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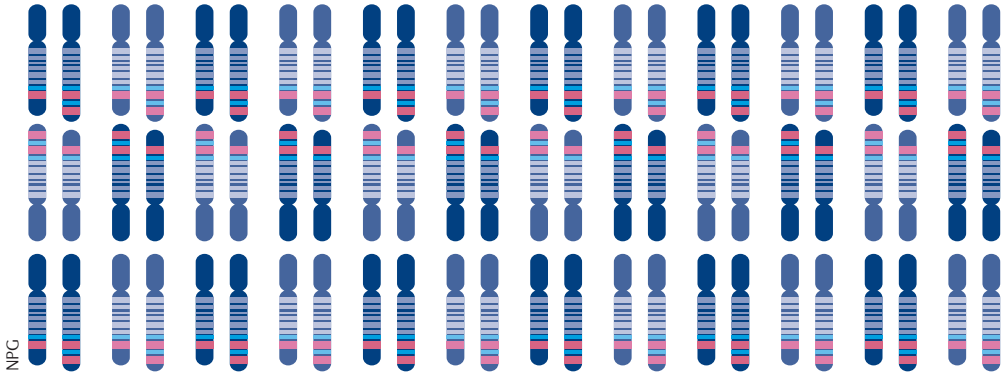
GENOME ORGANIZATION

Add a TAD of duplication



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Duplications of genomic regions are associated with various diseases, but the phenotypes, which are thought to arise from an increase in gene copy number, often cannot be explained by changes in gene dosage. A recent study by Franke, Ibrahim *et al.* reveals that genomic duplications that change the structure and function of topologically associated domains (TADs) can cause disease without altering gene copy number.

TADs are chromosomal regions with an elevated frequency of internal chromatin interactions, such as between genes and their distal regulatory elements. A genomic region of ~3 Mb on human chromosome 17 comprises two adjacent TADs: one includes the gene *SOX9*, which encodes a developmental transcription factor that regulates male sex determination; the other includes two genes encoding potassium channels, *KCNJ2* and *KCNJ16*. In humans, the duplication of a region between *SOX9* and *KCNJ2*, which lies within the *SOX9* TAD, leads to female to male sex reversal; however, an inter-TAD duplication that includes the sex reversal duplication and extends into the KCNJ TAD — but does not encompass the KCNJ genes — has no phenotype. A third duplication, which extends even further and includes the KCNJ genes, causes Cooks syndrome, which is characterized by congenital limb malformations.

> “the inter-TAD duplication formed a new, separate TAD”

To investigate the difference between the sex reversal (intra-TAD) duplication and the no-phenotype (inter-TAD) duplication, the authors performed circular chromosome conformation capture-sequencing (4C-seq) in duplication-containing fibroblasts. Compared with control fibroblasts, loci in the intra-TAD duplication — which potentially includes *SOX9* regulatory elements — had increased frequency of interactions with the entire *SOX9* TAD, including with *SOX9* itself; this could potentially increase *SOX9* expression and explain the sex reversal phenotype. Conversely, loci in the no-phenotype duplication formed interactions across the duplicated parts of both TADs, but not with loci outside the duplication, such as the *SOX9*, *KCNJ2* or *KCNJ16* genes. This explained the lack of associated pathology and suggested that the inter-TAD duplication formed a new, separate TAD, which was termed a ‘neo-TAD’.

Similar chromatin interactions were observed in the cells of mice engineered to carry the equivalent of the sex reversal or the no-phenotype duplication, including the formation of a neo-TAD in the latter case. Furthermore, the expression pattern of a *lacZ* reporter gene from the mouse neo-TAD was similar to that of endogenous *Sox9*, which indicated that the *Sox9* regulatory region in the neo-TAD was functional and sufficient to recapitulate physiological expression.

Next, the authors extended the inter-TAD duplication to include the *Kcnj2* and *Kcnj16* genes. Again, this resulted in the formation of a new TAD that corresponded to the duplication, with a strong increase in the frequency of interactions between the duplicated parts of the *Sox9* and Kcnj TADs. Importantly, in these mice, *Kcnj2* acquired a new expression pattern that was similar to that of *Sox9*, and the phenotype of the mice closely resembled Cooks syndrome. Thus, the inclusion of *Kcnj2* in the neo-TAD resulted in its activation by the *Sox9* TAD regulatory region, suggesting that misregulation of *KCNJ2* expression is the cause of Cooks syndrome.

These results indicate that duplications within TADs do not have a major effect on TAD structure but can increase interactions between (duplicated) regulatory elements and their target genes, which can cause changes in gene expression and consequently disease. Inter-TAD duplications, by contrast, result in the formation of new TADs, where new interactions between regulatory elements and genes can form. Such chromatin interactions can cause disease and could also potentially drive genes to acquire new functions during evolution.

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ORIGINAL ARTICLE Franke, M., Ibrahim, D. M. *et al.* Formation of new chromatin domains determines pathogenicity of genomic duplications. *Nature* **538**, 265–269 (2016)