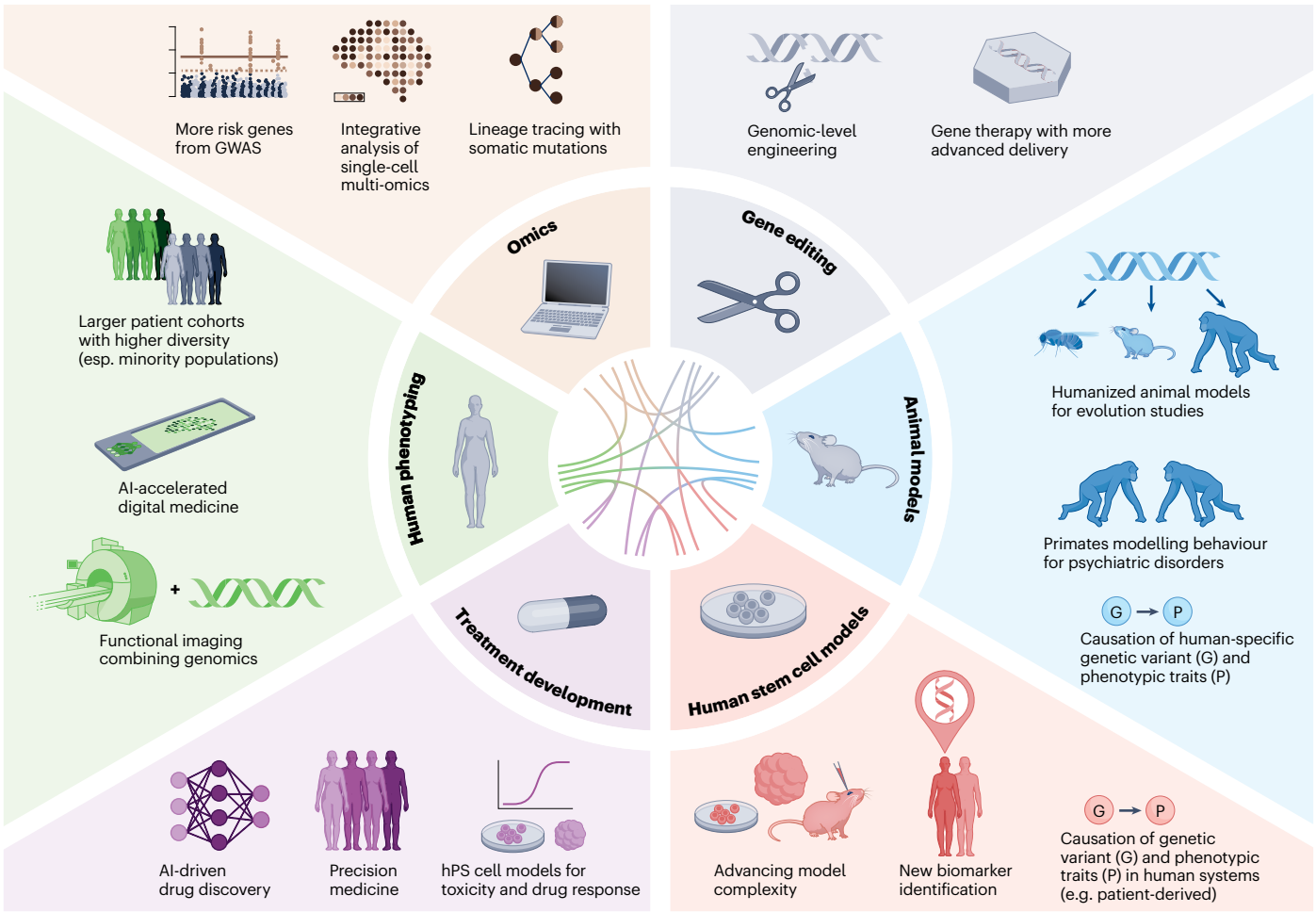
Single-cell multi-omic
Referring to high-throughput quantification of multiple types of biomolecules (for example, DNA, RNA, chromatin, protein and metabolites) from the same individual cell, aiming to achieve more biological insight than can be inferred by analysing each molecular layer from separate cells.

Xenografted
Refers to cells or tissue transplanted from a donor into a recipient of a different species.

were shown to be necessary in mice heterozygous for mutations in both *DVL1* and *DVL2*, and in mice and humans heterozygous for mutations in both *DVL* and *VANGL2*; these compound heterozygous individuals display functional redundancy and additive effects that resemble those of each homozygote[103]. The synergistic impact of common heterozygous variants of individually small effects is better represented in polygenic disorders with neurodevelopmental manifestations, such as ASD[11,137,141,181]. Interactions with non-coding genomic regions affecting gene expression through enhancers or other interactions in human disease traits are also critical. The advancement of single-cell molecular detection tools can reveal cell type-specific and developmental stage-specific consequences. For example, long non-coding RNAs, such as *DLX6-AS1*, *LINC00643* and *LINC01166*, which are dysregulated in multiple neurodevelopmental and neuropsychiatric disorders[182], modulate interneuron specification. Advances in genome-editing technologies allows systematic investigation of epistasis of genetic interactions during specific brain developmental steps using hPS cell models. For example, CRISPR-based editing of one putative (*FURIN rs4702*) and four top-ranked (*FURIN*, *SNAP91*, *TSNARE1* and *CLCN3*) common schizophrenia risk variants in isogenic iPS cell lines demonstrated their causative and synergistic effects in synapse modulation[177]. Overall, developmental process-specific genetic variations, their pleiotropy and regulation, and genetic interactions with each other and the environment demand technological advancement for understanding the complex genetic architecture of human brain development.

Opportunities and challenges

Tremendous progress has been made using tools from various technology domains to study the precise genetic control of human brain development, with the goal of advancing therapeutic development (Fig. 6a). Patient phenotyping studies, such as genetic studies and functional imaging[183–186], examine neuropathological traits during development and identify disease-causing genetic variants. Whole-genome-scale investigations can reveal disease-associated risk genes, which are critical for studying large-effect genes reliably associated with specific developmental processes or complex disorders such as ASD. In particular, single-cell omics[13,122,187–191] and analysis[192–195] at the genomic, transcriptomic and epigenomic levels have been widely applied to map a holistic,


a
More risk genes from GWAS
Integrative analysis of single-cell multi-omics
Lineage tracing with somatic mutations
Genomic-level engineering
Gene therapy with more advanced delivery
Omics
Gene editing
Larger patient cohorts with higher diversity (esp. minority populations)
Humanized animal models for evolution studies
Human phenotyping
Animal models
AI-accelerated digital medicine
Primates modelling behaviour for psychiatric disorders
Functional imaging combining genomics
G → P
Causation of human-specific genetic variant (G) and phenotypic traits (P)
Treatment development
Human stem cell models
AI-driven drug discovery
Precision medicine
hPS cell models for toxicity and drug response
Advancing model complexity
New biomarker identification
G → P
Causation of genetic variant (G) and phenotypic traits (P) in human systems (e.g. patient-derived)

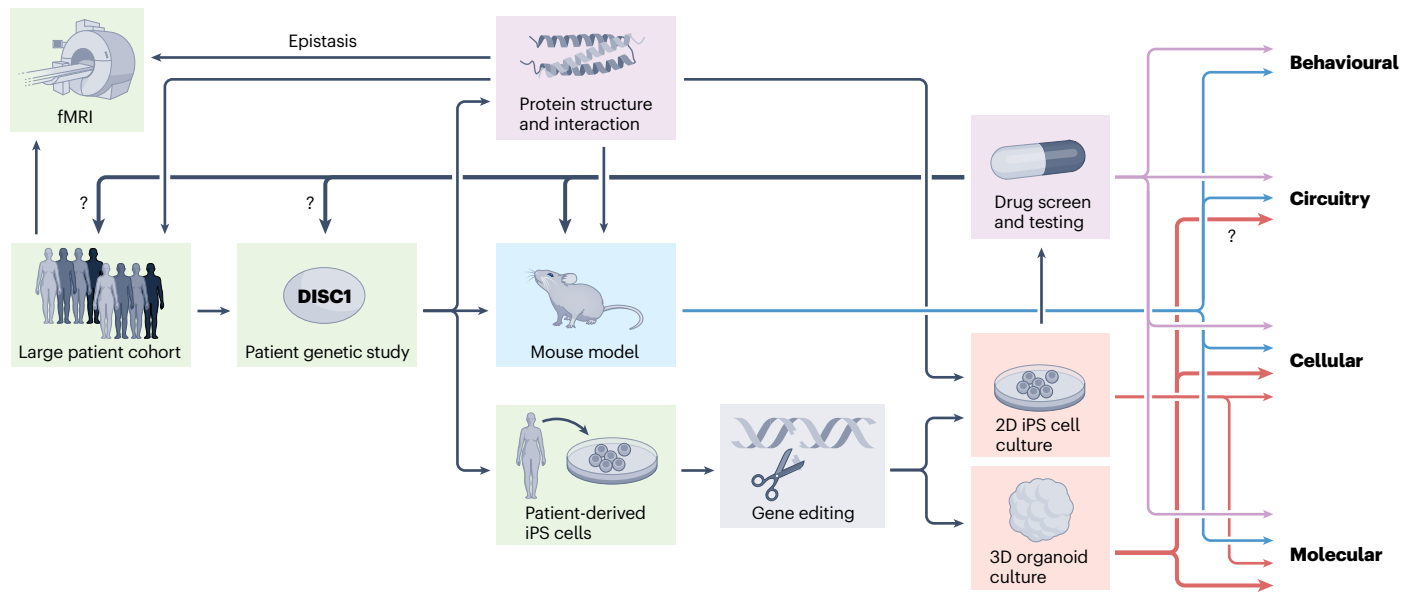

b
fMRI
Epistasis
Protein structure and interaction
Drug screen and testing
Behavioural
Circuitry
Cellular
Molecular
Large patient cohort
DISC1
Patient genetic study
Mouse model
2D iPS cell culture
Patient-derived iPS cells
Gene editing
3D organoid culture

Fig. 6 | Current and future approaches for studying the genetics of human brain development. a, Each section of the circle depicts a different biotechnology domain that empowers the investigation of the genetics of human brain development, including human phenotyping studies (such as patient genetics and functional imaging), multidimensional molecular detection and manipulation tools (such as single-cell multi-omics, genome-wide association studies (GWAS), CRISPR-based tools and prime editing), animal and human pluripotent stem cell (hPS cell)-based model systems (such as primate models, induced pluripotent stem cells (iPS cells) and 3D human brain organoids), and therapeutic development (such as compound screening, safety testing and efficacy testing). The lines in the centre indicate that different biotechnology domains can integrate to generate synergistic effects. For each biotechnology domain, the outermost part of the figure depicts several future technology developments that we believe will lead to substantial improvements. Notably, all technology use and advancement in the biomedicine space ought to be supervised and guided by proper ethics. **b**, An overview of a multidisciplinary study, using the *DISC1* gene as an example. We first examined the role of DISC1 in animal models and utilized human models based on patient mutations and isogenic iPS cell lines to determine the causative roles in specific neural developmental phenotypes. This was followed by mechanistic studies to identify druggable targets and drug testing, and we finally tested efficacy at functional and behavioural levels in a humanized mouse model with the same patient mutation (see the main text for details). AI, artificial intelligence; fMRI, functional magnetic resonance imaging. Structure of DISC1 protein adapted from ref. 205, Springer Nature Limited.

high-resolution picture of genetic architecture in a cell type-specific manner (Fig. 6a). On the other hand, mechanistic interrogation of the causality of genetic variants and their hypothesized functions and testing of drug responses during brain development are enabled by various animal models and the rise of hPS cell-derived 2D and 3D model systems, together with rapid iterations of genetic manipulation tools[14,142]. Although hPS cell-derived models have the unique advantage of modelling human-specific molecular and cellular features, animal models (particularly rodents and primates) with human mutations remain the primary approach to explore many higher-level functions, such as cognition and behaviours[50,52] (Fig. 6a). Integration of technologies in each domain allows for the convergence of human genetics and developmental neuroscience, systematically establishing genotype–phenotype links to offer insights into how the human brain develops, which in turn paves the way for disease diagnostic and treatment strategies (Fig. 6a).

Opportunities with combinatorial approaches

The power of combinatorial approaches will provide tremendous opportunities for a better understanding of the genetics of human brain development and for developing therapeutic strategies for brain disorders (Fig. 6a). A notable example is our multidisciplinary investigation of *DISC1*, which was initially identified as an ultra-rare risk gene for major psychiatric disorders in a large Scottish family[196] and later in a smaller American family (Pedigree H)[197] (Fig. 6b). Starting with rodent models, loss of function of *DISC1* in adult-born dentate granule neurons was shown to lead to deficits in neuronal morphology, migration, dendritic growth and synapse formation[198–200], as well as behavioural abnormalities[201]. Derivation of iPS cell lines from multiple members of Pedigree H[202] and generation of isogenic rescue and mutant iPS cell lines with genome editing, together with targeted differentiation into 2D cortical neurons, established a casual role of the *DISC1* mutation in dysregulated synapse formation and gene expression[203]. 3D brain organoid analysis further revealed deficits in cortical neuronal maturation and layer segregation, as well as its underlying molecular mechanism[133]. In addition, molecular and atomic structural analyses of DISC1 have identified its molecular binding partners and signalling pathways, and some of these interactions were shown to affect neuronal development in mice and hPS cell-derived 2D and 3D models[151,201,204–210], to exhibit epistasis in increased genetic risk for schizophrenia from multiple cohorts[208,209], and to affect hippocampal function and connectivity based on functional magnetic resonance imaging analysis in humans[211]. Mechanistic molecular analysis of iPS cell-derived 2D neurons further identified druggable targets, leading to pharmacological rescue of synaptic deficits in mutant human neurons. Finally, a humanized mouse model with the same mutation found in Pedigree H revealed that synaptic and behavioural deficits could be rescued in adult mice by the same pharmacological approach as in 2D human neurons[212]. Taken together, these studies illustrate an actionable pipeline starting with human models based on patient mutations with isogenic iPS cell lines to establish causative roles in specific phenotypes of neural development, followed by mechanistic studies to identify druggable targets and drug testing, and finally efficacy testing at functional and behavioural levels in a humanized mouse model with the same patient mutation (Fig. 6b).

Limitations of current hPS cell models and future improvements

Research using hPS cell models is still in its early stages, and many challenges remain to be addressed[213]. Unlike inbred mice that have practically identical genetic backgrounds, humans have a high degree of genetic diversity. Therefore, it is crucial to study the effects of specific genetic variants in the context of different genetic backgrounds to fully understand their impact in various human populations, especially minority groups[97,98]. In addition, substantial variability exists among hPS cell models, highlighting the need for improved reproducibility. iPS cell lines are highly variable because they are generated using different methods and from different patients, and therefore it may be necessary to customize differentiation protocols for each individual line[142].

Organoid models also need further optimization to incorporate a wider variety of cell types that better mimic the cell type heterogeneity found in vivo. For example, microglia[214,215] and endothelial cells[216], which are derived from different lineages than neural cells, can be incorporated into organoids through the use of co-culture techniques. Although current organoid models are improving in their ability to model early stages of brain development, such as neurogenesis, substantially more effort is needed to optimize these models to better replicate the cytoarchitecture and circuitry of the human brain, including distinct cortical layers and functional columns. Such efforts will lay a solid cellular foundation for modelling later stages of human brain development. Meanwhile, xenotransplantation of human cells and organoids into animal hosts provides another opportunity to study human-specific cell types or cell interactions (such as examining circuitry and behaviour of animal hosts) in an in vivo context[215,217,218]. More effort also needs to be invested in developing reproducible protocols for generating a wider variety of brain cell types and brain region-specific organoids to expand the range of brain regions that can be modelled[142]. Although most current studies focus on modelling a single brain region, the assembloid approach allows interactions between different brain regions to be studied, albeit with artificial boundaries. Developing methods to pattern brain organoids that span

multiple brain regions[219] holds promise for studying long-distance directed neuronal migration, axonal projections and the propagation of circuit activity.

Finally, high-throughput approaches that produce large quantities of organoids with consistent properties are needed to enable targeted or genome-wide CRISPR-based or prime editing screens for the functional investigation of specific genes or single-nucleotide variants in specific developmental steps, as well as the testing of drugs and treatment strategies on organoids with high-throughput readouts. Importantly, as organoid models become more advanced and are xenografted into animal models (potentially large animals), it is crucial to consider the ethical implications of all of these developments[217,218].

Overall, despite being in the early stages, promising hPS cell approaches will soon be broadly adopted and advanced for more systematic and ambitious screening and testing of human-specific variants, for example, using massively parallel reporter assays[10], or even testing every risk variant from GWAS to establish causative links between genotypes and their cellular and molecular consequences. Aligning with the goal of reducing the use of animals in research, this will help us to move towards a holistic understanding of the genetics of human brain development and pave the way for clinical applications.

Other future innovations

In addition to these developments in cell models, other technical advances will be required to enable a fuller understanding of the genetics underlying brain development (Fig. 6a). Better phenotyping of human traits will be essential. Improved acquisition of human brain tissue through global collaborations[55,189,191,220–224] will enable a higher number and wider diversity of human populations to be covered, which will reduce bias in genetic studies[97,98] and make polygenic scores for different diseases, many of which currently are based on populations with European ancestry, more applicable to a wider range of ethnicities[225]. Increasing the scale, depth and affordability of omics analyses will uncover previously unappreciated variants and their roles and reveal levels of human trait complexity through integrative analyses of different data modalities[12,101]. More advanced histological, pathological, functional and radiological imaging methods and artificial intelligence-accelerated analytic tools are needed that achieve higher accuracy, resolution, depth and throughput and offer more modalities[226] (Fig. 6a).

Better genetic causality studies of human traits will also be required. With the availability of modelling systems and an increasing number of confidently identified human-specific or disease risk gene candidates, the field is poised for larger-scale functional testing. Tools for basic research will continue to expand, such as gene editing tools that achieve genome-wide coverage, greater efficiency and fewer off-target effects[227,228], and those that enable the tracing and reconstruction of brain cell lineages[188] (such as virus-based lineage tracing tools and the use of somatic DNA mutations as a natural retrospective barcoding system)[122] (Fig. 6a).

Finally, better strategies are needed for developing treatments of neurodevelopmental and neuropsychiatric disorders. A deep understanding of genetics guides the identification of new biomarkers for diagnosis and novel targets for therapeutics. Although artificial intelligence-driven computational approaches will greatly expedite drug discovery[229], the use of hPS cell models should be scaled up to an industrial level to enable drug safety and efficacy to be evaluated at both population and individual levels. Moreover, genetic discoveries will hasten the improvement of novel and precise treatment methods, such as gene therapy with safer and more efficient delivery[230]. Taken together, these future developments promise to streamline discoveries from characterization and modelling of human traits to gene and drug discovery (Fig. 6a).

Conclusions

Our understanding of the genetic basis of human brain development largely comes from cross-species comparative studies that identify cellular and molecular features contributing to human-specific phenotypes and discoveries of genetic variants and interactions in patients with developmental brain disorders. Classic paradigms typically start by defining phenotypic traits of evolutionary human-unique features or brain diseases. This is followed by forward molecular genetics approaches, such as next-generation sequencing, to identify associated genetic variants hypothesized to be the genetic cause. Then, reverse genetics through gene manipulation in animal models allows for the establishment of causality between genotypes and phenotypes and subsequent studies of phylogenetics or pathogenesis. The growing accessibility of human tissue and rapid development of hPS cell-based model systems, along with advances in gene editing, sequencing analysis and functional assays, have enhanced our ability to tackle these problems. We can now apply the same pipeline to multiple human and animal model systems to directly identify and study genes at an unprecedented resolution and scale to establish genotype–phenotype causality. As more genes and variants are being identified in sequencing studies, it remains both a goal and a challenge to perform functional testing of anatomical structures and neuronal circuitry in later human developmental stages, for which reliable hPS cell-based models are still lacking. Importantly, the combination of these enabling technologies may soon lead to a paradigm shift in how we study the genetics of human brain development, especially for features unique to humans, mutations in non-coding regulatory regions, gene–gene interactions and mechanisms underlying complex polygenic diseases. Advancing our knowledge of the genetics of human brain development using integrated models and molecular detection tools may soon substantially transform our diagnostic architecture and accelerate therapeutic development for brain disorders.

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