Prenatal upper-limb mesomelia and 2q31.1 microdeletions affecting the regulatory genome

To the Editor: We read with great interest the article "Noncoding Copy-Number Variations Are Associated With Congenital Limb Malformation," by Flöttmann et al.[1] Their work represents the first large-scale study assessing copy-number variants (CNVs) in individuals with limb defects, showing that the majority of the pathogenic variants are noncoding CNVs affecting regulatory elements. Besides describing previously known disease-associated CNVs, the authors studied in detail four novel candidate loci, which caught our attention and encouraged us to share our experience with a prenatal case.

The patient was a male fetus—the first pregnancy of unrelated healthy parents with unremarkable family history. After an uneventful first trimester, ultrasound at 21 weeks and 1 day showed limb abnormalities: the radius and ulna were 19.8 and 15.1 mm, respectively (significantly less than the second percentile), with bowing of the radii and ulnar deviation of the hands. Occipital frontal circumference, humerus, femur, tibia, fibula, and foot measurements were within the normal range, and no other malformations were detected.

Array comparative genomic hybridization (SurePrint G3 Human CGH Microarray Kit; 60 K) performed on a chorionic villus sample showed a 998-kb microdeletion on 2q31.1 (175,904,189–176,902,313; GRch37/hg19), *de novo*. Higher-resolution array comparative genomic hybridization (180 K) confirmed this finding and detected a second, more telomeric *de novo* microdeletion of 153 kb on 2q31.1 (177,146,631–177,299,841).

The parents elected for termination. Fetal autopsy at 22 weeks showed length, weight, and occipital frontal circumference consistent with age, and confirmed upper-limb mesomelia: the humerus length was 4.4 cm (50th percentile), and the ulna length was 2.4 cm (less than the 2nd percentile). Diffuse cutaneous edema and minor facial anomalies were noted in the absence of other congenital malformations (**Supplementary Figure S1A** online). Mesomelic involvement confined to the upper limbs was confirmed on skeletal survey (**Supplementary Figure S1B**). The second pregnancy of the couple resulted in a healthy baby with normal chromosomes.

In humans, segmental identity and patterning along the body axis is specified by the spatiotemporal expression of four gene clusters that encode the evolutionarily conserved *HOX* genes: *HOXA–D*. The *HOXD* cluster on chromosome 2q31.1 is surrounded by two topologically associated domains (TADs) containing long-range enhancers and regulatory elements for the *HOXD* genes.[2] Mouse models showed that this cluster is regulated in a manner such that gene expression along the anterior–posterior body axis correlates with their physical order on 2q31.1—a phenomenon known as spatial and temporal collinearity.[2] Regulatory elements within the telomeric TAD are responsible for early proximal limb development (arm and forearm), while those within the centromeric TAD regulate later distal limb development (hands).[2]

The chromosomal microarray results in our patient excluded the involvement of any of the genes in the *HOXD* cluster, but rather the deletions impacted both TADs (**Supplementary Figure S1C** online). The 998-kb microdeletion involves *ATF2, ATP5G3* and the limb-expressed gene *KIAA1715* (*Lunapark*; *Lnp*), as well as a 40-kb enhancer that controls both *KIAA1715* and genes within the *HOXD* cluster itself. Notably, the second, 153-kb microdeletion involves the telomeric TAD. It also disrupts *MTX2*, which encodes a mitochondrial membrane protein not expressed in the limb bud, and thus unlikely to contribute to the phenotype in our patient.

Of note, a well-characterized mouse mutant, *Ulnaless*, exhibits markedly reduced ulna and radius, and small tibia and fibula as a result of a balanced paracentric inversion, whose centromeric breakpoint resides in *KIAA1715* and telomeric breakpoint is positioned 770 kb downstream from *MTX2*.[2] In contrast to the *Ulnaless* mouse mutant, the skeletal defect in our patient is confined to the upper limbs, inconsistent with a more generalized skeletal dysplasia. We therefore searched the literature for similar structural variants and mesomelic dysplasia (a heterogeneous group of skeletal dysplasias) in humans. Interestingly, whereas both *KIAA1715* and *MTX2* are duplicated in Kantaputra-type mesomelic dysplasia (OMIM 156232) and variably disrupted in patients with mesomelia, the sparing of the lower limbs distinguishes our patient from Kantaputra-type mesomelic dysplasia. Indeed, our patient more closely resembles those described by other authors, with normal height and sparing of the lower limbs (**Supplementary Table S1** online). One family was found to have a balanced translocation t(2;8) with the chromosome 2 breakpoint located 21 kb from *MTX2*.[3] Another patient had a duplication involving only *MTX2*, but not *KIAA1715*, and exclusive or predominant involvement of the upper limbs.[4] Although differences in resolution of the various cytogenetic tests used make it difficult to compare breakpoints, these cases, together with ours and the new findings by Flöttmann et al.,[1] point to deregulation of the spatiotemporal expression of the *HOXD* cluster as the underlying cause, thus representing one of only a few conditions so far ascribed to the disruption of TADs.

Specifically, given that the mesomelia in our patient is confined to the upper limbs, and the TAD telomeric to the *HOXD* cluster is responsible for regulation of more proximal limb morphogenesis, we conclude that the more distal 2q31.1 deletion seen in our patient using higher-resolution microarray is responsible for the phenotype. In accordance with linear and spatial collinearity, we speculate that the region containing *MTX2* includes *cis*-elements that specifically regulate the *HOXD* genes responsible for forearm development. Additional involvement of the lower limbs might depend on the variable localization of breakpoints around *MTX2*, and Kantaputra-type mesomelic dysplasia might therefore represent a spectrum ranging from isolated upper-limb involvement to more severe phenotypes involving both arms and legs.

In conclusion, our report contributes to the CNV morbidity map for limb malformations delineated by Flöttmann et al.[1] It also points out the importance of high-resolution chromosomal microarrays in selected cases, and the need to consider the effect of possible regulatory regions when the detected CNV does not contain genes associated with known diseases. Nevertheless, one should keep in mind that predicting the phenotype by only analyzing the CNV will remain challenging in many cases, since different types of structural variants can cause similar phenotypes.

We appreciate the work of Flöttmann et al.[1] in unraveling the complexity of this subject, and strongly agree with the authors that these mechanisms should be considered in the clinical interpretation of CNVs. We hope that further studies in humans with limb malformations will follow.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

DISCLOSURE

The authors declare no conflict of interest.

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