It all begins with the phenotype

The recent paper by Xu et al. investigated a Chinese four-generation family with a polydactylous limb anomaly.[1] Affected family members shared a sequence variant in an enhancer of Sonic Hedgehog (*SHH*), a gene essential for embryonic limb patterning. The reported variant (446T>A) in this well-known enhancer, termed the ZRS, has not been described in literature before. Additionally, the paper extensively evaluated the pathogenic effect of the variant with a combined approach, using CRISPR/Cas9 targeted transgenic mice models, electrophorectic mobility shift assays (EMSA), chromatin immunoprecipitation (ChIP) assays, and luciferase reporter assays.

As the most important conclusion, the authors claim that this is the first case of a preaxial polydactyly type 1 (PPD I) family associated with a variant in the ZRS and therefore adds PPD I to the spectrum of ZRS-associated phenotypes.

However, after reviewing the provided images and descriptions, we believe that the phenotypes of the affected members of this family should not be classified as preaxial polydactyly type I (PPD I), but as triphalangeal thumb (TPT), a limb anomaly commonly associated with variants in the ZRS. We will substantiate our argument with three explanations on the observations made in this paper:

1. The authors phenotyped hands of patients II-11, II-7, III-4, III-8, III-11, III-12, IV-1 as "incomplete duplication of distal phalanx" and therefore a PPD type I. Based on the images of Supplemental Figure 1b, these limb anomalies seem to depict a TPT phenotype, consisting of an additional deltaphalanx (TPT type 1 in both the classifications of Wood and Buck-Gramcko).[2,3] This phenotype is fairly common in TPT families. In our case series of 148 triphalangeal thumbs, we have operated on 41 thumbs with an additional deltaphalanx in Dutch TPT families.[4] The question on whether this deltaphalanx is a rudimentary remnant of either an additional distal phalanx or a complete midphalanx remains the subject of a developmental and rather philosophical discussion dating back to Galen in the second century AD.[5] The most important conclusion, however, is that it is widely accepted by clinicians and geneticists that this congenital thumb anomaly should be classified within the spectrum of TPT and not within the spectrum of preaxial polydactyly.[2,3]
2. The additional delta phalanges in the thumb are significantly different from the observed sesamoid ossifications at the interphalangeal (IP) joint of the thumb in some family members. As the authors already mentioned, sesamoid bones were found in family members without variants of the ZRS. They can be recognized as small ossicles on the volar side of the IP joint and, in contrast with TPT, do not interfere with the congruence of the joint. The prevalence of sesamoid bones at the IP joint of the thumb is high, as they can be observed in up to 67% of the radiographs in the adult population.[6] Therefore, an association between the presence of sesamoid bones and triphalangism cannot be made.
3. Patients II-11 and IV-1 were said to display a phenotype resembling PPD type I or type II following the Wassel classification for radial polydactyly. Although we were only able to evaluate the radiographs of patient II-11, we agree with the observation that the radiograph resembles a Wassel type II radial polydactyly.

However, an important feature that needs to be emphasized is that this radiograph was made at an adult age. In newborns with TPT, small delta phalanges are still visible on a radiograph. If these ossifications are not removed during childhood, they can fuse to the other phalanges over the course of several years. Once these patients reach adulthood, the additional phalanx will have disappeared on a radiograph. As an example, we provided radiographs of two young patients from our series with a similar phenotype as patient II-11, but with a small ossification in the IP (Supplementary figure). We believe that patient II-11 could have triphalangism at a younger age and could show a similar phenotype if a radiograph would have been taken.

As the authors have stated, convincing scientific evidence regarding a genetic locus of PPD type I is still lacking, even though it is one of the most common limb anomalies. The fact that PPD type 1 usually occurs in sporadic patients and unilaterally in 67% of the cases raises the question whether a single genetic substrate is the cause of this phenotype.[7]

In conclusion, we applaud the authors for their extensive work to obtain a better understanding of the regulatory mechanism of the ZRS on SHH expression in the embryonic limb. Furthermore, we encourage reporting families with aberrations in the ZRS to optimize genotype–phenotype correlation within ZRS-associated anomalies and gather more knowledge on the long-range regulation in embryonic limb development.

We would like to emphasize the critical importance of dedicated clinical geneticists or congenital upper limb surgeons being involved in (molecular) genetic research. Appropriate phenotyping of patients is essential as it establishes a mandatory foundation for all qualitative genetic research, improves the counseling of affected families, and increases the diagnostic yield in standard diagnostic genetic

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testing. Therefore, we advocate a combined effort by experts from multiple fields within genetic research of congenital upper limb anomalies.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-019-0724-6) contains supplementary material, which is available to authorized users.

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DISCLOSURE

The authors declare no conflicts of interest.

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