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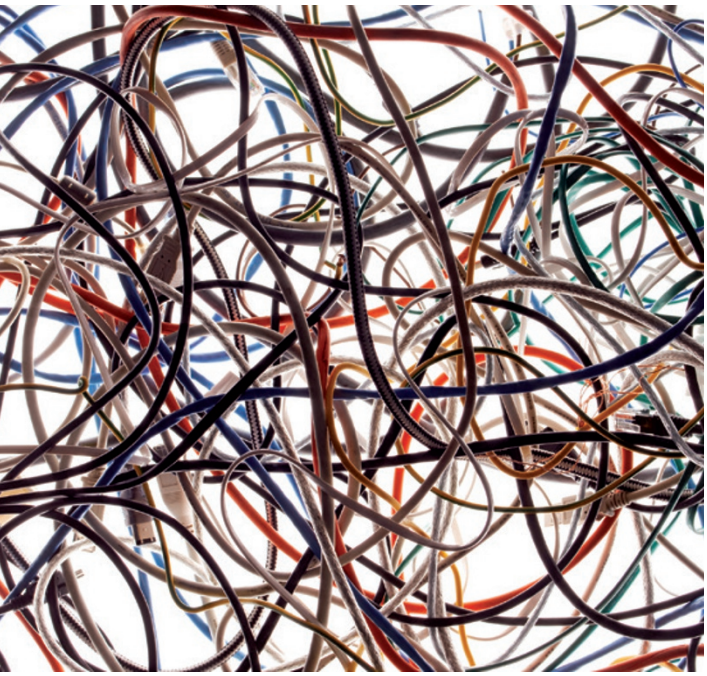
GENOME ORGANIZATION

Disorder — from chromatin to limb development

> “disruption of TAD boundary elements can lead to developmental disorders”

The mammalian genome is structured into topologically associated domains (TADs). These megabase-sized domains permit chromatin interactions that control gene regulation and are usually conserved across cell types and species. Lupiáñez *et al.* now report that disruption of TAD boundaries, which separate and prevent interactions between neighbouring TADs, can alter genome structure, leading to regulatory domain rewiring and developmental disorders.



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Structural variants in the *WNT6–IHH–EPHA4–PAX3* region, which is organized into three neighbouring TADs on chromosome 2, have previously been linked with polydactyly, brachydactyly and F syndrome — three rare developmental disorders that result in limb malformation. In the current study, high-resolution array comparative genomic hybridization and genome sequencing were first used to characterize the nature of the structural variation across the extended *WNT6–IHH–EPHA4–PAX3* region in several individuals with these disorders. Notably, at least one of the predicted TAD boundaries was disrupted in each of the individuals. The team then used an adapted clustered regularly interspaced short palindromic repeat (CRISPR)–Cas protocol for the introduction of large structural variants to engineer mouse models with the same disease alleles (for polydactyly and brachydactyly, these recapitulate the same limb malformations seen in humans). To examine any possible changes in chromatin interactions, a series of circularized chromosome conformation capture sequencing (4C-seq) experiments were performed in the distal limbs of mutant and wild-type mice.

The team report ectopic interaction of *Pax3*, *Wnt6* or *Ihh* with the *Epha4* TAD (which contains a cluster of limb enhancers that drive gene expression) in all of the mouse models with the disease alleles, confirming that structural changes can produce new regulatory interactions. Mouse models with similar sized structural alterations but with the TAD boundaries intact had normal limbs and digits. Regions of interaction, however, may include additional genes beyond these targets. In addition, 4C-seq on patient-derived fibroblasts showed the same reorganization and abnormal interactions as those in the mouse models, demonstrating that human fibroblasts can also be used to study the effects of TAD disruption.

It had previosuly been shown that disruption of TADs could lead to altered gene expression; however, this work provides the first evidence that disruption of TAD boundary elements can lead to developmental disorders. Although the current work is focused on one locus, with the increasing availability of TAD data for the human genome, the techniques used and the mechanistic details revealed in this study may benefit interpretation of the causal factors of many other developmental disorders.

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ORIGINAL RESEARCH PAPER Lupiáñez, D. G. *et al.* Disruptions of topological chromatin domains cause pathogenic rewiring of gene–enhancer interactions. *Cell* **5**, 1012–1025 (2015)

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