A novel GLI3 mutation affecting the zinc finger domain leads to preaxial-postaxial polydactyly-syndactyly complex

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Abstract

Background: Polydactyly is a highly common congenital limb defect. Extra digits may appear as an isolated anomaly or as a part of a syndrome. Mutations in GLI3 have been shown to cause Greig cephalopolysyndactyly, Pallister-Hall syndrome and non-syndromic polydactyly. Genotype-phenotype correlation studies of GLI3 mutations suggest a model by which mutations in the zinc-finger domain (ZFD) of GLI3 likely lead to syndromic polydactyly. Here we describe a rare case of autosomal dominant heterozygous missense mutation in the ZFD of GLI3 leading to a variable polydactyly-syndactyly complex.

Case presentation: A large Jewish Moroccan family presented with apparently autosomal dominant heredity of bilateral thumb polydactyly in hands and feet combined with post-axial polydactyly type B or type A. Syndactyly was evident in most patients’ hands and feet. Apart from head circumference beyond 90th percentile in some of the affected individuals, none had craniofacial dysmorphism. A novel GLI3 c.1802A > G (p.His601Arg) mutation was found in all affected individuals.

Conclusion: We demonstrate that a mutation in the ZFD domain of GLI3 leads to phenotypic variability, including an isolated limb phenotype. Thus, the variability in phenotypes caused by mutations in this master developmental regulator is more profound than has been previously suggested.

Keywords: Polydactyly, Syndactyly, GCPS, GLI3, Mutation, C2H2, Zinc finger
macrocephaly and hypertelorism [9]. Here we report a new heterozygous missense mutation in the zinc finger domain of \( GLI3 \) leading to a variable phenotype presenting in some cases as an isolated polydactyly-syndactyly complex.

**Case presentation**

A large Jewish Moroccan kindred presented with apparently autosomal dominant heredity of polydactyly (Figure 1A). Affected and unaffected family members underwent thorough clinical and molecular evaluation following Soroka Medical Center IRB approval and informed consent. Phenotypic variability among the 14 affected individuals was evident: most had hands and feet bilateral thumb polydactyly and post-axial polydactyly type B. Syndactyly was found in the feet of all affected individuals and in the hands of most (Figure 1D,E). A single case of thumb polydactyly combined with post-axial polydactyly type A (well developed separated digit) was evident (Figure 1A, IV:1; Figure 1B,C). None of the affected individuals had apparent craniofacial dysmorphism. Occipitofrontal head circumference (OFC) and interpupillary distance (IPD) were measured in 13 (8 affected and 5 unaffected) family members. The measurements were most variable (see Additional file 1). Five patients had an OFC at or above the 90th percentile, while all the healthy individuals showed OFC beneath 90th percentile. As to IPD measurements, 5 of 8 affected as well as 4 of 5 unaffected individuals were above 97th percentile.

**Methods and results**

Blood samples were obtained from 13 affected and 6 unaffected family members and genomic DNA was extracted by routine techniques. Linkage to genes known to be associated with non-syndromic polydactyly was tested using 2 polymorphic markers flanking each candidate gene. Association of the phenotype with \( HOXD13, ZPA \) regulatory sequence (ZRS) and \( FBLN1 \) was ruled out (data not shown). Polymorphic markers D7S1526 and D7S691 flanking \( GLI3 \) identified a heterozygous haplotype shared by all affected family members (data not shown). Sanger sequencing of all \( GLI3 \) exons and their flanking exon-intron boundaries and comparison (NCBI BLAST) to the published \( GLI3 \) sequence (GenBank reference number NM_000168.5), identified a single heterozygous missense mutation in exon 12: c.1802A > G, p.His601Arg (Figure 2A,B). This novel mutation was found to segregate within the kindred as expected, demonstrating full penetrance of the phenotype.
The GLI3 ZFD consists of 5 C2H2 type zinc fingers. The histidine to arginine substitution caused by the mutation is of the second histidine within one of the C2H2 zinc fingers comprising this domain. In zinc finger domains, a zinc ion forms four coordinate bonds generating a stable fold [10]. Since the GLI3 601His is one of the four residues directly interacting with the Zn$^{2+}$ ion in a C2H2 zinc finger motif, the p.His601Arg substitution is likely to have functional consequences. Using crystallographic structure of the consensus motif of C2H2 type zinc finger (PDB:3IUF), we used UCSF Chimera software [11] to construct a simplified model illustrating the change in interactions with the Zinc cation. As shown in Figure 2, the mutation is predicted to cause destabilization of the Zn$^{2+}$ ion, as the stable coordinative bond of histidine 232 (comparable to GLI3 histidine 601) with the metal ion (Figure 2C) is replaced in the mutated protein by a much longer unrealized bond with an arginine residue (Figure 2D). We speculate that the p.His601Arg mutation might alter the ability of GLI3 to bind its downstream targets within the SHH pathway, such as the posterior Hoxd genes [12].

**Conclusions**

In the kindred we describe, the OFC and IPD findings possibly stem from intrafamilial variability. The craniofacial manifestations of GCPS are highly variable and not all patients with GCPS have obvious macrocephaly [9] as observed in the family described here. In fact, the mild end of the GCPS spectrum is a continuum with isolated polydactyly [7]. Thus, in some cases there is unclear clinical delineation of GCPS versus non-syndromic polydactyly. The effects of truncating mutations (leading to loss of functional regions) on digit number and identity were largely discussed in the context of GLI3’s role as mediator of the hedgehog pathway, whereas connection between defect in the DNA binding domain (DBD) and the phenotype of our pedigree is more elusive. Besides the posterior Hoxd genes, other factors known to be downstream of GLI3 are FGF8, FGF4, GREM1, HAND2 and JAG1 [12]. It is plausible that changes in expression patterns of these GLI3 targets (as a result of an obstructed DBD) can contribute to the polydactyly phenotype. Both the precise mechanism through which the specific mutation leads to the disease phenotype, and the molecular mechanisms underlying the familial phenotypic variability are yet to be elucidated.

In summary, we describe a large kindred with a novel heterozygous GLI3 ZFD domain missense mutation leading to polydactyly-syndactyly complex. The phenotype described is within the wide range of phenotypic spectrum of GCPS established by Biesecker [13]. With recent emerging evidence of GLI3 mutations causing non-syndromic limb defects [14,15], our data highlight the fact that a GLI3 mutation within the ZFD domain can cause a mild form of GCPS with no prominent facial dysmorphism. This report strengthens the need to screen for GLI3 mutations in patients with polydactyly-syndactyly phenotype, even when syndromic features are not evident.

**Consent**

Written informed consent was obtained from the patients for publication of this Case report and any accompanying images.

**Additional file**

Additional file 1: Clinical data. Gender, affected (+/−), age, OFC, IPD and malformations data of family members.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

MV carried out the molecular genetic studies and drafted the manuscript. YL was involved in acquisition and analysis of clinical data and helped drafting the manuscript. OSB conceived and coordinated the study and took part in writing the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

This research was supported by the Israel Science Foundation (grant No. 1689/12). We deeply thank the Morris Kahn family foundation for the generous support of this study, and the patients and their families for kind participation. We would also like to acknowledge Dotan Amar for his assistance in drawing some of the figures. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081).

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**Received: 13 April 2014 Accepted: 24 September 2014 Published online: 30 September 2014**

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doi:10.1186/s12881-014-0110-9

Cite this article as: Volodarsky et al.: A novel GLI3 mutation affecting the zinc finger domain leads to preaxial-postaxial polydactyly-syndactyly complex. *BMC Medical Genetics* 2014 15:110.