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STATE-OF-THE-ART REVIEW

Soma-to-germline transformation in chromatin-linked neurodevelopmental disorders?

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Mutations in numerous chromatin regulators cause neurodevelopmental disorders (NDDs) with unknown mechanisms. Understandably, most research has focused on how chromatin regulators control gene expression that is directly relevant to brain development and function, such as synaptic genes. However, some NDD models surprisingly show ectopic expression of germline genes in the brain. These germline genes are usually expressed only in the primordial germ cells, testis, and ovaries for germ cell development and sexual reproduction. Such ectopic germline gene expression has been reported in several NDDs, including immunodeficiency, centromeric instability, facial anomalies syndrome 1; Kleefstra syndrome 1; MeCP2 duplication syndrome; and mental retardation, X-linked syndromic, Claes–Jensen type. The responsible genes, *DNMT3B*, *G9A*/*GLP*, *MECP2*, and *KDM5C*, all encode chromatin regulators for gene silencing. These mutations may therefore lead to germline gene derepression and, in turn, a severe identity crisis of brain cells—potentially interfering with normal brain development. Thus, the ectopic expression of germline genes is a unique hallmark defining this NDD subset and further implicates the importance of germline gene silencing during brain development. The functional impact of germline gene expression on brain development, however, remains undetermined. This perspective article explores how this apparent soma-to-germline transformation arises and how it may interfere with neurodevelopment through genomic instability and impaired sensory cilium formation. Furthermore, we also discuss how to test these hypotheses experimentally to ultimately determine the contribution of ectopic germline transcripts to chromatin-linked NDDs.

Abbreviations
AUB, aubergine; BMP4, bone morphogenetic protein 4; CYCT, cytochrome C, testis; D1PAS1, DNA segment, Chr 1, Pasteur Institute 1; DAZL, deleted in azoospermia like; DNAH1, dynein axonemal heavy chain 1; DNMT3B, DNA methyltransferase 3B; DP1, transcription factor Dp-1; dREAM/MMB, RBF, E2F, Myb/Myb-MuvB; E2F6, E2F transcription factor 6; EHMT1/2, euchromatic histone-lysine N-methyltransferase ½; ESC, embryonic stem cell; FOXJ1, forkhead box J1; GLP, G9A-like protein; H2AK119ub1, histone 2A lysine 119 monoubiquitination; H3K27, histone 3 lysine 27; H3K4me, histone 3 lysine 4 methylation; H3K9me, histone 3 lysine 9 methylation; KDM5C, lysine demethylase 5C; L3MBTL2, lethal(3)malignant brain tumor-like protein 2; MAEL, maelstrom; MAX, MYC-associated factor X; MECP2, methyl-CpG-binding protein 2; MGA, MAX gene associated; MVH, mammalian vasa homologue; NDD, neurodevelopmental disorder; PGCs, primordial germ cells; PIWI, P-element-induced wimpy testis; PRC1.6, polycomb repressive complex 1.6; Rb, retinoblastoma; REC8, REC8 meiotic recombination protein; REST, RE-1 silencing transcription factor; RNAi, RNA interference; RNF17, ring finger protein 17; SPAG6/16, sperm-associated antigen 6/16; SPATA1, spermatogenesis-associated 1; STRA8, stimulated by retinoic acid 8; SYCE1, synaptonemal complex central element protein 1; SYCP1-3, synaptonemal complex protein 1-3; TEX11/14, testis expressed 11/14; UPD, uniparental disomy; γH2AX, gamma histone 2AX.

Introduction

In the past decade, the genetic basis of neurodevelopmental disorders (NDDs)—including intellectual disability, autism spectrum disorders, and schizophrenia—became increasingly apparent, owing to the advent of next-generation sequencing. An unexpected outcome of such sequencing studies was the prevalence of chromatin regulator mutations responsible for monogenic NDDs [1–9]. While there is a well-established genetic connection between chromatin regulators and NDDs, the biology underlying this relationship is still unclear [3,9–11]. A widely accepted idea to explain this relationship is that these chromatin regulators control the expression of genes essential for brain function, such as synaptic genes. Studies on NDD-associated chromatin regulators typically involve genome-wide gene expression analyses with mRNA-seq in chromatin factor mutants and then further investigation of well-characterized dysregulated brain genes. This focus on synaptic genes is a logical approach, given that altered synaptic development and function are a hallmark of many NDDs [12,13].

As we now view chromatin regulators as general influencers of transcription, an essential aspect of chromatin regulation is relatively underappreciated in the NDD research, that is, chromatin's roles in cellular identity. Decades of research in model organisms across taxa have made it clear that chromatin regulation is central to the generation and maintenance of Waddington's 'epigenetic landscape' [14]. For example, histone methyltransferases were first discovered as the maintenance mechanism of discrete *Hox* gene expression that determines body segment identity [15]. Forward genetic screens in fruit flies revealed *Polycomb* blocks ectopic expression of posterior *Hox* genes in anterior segments [16], and *trithorax* was identified as being antagonistic to *Polycomb* soon after [17]. It is now known Polycomb group proteins place repressive histone 3 lysine 27 (H3K27) methylation or H2A lysine 119 (H2AK119) ubiquitination, whereas trithorax group proteins install activating histone 3 lysine 4 (H3K4) methylation [18–21]. While these chromatin modifiers were originally thought to be the factors that initiate the development of positional body segment identity, later studies indicated they instead act as maintenance factors [22,23].

Indeed, there are well-documented cases of chromatin-linked NDDs with cellular identity defects within the same cell lineage, such as alterations in neuron–glia transition [24] or positional neuronal identity [25]. Excellent reviews discuss these cellular identity deficits between brain cell types [26–29]. However, gene expression studies of NDD models often find differentially expressed genes that are not straightforward to postulate their impacts. In some cases, the mutant brain exhibits tissue-specific mRNAs normally absent in the brain [30–35]. Such ectopic gene expression suggests a fundamental impairment of cellular and even tissue identity, yet this phenomenon has mostly evaded experimental scrutiny to determine its consequences. This review will focus on such gross deviations in cellular identity, namely, the ectopic expression of germline genes in the brain.

Germline genes are those highly expressed in the germ cells, that is, sperm and eggs, within the testis and ovary and are absent or lowly expressed in somatic tissues—including the brain. We found several mouse models of human NDDs exhibiting ectopic expression of germline genes in the brain through a literature survey. Although these models display neurological impairments reminiscent of human NDDs, the expression of neuron-specific genes is largely preserved. The genes responsible for these neurodevelopmental conditions encode chromatin regulators involved in gene silencing. Thus, silencing germline genes in somatic cells is an inherent mission of these chromatin regulators, and ectopic germline gene expression can define a subset of chromatin-linked NDDs. Furthermore, the expression of germline genes in the brain suggests neurons lacking these epigenetic regulators fail to fully distinguish between neuronal and germline identity during development (Fig. 1). As a result, this partial soma-to-germline transformation may impair neuronal development and function.

This perspective article aims to define a group of neurodevelopmental conditions that exhibit germline gene expression in somatic cells and discuss experimental approaches to address outstanding questions. This largely unexplored NDD research area may reveal novel disease pathology, opening new avenues for diagnostics and therapeutics.

Chromatin-linked NDDs with ectopic germline gene expression in the brain

We initially recognized ectopic germline gene expression in the RNA-seq data generated from mutant mouse brains deficient for a histone H3K4 demethylase, KDM5C [30,31,33]. This unexpected observation motivated us to search the literature to find other cases. As a result, we found four monogenic NDD conditions involving ectopic germline gene expression (Table 1). These are immunodeficiency, centromeric instability, facial anomalies syndrome 1 (ICF1, MIM:

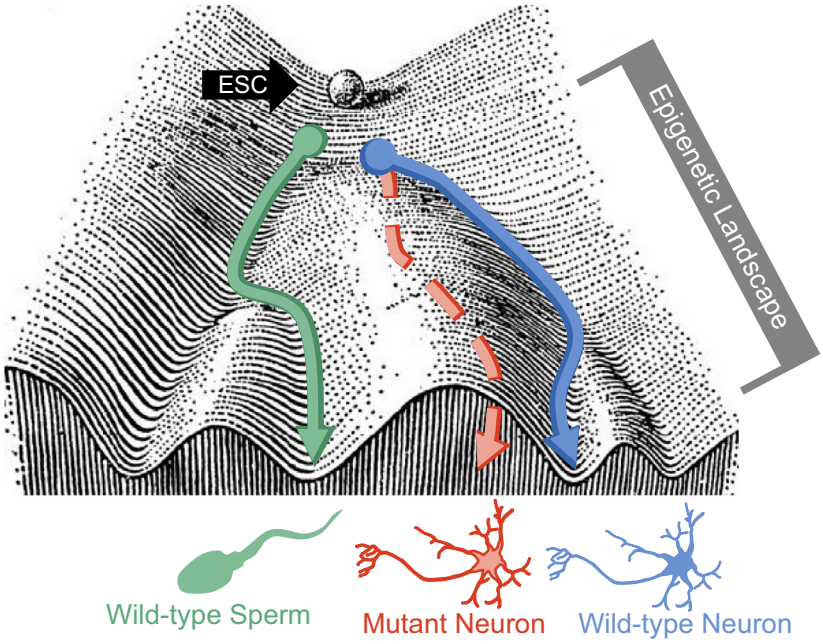

Fig 1. Visualizing cellular identity defects in chromatin-linked neurodevelopmental disorders through Waddington's landscape. Waddington's landscape depicts how a cell containing a single genome can develop into a variety of phenotypes. As a pluripotent ESC differentiates, the epigenetic landscape influences what genes are expressed and what type of cell it ultimately becomes. For example, cells traveling down the green path on the left would become sperm, while cells traveling down the blue path on the right become neurons. Ectopic expression of germline genes in the brain with loss of these chromatin regulators suggests that there is a shift in the epigenetic environment, resulting in a failure to fully establish neuronal identity and a shift toward a germ cell fate (red path).

#242860); Kleefstra syndrome 1 (MIM: #610253); MeCP2 duplication syndrome (MDS, MIM: #300260); and mental retardation, X-linked syndromic, Claes–Jensen type (MRXSCJ, MIM: #300534). The responsible genes for these conditions all encode chromatin regulators that primarily silence gene expression; DNA methyltransferase 3b (DNMT3B), H3K9me1/2 methyltransferases G9A/GLP, methyl-CpG-binding protein 2 (MECP2), and lysine demethylase 5C (KDM5C). Thus, the illegitimate expression of germline genes defines a unique subset of chromatin-linked NDDs.

Of note, we define the 'germline genes' discussed in this review as transcripts that are heavily enriched in germ cells with little to no expression outside the testis or ovary in adulthood. While some of these derepressed germline genes are exclusively expressed in germ cells (e.g., *Mvh* and *Dazl*), others are lowly expressed in somatic tissues but have overwhelmingly biased expression toward the adult gonads (e.g., *Spag6* and *Dnah1*) [36–38]. Additionally, the translation and function of almost all discussed genes have been solely investigated in the context of the germline. Altogether, the impact of these germline transcripts on somatic tissues such as the brain is ultimately unknown.

DNMT3B

In mammalian genomes, CpG-dense DNA segments, referred to as CpG islands, signify developmental and housekeeping gene promoters [39]. Cytosines within CpG islands are rarely methylated [40,41]. However, germline genes are an exception and are highly methylated in nongermline tissues [40,41]. DNA methyltransferase 3 beta (DNMT3B) is the *de novo* CpG methylation enzyme responsible for germline gene methylation. Loss-of-function mutations in *DNMT3B* cause immunodeficiency, centromeric instability, facial anomalies syndrome 1 (ICF1) [42]. Phenotypes of ICF1 are highly heterogeneous with varying degrees of penetrance but commonly include facial dysmorphism, recurrent infections, and intellectual disability [43,44]. Mouse models of ICF syndrome have ectopic germline gene expression throughout the body, including the brain [34]. Loss of CpG methylation at the promoters of these ectopic germline genes suggests their expression is a direct result of impaired DNMT3B enzymatic activity [34]. Blood cells from ICF1 patients display hypomethylation and ectopic transcription of several genes involved in meiosis, such as MAELSTROM (*MAEL*), a repressor of transposable elements, and synaptonemal complex central element protein 1 (*SYCE1*), a meiosis-specific structural protein within the synaptonemal complex [45]. Thus, ectopic germline genes are a key phenotype of both humans and murine models of ICF1. Additionally, the work on ICF1 patient blood pioneers the concept that germline gene expression can be used as a diagnostic marker.

MeCP2

Once methylated, CpG dinucleotides are recognized by reader proteins to transmit the epigenetic information

Table 1. Chromatin-linked neurodevelopmental disorders that display ectopic transcription of germline genes in the brain.

Gene name	Alternative names	Major chromatin function	Associated NDD(s)	Reports of ectopic germline genes in brain	Example germline genes
Dnmt3b (DNA methyltransferase 3 beta)	*Icf1*	*De novo* DNA CpG methylation	Immunodeficiency, centromeric instability, facial anomalies syndrome 1 (ICF1)	Velasco *et al.* [34]	*Mvh (Ddx4), Tex11, Mael, Syce1*
Mecp2 (methyl-CpG-binding protein 2)	*Autsx3*	Binds methylated CpG DNA	Rett syndrome (RTT) MeCP2 duplication syndrome (MDS)	Ben-Shachar *et al.* [32] Samaco *et al.* [35]	*Spag6, Spata1*
G9a/Glp (G9a and G9a like protein)	*Ehmt2/ Ehmt1*	H3K9me1/2 methyltransferase	Kleefstra syndrome 1	Schaefer *et al.* [64]	*Dnah1, Dazl, Spag6*
Kdm5c (lysine demethylase 5c)	*Smcx, Jarid1c*	H3K4me2/3 demethylase	Mental retardation, X-linked syndromic, Claes–Jensen type (MRXSCJ)	Iwase *et al.* [30] Scandaglia *et al.* [33] Vallianatos *et al.* [31]	*Tex14, Cyct, Mvh (Ddx4), Dnah1, Rnf17, D1Pas1, Spag16*

into gene silencing. Methyl-CpG-binding protein 2 (MeCP2) belongs to a family of such reader proteins [46]. Loss-of-function mutations in the X-linked *MeCP2* cause Rett syndrome (RTT), while duplications and overexpression of *MeCP2* result in MeCP2 duplication syndrome (MDS) [47–49]. RTT and MDS share similar neurological phenotypes, such as developmental delay, intellectual disability, and autistic features [47–49]. Of note, more recently, MeCP2 has been shown to recognize non-CpG methylation in the brain, namely CpA methylation (mCAC) [50–53]. Importantly, selective loss of MeCP2's ability to bind mCAC recapitulates RTT phenotypes and therefore appears to be a crucial function of MeCP2 [54]. MeCP2 has been classically viewed as a transcriptional repressor, given that CpG methylation is inhibitory to transcription. However, RTT and MDS mouse models suggest MECP2 can act as either an activator or suppressor for gene expression [55–57]. One study comparing gene expression between MDS and RTT mouse models found some germline genes appear to be activated by MeCP2 based on the gene ontology terms such as 'sexual reproduction' and 'reproduction' [32]. MeCP2-induced genes from another microarray study on the amygdala of the MDS mice also contain germline genes, such as sperm-associated antigen 6 (*Spag6*) and spermatogenesis-associated 1 (*Spata1*) [35]. Thus, albeit counterintuitive, a higher MeCP2 dose can cause illegitimate expression of germline genes in the brain with unknown mechanisms.

G9a/GLP

Histone 3 lysine 9 methylation is a repressive chromatin modification that can silence gene expression in concert with CpG methylation [58]. G9a and G9a-like protein (G9a/GLP) dimerize to catalyze H3K9 mono- and dimethylation (H3K9me1/2) [59,60]. The G9a/GLP heteromeric complex is responsible for installing the bulk of H3K9me1/2 at euchromatic regions [61]. The heterozygous loss of GLP (*EHMT1*) was found to explain a neurodevelopmental disorder, Kleefstra syndrome 1 [62,63]. Kleefstra syndrome 1 patients display severe intellectual disability, hypotonia, and epilepsy [62,63]. In mice, conditional deletion of the enzymatic domain of G9a or GLP in postnatal, excitatory forebrain neurons results in the ectopic expression of many non-neuronal genes in the hippocampus, striatum, and hypothalamus [64]. This study found genes unique to the liver and muscular system were the most robustly dysregulated across brain regions but also consistently identified dysregulated germline genes, such as *Dazl*—a key factor for developing germline identity and initiating meiosis [64–67].

KDM5C

In contrast to CpG and H3K9 methylation, H3K4 methylation decorates transcriptionally engaged regulatory regions [68]. Lysine demethylase 5c (KDM5C) is an eraser enzyme for H3K4 di- and trimethylation (H3K4me2/3), marks enriched at active gene promoters [69–71]. Loss of *KDM5C* results in mental retardation, X-linked syndromic, Claes–Jensen type (MRXSCJ), a neurodevelopmental disorder with intellectual disability, aberrant aggression, and autistic features [72,73]. *Kdm5c* knockout (-KO) mice recapitulate behavioral and morphological phenotypes seen in human patients, thereby serving as a model of MRXSCJ [30,33]. The seminal work on fruit fly KDM5 demonstrates this enzyme

family's evolutionary-conserved roles in brain development and function [74–80]. Multiple studies in the mouse model have identified ectopically expressed germline genes in the *Kdm5c*-KO amygdala, prefrontal cortex, and hippocampus [30,31,33]. Some germline genes identified across these mouse studies include a testis-specific RNA helicase (*D1Pas1*) and a testis-specific cytochrome C (*Cyct*) [30,31,33]. These germline gene promoters appear to lack CpG methylation in the *Kdm5c*-KO hippocampus. H3K4me removal therefore appears to be a requisite for DNMT3B-mediated DNA methylation [33], highlighting a mechanistic link between the chromatin regulators involved in faithful germline gene silencing.

In summary, these four genes represent a group of NDDs with unique germline gene derepression in the brain. These four chromatin regulators appear to be linked mechanistically in generating a repressive chromatin environment to prevent ectopic expression of germline genes, yet their detailed molecular interplay remains elusive. Furthermore, derepression of germline genes is unlikely to be unique to these four NDDs because numerous chromatin modifiers act in concert to regulate higher-order chromatin structure.

Many outstanding questions remain unanswered. When in development are germline genes derepressed? What are the molecular mechanisms for the derepression? Does the ectopic germline gene expression contribute to defective neural development and functions? If so, how? Investigating these questions will provide insight into whether and how ectopic germline genes contribute to NDD pathology and ultimately dictate how feasible it is to develop novel diagnostics and therapeutics around this phenotype. We explore these questions below.

The developmental time window for germline gene silencing

Currently, the dynamics behind ectopic transcription of germline genes are poorly understood. During early embryogenesis, the somatic and germline trajectories are distinguished soon after embryonic stem cells (ESCs) of the inner cell mass differentiate into epiblast stem cells [81,82]. By approximately embryonic day 6.5 (E6.5) in mice, epiblast cells proximal to the extra-embryonic ectoderm receive bone morphogenetic protein signals, such as BMP4, that initiate their development into primordial germ cells (PGCs) that will eventually form the entire germline [81,82]. Many crucial chromatin changes occur during this developmental window to shape the embryonic transcriptome and distinguish somatic cells from germ cells, including silencing germline genes [83]. Thus, these chromatin regulators may be required during embryogenesis to establish this soma–germline boundary.

Some of the chromatin regulators discussed above appear to participate in the early demarcation of somatic and germline identity. *Dnmt3b*-deficient mouse embryos exhibit hypomethylated germline gene promoters and ectopic transcription of germline genes as early as E9.5 [84]. Consequently, adult *Dnmt3b*-deficient mice show germline gene expression in many somatic tissues [34]. By E6.5, *G9a* mutant embryos ectopically express germline genes such as *Sycp2* and *Bmp4,* which also lose H3K9me2 [85]. Additionally, ESCs lacking G9a or GLP express melanoma-associated antigen-A (*Mage-a*), a family of germline genes [59,60]. The presence of ectopic germline transcripts in such early stages indicates their expression in the mature brain is due to a failure to decommission germline genes during embryogenesis.

Alternatively, these chromatin regulators could act like Drosophila's *Polycomb* (discussed above) and maintain cellular identity by continuously suppressing germline genes throughout life. In support of this, deletion of GLP and G9a only in adult neurons still derepresses liver and germline genes [64]. In contrast to the continuous requirement of G9a/GLP, loss of KDM5C in mature cortical neuron cultures does not derepress the germline genes found in the constitutive *Kdm5c*-KO brain [33]. Furthermore, re-expression of KDM5C in mature *Kdm5c*-KO neurons does not restore germline gene silencing, suggesting KDM5C is only necessary for silencing during development [33]. These contrasting observations suggest that some chromatin regulators may have a stage-specific role in repressing germline genes.

Molecular machinery of germline gene silencing

As discussed, the four genes associated with illegitimate germline gene expression appear mechanistically linked. DNMT3B and MeCP2 are a writer and reader of DNA methylation, while G9a and KDM5C can exist in the same protein complex [34,71,86]. CpG methylation of germline gene promoters seems to require KDM5C-mediated H3K4me removal [33]. Thus, an emerging model of germline gene decommissioning is that KDM5C and G9a/GLP establish the repressive histone modification environment, which in turn recruits DNMT3B for stable silencing (Fig. 2). The interplay and order of action between KDM5C and G9a/GLP remain to be determined. Additionally, given that the above studies found

MeCP2 duplication activates germline gene expression, MECP2 may act independently from the repressive GLP/G9a, DNMT3B, and KDM5C.

However, these prior studies do not address how the four factors are specifically recruited to target germline gene promoters. One candidate is the polycomb repressive complex 1.6 (PRC1.6). PRC1.6 is a noncanonical PRC1 complex that contains PCGF6 and harbors H2AK119 monoubiquitination activity catalyzed by RING1A and RING1B [87,88]. PRC1.6 also contains sequence-specific DNA-binding factors, MAX-MGA and E2F6-DP1 heterodimers [86,89–91]. An shRNA screening for silencers of a germline gene reporter identified MAX as a crucial germline gene repressor in ESCs [92]. Subsequent work demonstrated that these DNA-binding factors are essential for recruiting the PRC1.6 components to germline genes [86,89–91]. Independent line of investigations uncovered the requirement of E2F6 in germline silencing [34,93,94]. Importantly, PRC1.6 components are known interaction partners for DNMT3B, G9a/GLP, and KDM5C [34,71,86]. This is supported by a recent study that unexpectedly found no significant changes in H2AK119ub1 at derepressed germline gene promoters of *Pcgf6*-KO ESCs, suggesting that this mark is dispensable for germline gene silencing [86]. Notably, these germline gene promoters did lose G9a/GLP binding and H3K9me1/2, indicating G9a/GLP may be a key component enabling PRC1.6-mediated repression [86]. PRC1.6 therefore can be seen as a central axis governing germline gene silencing by these chromatin regulators in somatic cells (Fig. 2).

However, PRC1.6 is not the only complex reported to suppress germline genes. In fruit flies and worms, a chromatin-regulatory complex called Drosophila RBF, E2F, and Myb (dREAM) or Myb-MuvB (MMB) is crucial for repressing germline transcription in somatic cells [95]. The dREAM/MMB complex consists of retinoblastoma (Rb) proteins, E2F transcription factors (E2F1-E2F4), and a family of transcriptional cofactors [96–99]. Though classically known as cell cycle regulators, E2F and Rb exert their additional roles in silencing germline genes within the dREAM/MMB complex [95]. Loss of *Rb* homologs in *Caenorhabditis elegans* results in a somatic expression of genes unique to P granules, a structure of RNA and protein only found in the germline [100,101]. Somatic cells of *Rb* mutants also displayed germline-like features such as enhanced RNA interference (RNAi) activity [100]. Interestingly, they found RNAi activity was especially enhanced in neurons, perhaps suggesting neurons may be more sensitive to a soma-to-germline transformation [100]. Loss of

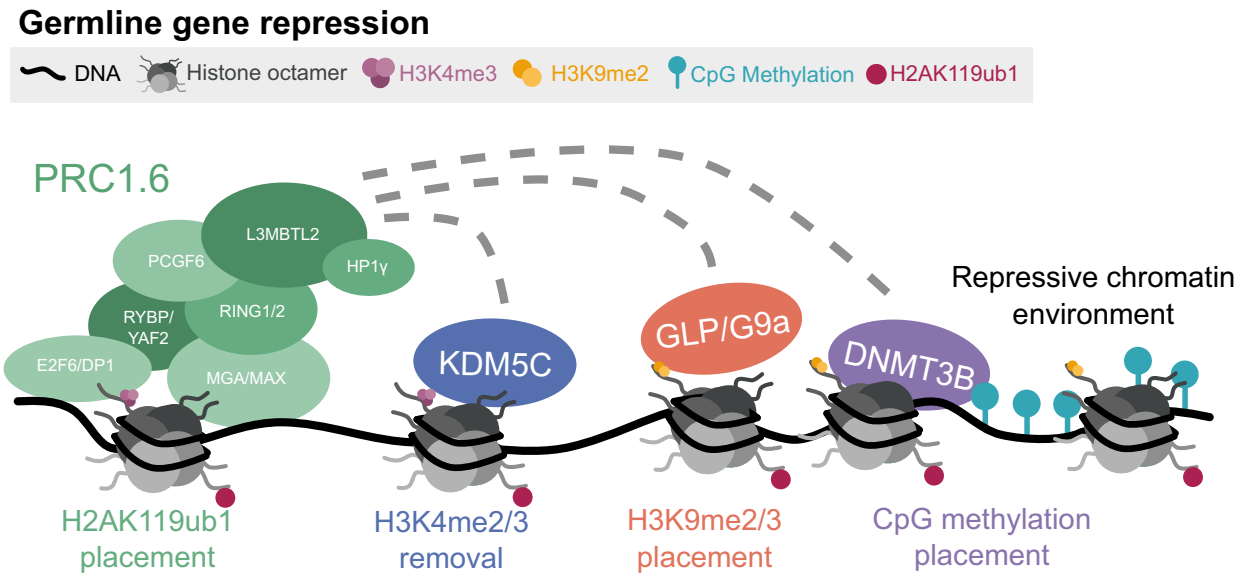

Fig 2. Model for cooperative repression of germline genes through PRC1.6, KDM5C, GLP/G9a, and DNMT3B. PRC1.6 (polycomb repressive complex 1.6) binds to DNA through two heterodimers, E2F6/DP1 (E2F transcription factor 6, transcription factor Dp-1) and MGA/MAX (MAX dimerization protein MGA, MYC-associated factor X) and places histone 2A lysine 119 monoubiquitination (H2AK119ub1). KDM5C (lysine demethylase 5C) removes activating histone 3 lysine 4 di- and trimethylation (H3K4me2/3). G9a and G9a-like protein (G9a/GLP) dimerize to place repressive H3K9 mono- and dimethylation (H3K9me1/2). DNMT3B (DNA methyltransferase 3 beta) likely binds once H3K4 is unmethylated to place the final repressive CpG methylation. PRC1.6 may facilitate the recruitment of KDM5C, GLP/G9a, and DNMT3B to germline genes through its DNA-binding heterodimers (dashed lines).

Drosophila lethal (3) malignant brain tumor (*l(3)mbt*), a substoichiometric component of dREAM/MMB, results in brain tumors that express the germline proteins PIWI, VASA, and AUB that promote tumor growth [102].

What is the relationship between PRC1.6 and dREAM/MMB complexes? A homologous complex to dREAM/MMB, referred to as DP, RB-like, E2F, and MuvB (DREAM), is present in human cells [98]. PRC1.6 and DREAM consist of related yet distinct components. While E2F6 is unique to PRC1.6, E2F1-4 are the DNA sequence recognition components of dREAM/MMB. Likewise, L3MBTL2 is a PRC1.6 member, whereas L3MBTL participates in dREAM/MMB [87,88,98]. In contrast to the dREAM/MMB's evolutionary conservation, PRC1.6 is unique to vertebrates [103]. Hence, dREAM/MMB is presumably the ancient form that suppresses both cell cycle genes and developmental genes, and gene duplications led to the emergence of PRC1.6 assigned with specialized function in germline gene silencing of vertebrates. Whether or not the vertebrate DREAM complex retains function in germline gene silencing remains to be determined. Broad cancer types frequently include loss of Rb and ectopic expression of germline genes [104], suggesting DREAM may still have a role in vertebrate germline gene silencing. Though it is not a germline gene, the oncogene APOBEC3B is cooperatively suppressed by DREAM/MMB and PRC1.6 [105]. Thus, the two vertebrate complexes could cooperate for complete germline gene silencing. However, DNMT3B, G9a/GLP, KDM5C, and MECP2 are not currently known to associate with dREAM/MMB [98,106]. Future study is warranted to determine whether distinct molecular machinery operates germline gene silencing. Lastly, based on these mechanistic insights, we postulate that human genetic screens may identify additional NDDs associated with components of PRC1.6 and possibly DREAM complexes with soma-to-germline transformation.

Potential neurodevelopmental consequences of ectopic germline genes

'Structure dictates function' is a foundational principle of biology that also holds true for the chromatin landscape. Chromatin marks across the genome influence which genes are transcribed, ultimately determining what tasks a cell can perform and how a cell responds to external stimuli [107,108]. Germ cells have a highly specialized transcriptome because of their unique functions, including maintaining immortality in spermatogonial stem cells, the reorganization of genetic material in meiosis, and the motility of sperm in a foreign environment [109,110]. Ectopic transcription of germline genes with the loss of the above chromatin regulators suggests the chromatin landscape in these disorders has fundamentally shifted toward a germline fate (Fig. 1). Therefore, this deviation in chromatin structure opens the possibility of altered functions in cells that aberrantly express germline genes. While the vast majority of germline genes have never been studied in the context of neurodevelopment, some studies have investigated the consequences of germline genes outside of their traditional context. The studies described below illuminate the potential avenues by which ectopic germline transcription can disrupt neurodevelopment and contribute to NDDs.

Meiosis and genomic instability

A subset of germline genes transcribed with loss of the chromatin regulators above are unique to meiotic structures. Meiosis is a fundamental process of germ cells to prepare the genome for fertilization. Meiosis begins with meiotic recombination in which homologous chromosomes exchange genetic material during prophase 1 [111,112]. Meiotic recombination includes the following distinct stages: (a) leptotene—the duplicated chromosomes condense and associate with the nuclear envelope. The first programmed double-strand breaks (DSBs) are formed; (b) zygotene—the number of DSBs increase to promote homologous pairing. Synapsis begins, and the synaptonemal complex forms between homologous chromosomes; (c) pachytene—the synaptonemal complex is fully formed and crossing over occurs; and (d) diplotene—the synaptonemal complex disassembles [111,112]. After meiotic recombination, cells undergo a reductional division that separates homologous chromosomes. In germ cells, the master regulators *Dazl* and *Stra8* activate the meiotic gene expression program (Fig. 3, left) [65,66,113]. Some studies have found *Dazl, Stra8*, and their downstream targets—*Rec8, Sycp1, Sycp2,* and *Sycp3*—are ectopically expressed in embryos lacking the above chromatin regulators [84]. Could the ectopic expression of these master regulators trigger meiosis outside of the germline?

One study investigating Myc-associated factor X (MAX) suggests that at least part of the meiotic progression can occur in mouse ESCs. MAX is a transcription factor that dimerizes with Max-gene associated protein (MGA) in the complex PRC1.6 discussed above [89]. *Max*-null ESCs express germline genes—including the meiosis-specific genes *Stra8*, *Dazl*, *Rec8,* and *Sycp3* [114]. Even in stringent culture conditions to maintain ESC

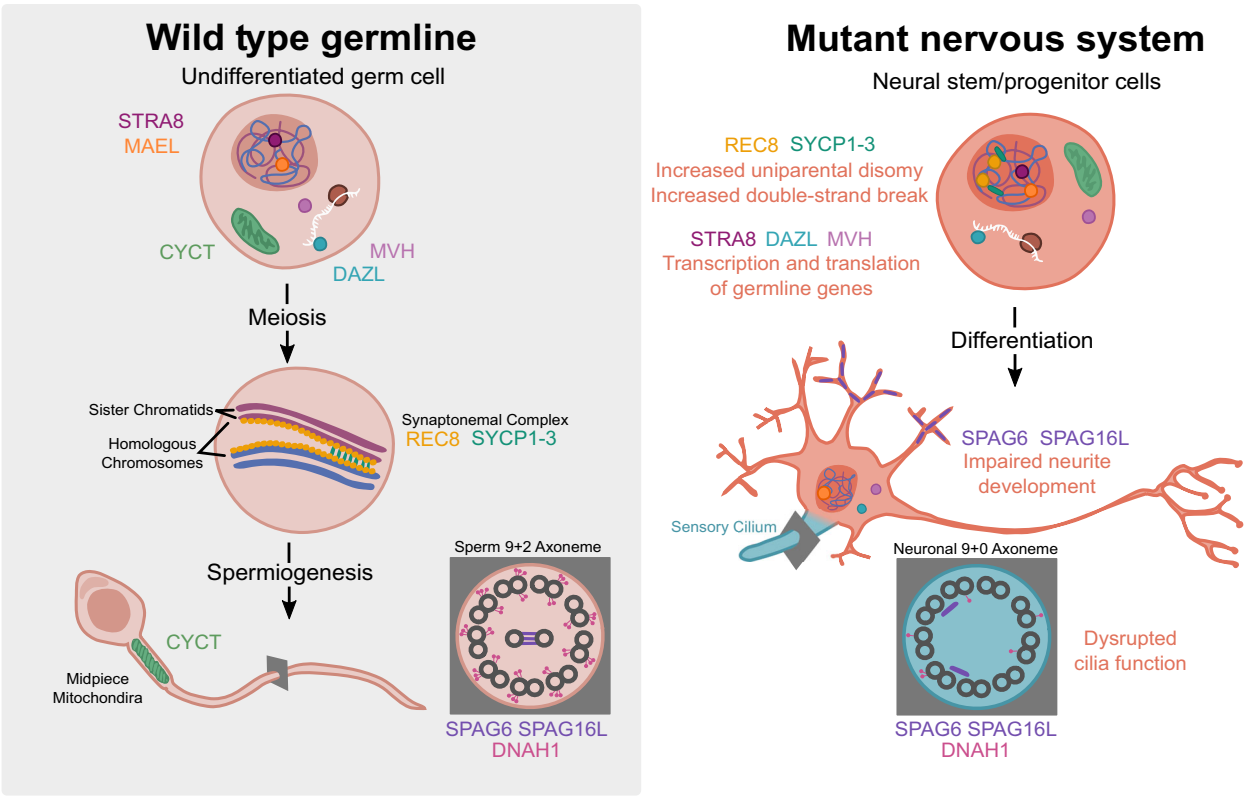

Fig 3. Functions of germline genes in the wild-type germline and the potential consequences when ectopically expressed in mutant neurons. Left panel: Wild-type germ cells express RNA-binding proteins DAZL (deleted in azoospermia like) and MVH (mammalian vasa homologue) and the transcription factor STRA8 (stimulated by retinoic acid 8), which promote the transcription and translation of other germline genes. Germ cells also express MAEL (maelstrom), which represses transposable elements. During meiosis, REC8 (REC8 meiotic recombination protein) and SYCP1-3 (synaptonemal complex protein 1–3) form the synaptonemal complex for homologous recombination. Mature spermatozoa are highly specialized for motility and therefore contain many mitochondria, as well as a motile 9 + 2 axoneme. Sperm express CYCT (cytochrome C, testis) in the mitochondria, DNAH1 (dynein axonemal heavy chain 1) in dynein motors, SPAG6 (sperm-associated antigen 6), and SPAG16L (sperm-associated antigen 16, long isoform) in the central pair of microtubules. Dynein motors within the sperm axoneme bind to microtubules to generate the force for the flagellar beating. Right panel: Ectopic expression of STRA8, DAZL, and MVH in developing and mature neurons likely promotes aberrant transcription and translation of germline genes. Expression REC8 and SYCP1-3 in developing neurons can result in genomic instability by increasing the frequency of uniparental disomy and double-strand break during mitosis. Overexpression of SPAG6 impairs dendritic development, and incorporation of SPAG6, SPAG16L, and DNAH1 in the neuronal axoneme could disrupt the development and function of neuronal cilia.

identity, *Max*-null ESCs displayed leptotene and zygotene-like SYCP3 staining [114]. The aberrant presence of leptotene- and zygotene-like morphology, the increase in a DSB marker γH2AX, and the rise in apoptosis indicate *Max*-null ESCs enter an anomalous meiosis-like state. Furthermore, double loss of *Max* with *Stra8* rescues both meiotic morphology and cell survival [114]. This study ultimately demonstrates not only can the meiotic program be activated in somatic cells, but also modulating a master regulator of meiosis can mitigate the deleterious effect of the ectopic meiotic program.

The presence of meiotic machinery can also interfere with mitotic chromosome segregation. Such an example has been demonstrated in fission yeast, in which expression of meiotic factors in their mitotic phase causes uniparental disomy (UPD) [115], a form of genomic instability in which homologous chromosomes from only one parent are inherited to a daughter cell. In this study, using a UPD reporter system, the authors found that the loss of *mmi1*, a component of the RNAi machinery, led to ectopic expression of meiotic genes and an increased rate of UPD [115]. Because RNAi also regulates pericentromeric heterochromatin formation, impaired centromeric integrity could be responsible for the UPD [116,117]. However, they found that deleting *rec8*, one of the ectopically expressed meiotic genes, suppressed UPD in the RNAi-deficient cells. Importantly, overexpression of

rec8 alone was sufficient to drive UPD in wild-type cells [115]. In mammalian germ cells, REC8 expression is induced by DAZL and drives the formation of the meiosis-specific synaptonemal complex [65,113,118]. Thus, it is plausible that ectopic germline gene expression in early embryogenesis leads to chromosomal instability, including UPD in mammals. UPD underlies many NDDs in humans due to the consequential loss of imprinted genes, illuminating a potential avenue by which ectopic germline transcripts may impair neuronal function [119].

Ectopic expression of the meiotic machinery can also be coupled with changes in the chromatin landscape to promote genomic instability. For example, H3K4me3, a substrate of KDM5C, is typically enriched in active gene promoters [70,120,121]. During meiosis, colocalization of H3K4me3 with H3K36me3—a mark typically found in the gene bodies of actively transcribed genes—serves as a platform for DSB machinery recruitment [122]. Because loss of KDM5C increases the number and width of H3K4me3 peaks [30,31], this might produce aberrant colocalization of H3K4me3 and H3K36me3, which in turn results in ectopic DSB formation.

Genomic instability caused by ectopic meiotic genes, such as UPD and DSBs, can generate somatic mosaicism. Somatic mosaicism renders a population of cells genetically distinct from the rest of the body and is associated with many NDDs, such as autism spectrum disorder and intellectual disability [123]. Synaptic genes are particularly vulnerable to somatic mutations because they are, on average, longer than other genes [124,125]. Thus, the brain would be particularly sensitive to genomic instability arising from ectopic meiotic genes, potentially linking ectopic germline genes and NDD phenotypes (Fig. 3, right).

Cilia and flagella

Another major category of genes ectopically expressed in these chromatin-linked NDDs is cilia and sperm flagellar genes (Table 1). The sperm flagellum is a specialized motile cilium that enables the sperm to reach the oocyte. In contrast, neurons and astrocytes do have a cilium, it is instead a nonmotile primary sensory cilium [126,127]. Both motile and nonmotile cilia have a core structure called the axoneme, which consists of an outer ring of nine microtubule pairs [128]. Like most motile cilia, the sperm flagellum has a 9 + 2 axoneme, which contains an additional central pair of microtubules, as well as dynein motors to generate the force to beat (Fig. 3, left) [128,129]. The sensory cilia of neurons and astrocytes instead have a 9 + 0 axoneme with no dynein motors and are therefore nonmotile [126,127]. These nonmotile cilia are often dubbed 'the cell's antenna' as they act as a platform for sensing regulatory molecules that influence development, such as sonic hedgehog signaling [126,127]. Genes encoding motile cilium proteins generally have an expression pattern heavily biased toward the testes due to the high concentration of spermatozoa. Aside from sperm, one of the few other cells with motile cilia in mammals is ependymal cells lining the brain ventricles that regulate the flow of cerebrospinal fluid [128].

What could be the impact of ectopic sperm flagellar genes on neuronal cilia and the developing brain? Given that the signaling cascades promoting flagellar beating are highly complex and intricately regulated [128,130], it is unlikely that a handful of ectopic flagellar proteins in neurons would induce motility in neuronal sensory cilia. However, the shared basic structure of motile and nonmotile cilia does open the possibility of ectopic flagellar proteins being incorporated into the neuronal cilium axoneme and impairing their sensory functions. In support of this, a study in zebrafish found overexpression of *Foxj1*, a master transcription factor for motile ciliogenesis, induced the formation of ectopic motile-like cilia with a 9 + 2 axoneme in cells that normally equip sensory cilia [131].

Foxj1 and dynein genes for motile cilia, such as *Dnah1,* are overexpressed in the brain of KDM5C- or GLP/G9a-deficient mice [130,132]. Because these studies employ bulk mRNA-seq, it is currently unclear whether cells with nonmotile cilia—that is, neurons or astrocytes—ectopically express these motile cilium genes or whether the ependymal cells elevate their expression outside normal levels. If these motile cilium genes are expressed in neurons or astrocytes, they may be aberrantly incorporated into neuronal sensory cilia and thereby impair their developmental functions (Fig. 3, right). Since impaired development of both motile and nonmotile cilia is linked to many neurodevelopmental phenotypes, such as intellectual disability and microcephaly [133], further investigation on these genes might provide insight into the mechanisms of KDM5C and GLP/G9a deficiencies.

Interestingly, some motile cilium genes, such as *Spag6* and *Spag16*, are expressed in wild-type neurons at a low level [38]. In sperm flagella, SPAG6 and the long isoform of SPAG16 (SPAG16L) associate with each other and the central microtubules of the 9 + 2 axoneme [134,135]. While expression of SPAG6 and SPAG16L is greatly biased toward the testis, one study surprisingly detected their proteins in the cytoplasm of wild-type neurons [38]. The short isoform of SPAG16 (SPAG16S) was originally thought to be exclusively

expressed in male germ cell nuclei and to induce the transcription of *Spag16L* and other male-specific germline genes [136,137]. However, SPAG16S was also found in wild-type hippocampal neuronal nuclei, suggesting it may also have a transcriptional role in the brain although the targets are unknown [38]. While the function of *Spag6* and *Spag16* in the wild-type brain is currently unclear, some studies found overexpression of *Spag6* in developing cortical neurons slows neuronal migration and decreases the number and length of neurites [138,139]. These findings led to the hypothesis that, similar to its function in sperm, SPAG6 regulates microtubule stability in neurons. Some NDD models that overexpress *Spag6* or *Spag16*, such as MRXSCJ, also display decreased dendritic complexity, illuminating a potential impact of ectopic flagellar factors in NDD pathology (Fig. 3, right) [30,31].

Routes to test the impact of ectopic germline transcription

In this review, we hypothesize that the ectopic expression of germline genes in the brain impairs neurodevelopment. However, germline genes are not the only dysregulated genes observed in these chromatin modifier mutants. For example, models of these NDDs also show dysregulation of some synaptic genes, such as GABA-A receptor subunits and voltage-gated potassium channels [7,31–33,64]. Therefore, to test this hypothesis, experiments must isolate the impact of germline gene dysregulation in the brain.

Two major experiments [140] should be done to test whether ectopic expression of germline genes is necessary and sufficient to impair neurodevelopment in chromatin-linked NDDs. The first experiment tests the necessity by selectively preventing the ectopic germline gene expression and then assessing whether it restores normal neurodevelopment in the chromatin modifier mutants. The second experiment tests the sufficiency by overexpressing only germline genes in the brain and examining whether it phenocopies chromatin regulator mutants.

Two approaches can test the necessity of germline genes for neuronal impairments. Firstly, one can ablate individual germline genes with predicted harmful outcomes—such as meiotic or sperm flagellar genes—in chromatin regulator mutants and test whether this ameliorates their neuronal impairments. However, this approach is limited to testing the impact of only one gene. To test the necessity of germline genes as a whole, one can instead target upstream master regulators of germline transcription and translation to blunt the ectopic expression of multiple germline genes at once. For example, *Dazl, Stra8*, and their downstream targets—for example, *Rec8, Sycp1, Sycp2, Sycp3*—are ectopically expressed in the brain [64] and early embryos [84] of some chromatin regulator mutants. DAZL and STRA8 initiate the cascade of germline-specific transcription and translation crucial for germ cell identity and meiosis and are also known to regulate the expression of many ectopic germline genes found in the chromatin regulator mutant brains [36,65,66,113,141,142]. Thus, deletion of these master regulators during development should blunt the aberrantly expressed germline and meiotic programs in the NDD mouse models. This approach to block global ectopic germline transcription is known to be effective in *Max*-null ESCs, in which additional *Stra8* loss decreased expression of meiotic proteins, rescued cell survival, and improved genomic stability [114].

There are also two experimental avenues to test whether ectopic expression of germline genes is sufficient to impair neurodevelopment. Using the same principles discussed above, one can mimic germline gene dysregulation by overexpressing either individual genes of interest or master regulators of germline gene expression in the wild-type brain. An alternative approach is to instead generate chromatin regulator mutants that are only deficient for their ability to silence germline genes while preserving their other functions. This can be accomplished by point mutations that selectively interfere with recruiting the chromatin regulator to germline genes. However, a major challenge of this approach is that how these chromatin regulators are recruited to germline genes is poorly understood as they do not intrinsically possess sequence-specific binding. As discussed above, one promising recruiting candidate is PRC1.6, a repressive complex that binds to germline genes by two heterodimeric DNA-binding factors, MAX/MGA and E2F6/DP1 [89,91]. Currently, some studies suggest DNMT3B, G9a/GLP, and KDM5C associate with PRC1.6 members [34,71,86]. For example, cells lacking E2F6 also lose DNMT3B binding at the promoters of germline genes, suggesting PRC1.6 is a DNMT3B recruiting factor for these genes [34]. At this time, more research is required to determine the exact interaction interface between PRC1.6 and these chromatin regulators to ultimately identify mutations that isolate the germline gene-suppressing function.

After generating animals or cells carrying the genetic manipulations discussed above, we can then assess their impact on behavioral and neuronal phenotypes. These neurological traits should be ameliorated in the necessity test and phenocopied in the sufficiency test

compared with the original chromatin regulator mutants. Ultimately, these studies are essential to illuminate the degree to which germline genes contribute to the neurological traits of the associated NDD.

Conclusions and perspectives

Although chromatin regulators were originally found to maintain cellular identity, brain-expressed genes have attracted the most attention when investigating chromatin-linked NDDs. Thus, the impact of derailed cellular identity during brain development remains mostly unknown. This review has discussed four examples of chromatin-linked NDDs with severe identity crises in the brain due to ectopic germline gene expression. Expression of aberrant germline transcripts in ESCs lacking these chromatin regulators indicates the onset of the gene misregulation occurs very early in the life span [84,85]. The germline gene expression in pluripotent cells implies that the ectopic expression may occur in somatic tissues broadly, not just in the brain. Indeed, this has been found to be the case for DNMT3B [34,45]. Why, then, would the brain be particularly sensitive to ectopic germline transcripts during development?

One key to this relationship might be the highly specialized structure and functions of neurons and germ cells. Interestingly, the brain and the testis are currently the only known tissues with a specific mechanism to prevent their genes' ectopic transcription. Analogous to PRC1.6's role in decommissioning germline genes, neuronal genes are actively repressed outside the nervous system by REST (RE-1 silencing transcription factor) [143–145]. The importance of decommissioning neuronal and germline genes may lie in the fact that the brain and the testes have the two most specialized transcriptomes in the body [109,110], and thus, all the other organs may suppress these germline or neuronal genes for proper tissue function. However, normal brain function may be at a greater risk compared with other organs when the brain encounters germline gene products, which impose gross deviations in neuronal identity. We discussed two such scenarios by which ectopic germline transcripts may damage neuronal development —impaired genomic stability and cilium function—and further offered a framework for experimentally isolating germline genes' contributions to NDD phenotypes.

Another commonality between the germline and brain is an immune privilege; several organs, including the testis, brain, and placenta, can evade full immune surveillance [146,147]. Immune privilege is believed to grant protection from inflammation that could permanently damage these vulnerable tissues. Since the immune system never encounters the gene products of privileged tissues, misexpression of such genes in nonprivileged tissues can elicit an uncontrolled immune response and lead to tissue destruction. The active silencing mechanisms of the brain and germline genes might have evolved to hedge this fundamental risk. There are, however, no reports of increased inflammation in nonprivileged tissues of the animal models or human patients discussed above. Immune cells in the mutant organisms might have been exposed to germline proteins throughout development and thus recognize them as a part of 'self'. Alternatively, closer examinations may reveal aberrant inflammation elicited by germline gene products. Further investigations on the ectopic expression of germline genes in NDD models may explain why unique mechanisms emerged to block soma-to-germline transformation and inform us how to improve the diagnosis and treatment of chromatin-linked NDDs.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

KMB and SI outlined and cowrote this paper. KMB prepared the figures.

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