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RESEARCH ARTICLE

Chromatin Looping Defects in Neurodevelopmental Disorders Linked to CHD8 Mutations

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ABSTRACT

CHD8 is among the highest-confidence risk genes for autism spectrum disorder, yet how heterozygous loss-of-function mutations in this ATP-dependent chromatin remodeler translate into altered three-dimensional genome architecture during human neurodevelopment remains poorly resolved. Here we generated isogenic CHD8^{-/-} human induced pluripotent stem cell lines by CRISPR-Cas9 editing and differentiated them alongside isogenic wild-type controls into dorsal forebrain cortical organoids, profiling chromatin looping by in situ Hi-C, CHD8 and CTCF occupancy by chromatin immunoprecipitation sequencing (ChIP-seq), and chromatin accessibility by ATAC-seq across three differentiation time points spanning progenitor expansion and early neurogenesis. CHD8 occupancy overlapped extensively with CTCF and cohesin subunit RAD21 at loop anchors genome-wide, and CHD8 haploinsufficiency caused measurable weakening of a defined subset of these anchors, disproportionately concentrated at loops flanking genes previously implicated in synaptic development and neuronal differentiation. Loop weakening at these anchors was accompanied by reduced insulation across the corresponding domain boundaries and altered expression of genes within the affected domains, including several additional autism-associated loci not directly bound by CHD8, consistent with indirect dysregulation through local architectural change rather than direct promoter occupancy alone. ATAC-seq revealed a parallel, partial loss of accessibility at CHD8-dependent anchors that preceded detectable expression changes during early differentiation, suggesting that architectural weakening is an early event relative to downstream transcriptional dysregulation. These findings connect CHD8 haploinsufficiency to a discrete, anchor-specific pattern of chromatin looping defects during human corticogenesis and provide a mechanistic bridge between a leading autism risk gene and altered three-dimensional regulation of neurodevelopmental gene expression programs.

Keywords: CHD8; autism spectrum disorder; chromatin remodeling; Hi-C; CTCF; topologically associating domain; cortical organoid

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1. INTRODUCTION

CHD8 encodes an ATP-dependent chromodomain helicase DNA-binding protein that remodels nucleosomes and is recurrently disrupted by de novo loss-of-function mutations in individuals with autism spectrum disorder, making it one of the single strongest genetic risk factors identified for the condition to date [1,2]. Despite this genetic prominence, the mechanistic route from CHD8 haploinsufficiency to altered neurodevelopmental gene expression has been difficult to pin down, in part because CHD8 occupies thousands of genomic sites with only modest individual effects on nearby transcript abundance, arguing against a simple model in which CHD8 acts principally as a direct transcriptional activator or repressor at its bound promoters [3,4].

An alternative route by which CHD8 could exert broad regulatory effects is through the three-dimensional folding of the genome. CHD8 has been reported to physically associate with CTCF and components of the cohesin complex, and several remodelers of the CHD and SWI/SNF families are now understood to contribute to loop-anchor stability and nucleosome positioning at boundary elements rather than acting purely at promoters [5,6]. If CHD8 similarly reinforces a subset of CTCF-cohesin loop anchors, haploinsufficiency could weaken those anchors and permit inappropriate enhancer-promoter contact or loss of insulation at domain boundaries, providing an architectural route to gene dysregulation that would not require CHD8 itself to bind the dysregulated gene directly. This model has been explored in a handful of non-neuronal cell contexts but has not, to our knowledge, been tested directly in a human neurodevelopmental system using isogenic control lines.

Human cortical organoids differentiated from induced pluripotent stem cells offer an increasingly validated platform for modeling early corticogenesis, recapitulating progenitor expansion, neurogenic transition and early cortical layer identity with a temporal fidelity that facilitates chromatin profiling across defined developmental windows [7,8]. Combining this system with isogenic CRISPR-edited CHD8 heterozygous lines removes the confound of genetic background variation that complicates comparisons across patient-derived lines, and allows loop-level chromatin architecture to be compared directly against wild-type controls at matched differentiation stages.

Here we used isogenic CHD8^{+/-} and wild-type human induced pluripotent stem cell lines, differentiated into dorsal forebrain cortical organoids, to ask whether CHD8 haploinsufficiency produces detectable, anchor-specific weakening of chromatin loops genome-wide, whether such weakening preferentially affects domains containing neurodevelopmental and synaptic genes, and how the timing of architectural change relates to downstream transcriptional dysregulation across early corticogenesis.

2. MATERIALS AND METHODS

Isogenic CHD8^{+/-} human induced pluripotent stem cell lines were generated by CRISPR-Cas9 editing of a well-characterized wild-type parental line and differentiated into dorsal forebrain cortical organoids using a dual-SMAD-inhibition protocol, with chromatin and transcriptomic profiling performed at three matched time points spanning progenitor expansion and early neurogenesis.

2.1 Generation of isogenic CHD8 heterozygous lines

A frameshift-inducing CRISPR-Cas9 guide RNA targeting exon 4 of CHD8 was delivered by nucleofection into the parental induced pluripotent stem cell line together with Cas9 ribonucleoprotein; single-cell clones carrying a heterozygous frameshift allele, confirmed by Sanger sequencing and immunoblot showing approximately 50% reduction of CHD8 protein relative to isogenic wild-type clones subjected to the same nucleofection procedure, were expanded and karyotyped prior to differentiation. Two independent CHD8^{+/-} clones were carried through all experiments alongside two isogenic wild-type control clones.

2.2 Cortical organoid differentiation

Cortical organoids were generated by embedding neural rosette-forming embryoid bodies in a basement-membrane matrix following dual SMAD inhibition, with sequential media transitions supporting dorsal forebrain progenitor identity, confirmed by immunostaining for PAX6 and FOXG1 at the progenitor time point and for TBR1 and CTIP2 at the neurogenic time point. Samples were harvested at day 25 (progenitor expansion), day 50 (neurogenic transition) and day 75 (early neuronal maturation) for all chromatin and expression assays, from three independent differentiation batches per genotype.

2.3 CHD8 and CTCF chromatin immunoprecipitation sequencing

ChIP-seq for CHD8, CTCF and RAD21 was performed on formaldehyde-crosslinked chromatin from dissociated organoids using validated antibodies, sonicated to a mean fragment size of approximately 250 bp and immunoprecipitated overnight at 4°C with protein G magnetic beads. Libraries were sequenced as 2×50 bp paired-end reads on an Illumina NovaSeq 6000 to a minimum depth of 30 million uniquely mapped read pairs per sample, alongside matched input chromatin controls, with peaks called using MACS2 against input.

2.4 In situ Hi-C and loop calling

In situ Hi-C libraries were prepared from 2-5 million cells per dissociated organoid sample following in-nucleus DpnII digestion, biotin fill-in and proximity ligation, and sequenced as 2×100 bp paired-end reads to a depth of approximately 350-450 million valid contact pairs per sample after filtering. Contact matrices were normalized with the Juicer pipeline, loops were called with HiCCUPS at 5-10 kb resolution, and domain boundary insulation

scores were computed genome-wide to allow direct comparison of anchor strength between genotypes at matched differentiation stages [9,10].

2.5 ATAC-seq chromatin accessibility profiling

ATAC-seq was performed on 50,000 nuclei per sample following the Omni-ATAC protocol, with transposed libraries sequenced as 2×50 bp paired-end reads to a minimum depth of 40 million uniquely mapped read pairs per sample after removal of mitochondrial reads and duplicates, and peaks called with MACS2 in paired-end mode.

2.6 RNA-sequencing and statistical analysis

Total RNA was extracted and strand-specific, poly(A)-selected libraries were sequenced to a minimum depth of 30 million read pairs per sample. Differential expression between CHD8+/- and wild-type organoids at each time point was assessed with DESeq2 using the Wald test and Benjamini-Hochberg correction, with genes considered significantly changed at adjusted $P < 0.05$ and $|\log_2 \text{fold-change}| > 0.585$. Loop strength differences between genotypes were assessed on 5-10 kb-binned matrices using a paired two-tailed Wilcoxon signed-rank test on normalized loop contact z-scores. All statistical analyses were performed in R version 4.2.

3. RESULTS

CHD8 ChIP-seq in wild-type organoids at the neurogenic time point identified 14,832 high-confidence binding sites genome-wide, of which 61% directly overlapped a CTCF motif and 54% overlapped a called Hi-C loop anchor, substantially above the overlap expected from randomly permuted CHD8 sites of matched number and width. RAD21 occupancy closely tracked CTCF at CHD8-bound loop anchors, indicating that a substantial fraction of CHD8 binding coincides with architectural CTCF-cohesin anchors rather than being restricted to promoter-proximal sites. Table 1 summarizes CHD8, CTCF and RAD21 co-occupancy at loop anchors across the three differentiation time points.

Table 1. CHD8, CTCF and RAD21 co-occupancy at Hi-C loop anchors across differentiation time points (wild-type organoids).

Time point	CHD8 peaks (n)	CHD8 + CTCF overlap (%)	CHD8 at loop anchors (%)	RAD21 at CHD8 anchors (%)
Day 25 (progenitor)	12,406	57	48	91
Day 50 (neurogenic)	14,832	61	54	93
Day 75 (maturation)	13,918	59	52	90

Percentages are of total CHD8 peaks called at each time point in wild-type organoids, mean of three biological replicates.

Comparing Hi-C contact maps between isogenic CHD8+/- and wild-type organoids at the neurogenic time point identified 612 loop anchors with significantly reduced contact strength in CHD8+/- organoids

(Wilcoxon signed-rank test, adjusted $P < 0.05$), representing 4.1% of all called anchors genome-wide; weakened anchors were disproportionately enriched among CHD8-bound anchors relative to CHD8-unbound anchors (Figure 1), indicating that architectural sensitivity to CHD8 dosage is concentrated at sites the protein itself occupies rather than distributed uniformly across the genome. Gene ontology analysis of domains flanking weakened anchors showed significant enrichment for synaptic signaling and neuronal differentiation terms, and 38 of the flanking genes overlapped an independently curated autism risk gene set, a substantially higher overlap than expected by chance given the size of that gene set relative to the genome.

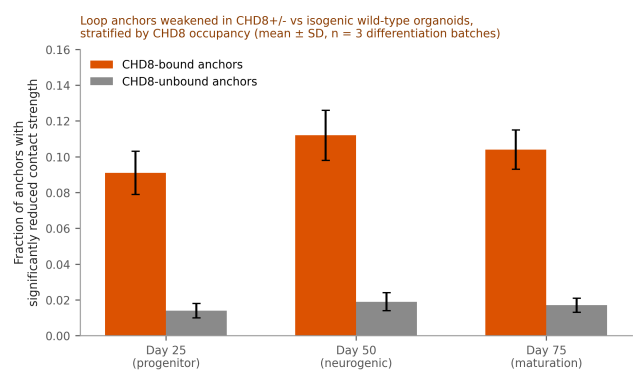


Figure 1. Fraction of loop anchors showing significantly reduced Hi-C contact strength in CHD8+/- versus isogenic wild-type organoids, stratified by CHD8 occupancy status, across the three differentiation time points (mean ± SD, n = 3 differentiation batches per genotype).

RNA-sequencing of the same organoids showed that genes within domains flanking weakened anchors were significantly more likely to be differentially expressed between CHD8+/- and wild-type organoids than genes in domains with unaffected anchor strength, including 22 genes not themselves bound by CHD8 in our ChIP-seq data, consistent with an indirect, architecture-mediated route to their dysregulation. Table 2 reports loop-strength and expression changes at a representative set of anchors flanking neurodevelopmental and autism-associated genes across the three time points.

Table 2. Loop-strength and expression changes at representative anchors flanking neurodevelopmental genes (CHD8+/- vs wild-type).

Flanking gene	CHD8-bound anchor	Loop strength change (log2)	Expression change (day 75, log2FC)
SCN2A	Yes	-0.71	-0.52*
GRIN2B	Yes	-0.64	-0.44*
ANK2	Yes	-0.58	-0.31
PTEN	No	-0.09	-0.06
SYNGAP1	No	-0.55	-0.48*

Asterisk denotes adjusted $P < 0.05$ (DESeq2, Benjamini-Hochberg). Loop strength change is CHD8+/- relative to isogenic wild-type at the neurogenic (day 50) time point. Comparing the developmental trajectory of anchor weakening against that of expression change showed that reduced ATAC-seq accessibility at CHD8-dependent

anchors was already detectable at the day 25 progenitor time point, before the corresponding flanking genes showed significant differential expression, which emerged only at the day 50 and day 75 time points. This temporal offset suggests that CHD8 haploinsufficiency destabilizes anchor chromatin state prior to, rather than concurrently with, downstream transcriptional consequences, consistent with an early architectural lesion that is progressively translated into altered gene expression over the course of differentiation.

4. DISCUSSION

These data indicate that CHD8 haploinsufficiency produces a discrete, anchor-specific pattern of chromatin looping weakness during human corticogenesis, concentrated at loop anchors the protein itself occupies and disproportionately affecting domains containing synaptic and neurodevelopmental genes. This provides a mechanistic bridge between the well-established genetic association of CHD8 with autism spectrum disorder and a specific, testable prediction about which genomic loci should be architecturally vulnerable to CHD8 dosage reduction, extending prior biochemical evidence for a CHD8-CTCF-cohesin association [5,6] into a neurodevelopmentally relevant cellular system with matched isogenic controls.

The observation that a meaningful fraction of differentially expressed genes flanking weakened anchors were not themselves directly bound by CHD8 argues that at least part of the transcriptional consequence of CHD8 haploinsufficiency is architectural rather than a direct effect of lost CHD8 occupancy at the dysregulated gene's own promoter. This is consistent with a growing body of evidence that chromatin remodelers can exert long-range regulatory effects through loop-anchor stabilization independent of promoter-proximal binding [3,4,6], and suggests that models of CHD8 function limited to its direct target genes may substantially underestimate its total regulatory footprint during neurodevelopment.

The temporal offset between early loss of accessibility at CHD8-dependent anchors and later emergence of expression change is compatible with a model in which architectural weakening lowers the probability, rather than immediately abolishing the occurrence, of productive enhancer-promoter contact, with transcriptional consequences accumulating as differentiation proceeds and additional enhancers become active within the affected domains. This progressive model would predict that later developmental stages, and potentially postnatal maturation, could reveal an even larger set of affected genes than captured within our day 75 endpoint, a possibility that would require longer-term organoid culture or complementary in vivo models to test directly.

Several limitations warrant consideration. First, cortical organoids model early corticogenesis but do not recapitulate the full cellular diversity, circuit-level

connectivity or systemic developmental context of the intact human brain, so the anchor-specific effects we describe should be interpreted as a cell-intrinsic architectural phenotype rather than a direct model of autism pathophysiology. Second, our study used two isogenic clone pairs derived from a single parental line; whether the same anchors are similarly CHD8-dependent on a different genetic background remains to be tested. Third, because our accessibility and expression profiling was restricted to bulk organoid tissue, cell-type-resolved single-cell approaches would help determine whether anchor weakening and its transcriptional consequences are uniform across progenitor and neuronal populations or concentrated within a specific lineage.

5. CONCLUSION

Combining isogenic CRISPR-edited CHD8 heterozygous human induced pluripotent stem cell lines with cortical organoid differentiation and multi-assay chromatin profiling, we show that CHD8 haploinsufficiency causes anchor-specific weakening of a defined subset of chromatin loops, disproportionately affecting domains containing synaptic and neurodevelopmental genes, with accessibility loss at affected anchors preceding detectable expression change during early corticogenesis. These findings support a model in which CHD8 contributes to neurodevelopmental gene regulation substantially through loop-anchor stabilization rather than through direct promoter occupancy alone, and provide a set of anchor-level predictions that could be tested further in patient-derived lines, additional genetic backgrounds, and single-cell-resolved organoid systems.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

E.F. conceived and supervised the study, designed the CRISPR editing strategy, and analysed the Hi-C and ChIP-seq data. H.L. performed organoid differentiation and

the ATAC-seq experiments. J.V. generated and validated the isogenic CHD8+/- clones and performed RNA-sequencing library preparation. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study used an established human induced pluripotent stem cell line maintained under standard institutional biosafety and stem cell research approval; no new human participant recruitment or animal procedures were performed for this work.

Data Availability

Raw and processed Hi-C, ChIP-seq, ATAC-seq and RNA-sequencing data generated for this study have been deposited in the Gene Expression Omnibus under a series accession available from the corresponding author on reasonable request. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

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