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A Novel ZRS Mutation in a Balochi Tribal Family with Triphalangeal Thumb, Pre-axial Polydactyly, Postaxial Polydactyly and Syndactyly

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Abstract

Limb malformations are one of the most common types of human congenital malformations. Mutations in the ZRS enhancer of Sonic Hedgehog are thought to be responsible for preaxial polydactyly in multiple independent families. Here, we describe a large Balochi tribal family from Southern Punjab, Pakistan, with a variable set of limb malformations and a novel ZRS mutation. The family has a limb phenotype characterized by triphalangeal thumb, preaxial polydactyly and postaxial polydactyly. There is also a high degree of phenotypic heterogeneity with less common clinical findings in the affected family members that include osseous syndactyly of forth-fifth fingers, clinodactyly, hypoplasia of mesoaxial fingers, and bifid halluces. The presentation in most of the affected patients was bilateral and symmetrical. A heterozygous C>A mutation at position 287 of the ZRS enhancer (chr7:156,584,283; hg19) was detected in all affected subjects and is absent from four unaffected family members, 42 unrelated samples and multiple databases of human variation. Combined, these results identify a novel ZRS287 C>A mutation which leads to a variable spectrum of limb phenotypes.

Keywords

Polydactyly; triphalangeal thumb; syndactyly; ZRS

INTRODUCTION

Preaxial polydactyly is caused by disruptions to the developmental patterning of the limb along the anterior-posterior (AP; thumb to pinky) axis that lead to changes in digit number and identity. The AP axis is specified by a small population of cells in the posterior limb bud that form the zone of polarizing activity (ZPA). These cells express the gene Sonic Hedgehog (*SHH*; OMIM *600725) which defines the posterior side of the limb. The expression of *SHH* in cells of the ZPA is controlled by a long range *cis*-regulatory enhancer

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called the ZPA regulatory sequence (ZRS; OMIM #605522). The ZRS is located nearly 1 megabase away from *SHH*, within intron 5 of the limb region 1 homolog (*LMBR1*; OMIM *605522) gene. This enhancer is required for *Shh* expression in the limb and is highly conserved from humans to fish [Lettice et al., 2003; Sagai et al., 2005]. Mutations in the ZRS have been shown to cause preaxial polydactyly in many animals including mice, dogs, cats, chickens and humans (reviewed in VanderMeer and Ahituv 2011).

In humans, 13 different point mutations and 10 duplications involving the ZRS have been shown to cause human limb malformations [VanderMeer and Ahituv, 2011]. Large duplications that encompass the ZRS and its surrounding sequence usually cause complex Haas-type polysyndactyly (webbing between digits and the presence of extra digits) and point mutations in the ZRS have been shown to cause preaxial polydactyly with or without triphalangeal thumbs and Werner mesomelic syndrome.

Here we report on a new point mutation within the ZRS in a family with a variety of distinct digit malformations including triphalangeal thumb, pre-axial polydactyly and postaxial polydactyly.

CLINICAL REPORT

A highly inbred Balochi tribal family was identified in a remote village of Southern Punjab, Pakistan. The pedigree comprised six generations with the trait segregating in three consecutive generations (IV–VI; Fig. 1A). The trait appeared *de novo* in subject IV-5 and was transmitted to 10 of his descendants in the next two generations (Fig. 1A). The affected family subjects have isolated limb phenotypes primarily affecting the hands with minimal involvement of the feet. There were no symptoms in any other organ system. A total of 11 subjects (seven males, four females) were affected (Fig. 1A). Photographs and X-rays of seven affected and one unaffected subjects were obtained.

The phenotype in this family is characterized by triphalangeal thumb, pre-axial polydactyly and postaxial polydactyly. Bilateral triphalangeal thumbs were observed in all of the affected subjects. The first digit was usually weak and had varus inclinations (Fig. 2). Three phalanges were observed in radiographs with a hypoplastic terminal phalanx visible on examination. Six of the nine affected subjects also had a supernumerary preaxial digit (Fig. 2; Table I). The additional digit was usually well-established with bony elements and a dorsal nail, but was non-functional. Roentgenograms revealed two or three phalangeal segments and symphalangism at the proximal inter-phalangeal (PIP) joint of the first digit (Fig. 2). Some degree of postaxial polydactyly was observed in all affected patients. In most individuals, this presented as a small cutaneous tag without nail or any bony element juxtaposed to the ulnar aspect of the distal inter-phalangeal-joint the 5^{th} finger (Fig. 2, Table I). One individual had more severe postaxial polydactyly with a complete digit that was syndactylous with digits 4–5 (Table I, subject V-7).0

Additional distinct clinical variants were identifiable in some patients. These include syndactyly of the postaxial digits (Fig. 2D; Table I). In these patients, syndactyly was bilateral and complete; nails of the syndactlyous fingers were intimately fused, separable only by a median fissure. In the radiographs, terminal phalanges of the webbed digits depicted osseous fusion and symphalangism. Three patients had bilateral clinodactyly of 5^{th} fingers (Table I). Another three patients had distal phalangeal hypoplasia of mesoaxial fingers (Fig. 1B,D; Table I). One patient had bifid halluces with fused nails and valgus deviation (V-5) (Fig. 2C). Generally, patients showed crowding of carpals in the affected hands (Fig. 2A, D). The distal heads of the radius and ulna were unremarkable.

MATERIALS AND METHODS

Ten samples were available for sequencing. Genomic DNA was extracted from peripheral blood using standard methods [Sambrook and Russel, 2001]. Primers were designed to cover the ZRS region [Lettice et al., 2003] and the pZRS [Park et al., 2008]. Primer sequences can be found in Supplementary eTable SI (See Supporting Information online). Sequencing was performed by Quintara Biosciences (Quintara Biosciences, Albany CA USA) according to standard procedure and sequences were analyzed using Sequencher (Gene Codes Corporation, Ann Arbor, MI USA).

RESULTS

In sequencing the proband (Patient IV-5, Fig 2A), we identified a heterozygous single base pair mutation in the ZRS. This mutation is a C>A substitution at position 287 according to conventional ZRS mutation numbering [Lettice et al., 2002] and is at chr7:156,584,283 (hg19) (Fig. 2B). This mutation was present in all affected subjects (n=6) and absent in all unaffected subjects (n=4), and perfectly segregated with the limb anomaly in three generations. This mutation has not been reported in dbSNP (build 135; http://www.ncbi.nlm.nih.gov/projects/SNP) nor in the current available data from the 1,000 Genomes Project (http://www.1000genomes.org). Additionally, this mutation has not been observed in 42 unrelated subjects, including 19 unrelated Pakistani subjects, sequenced in our laboratory (JV and NA, unpublished data).

DISCUSSION

There is strong evidence to suggest that the C>A mutation at ZRS287 could be the cause of some or all of the limb malformations in this extended family. This mutation is adjacent to the largest cluster of reported human ZRS mutations, which fall between ZRS295 and ZRS460 [VanderMeer and Ahituv, 2011]. It is within a highly conserved portion of the ZRS enhancer and the C allele at this location is conserved in all of the 46 vertebrate genomes that are currently available in the UCSC Genome Browser (http://genome.ucsc.edu). No other reported human sequences show any mutation at this site.

The phenotype of this family is striking compared to other examples of ZRS point mutations. Point mutations in the ZRS usually cause consistent and fully penetrant phenotypes of either triphalangeal thumb or triphalangeal thumb with preaxial polydactyly [Albuisson et al., 2010; Farooq et al., 2010; Semerci et al., 2009], but some variation has also been seen among individuals [Gurnett et al., 2007; Lettice et al., 2003] and one family appears to show reduced penetrance with phenotypically normal carriers of the mutation [Gurnett et al., 2007]. The family described here shows high phenotypic variability with regard to the severity of the anomalies and the limbs affected. Additionally, the clinical presentation of at least eight distinct phenotypic entities in this family expands the range of phenotypic variants associated with point mutations in the ZRS. While large duplications that include the ZRS have been shown to cause postaxial synpolydactyly [Klopocki et al., 2009; Sun et al., 2008; Wieczorek et al., 2009], this has not yet been reported for single base changes. The mechanism by which ZRS mutations cause digit malformations is not fully understood and it is possible that the postaxial polydactyly and syndactyly phenotypes in this family indicate that the site of their mutation within the ZRS is functionally distinct from other previously reported ZRS mutations.

In conclusion, the single base mutation found in this family identifies an additional site in the ZRS where mutations cause a wide spectrum of human congenital digit malformations.

Further study of this type of mutation will lead to a deeper understanding of ZRS function and the genetic mechanisms of embryonic patterning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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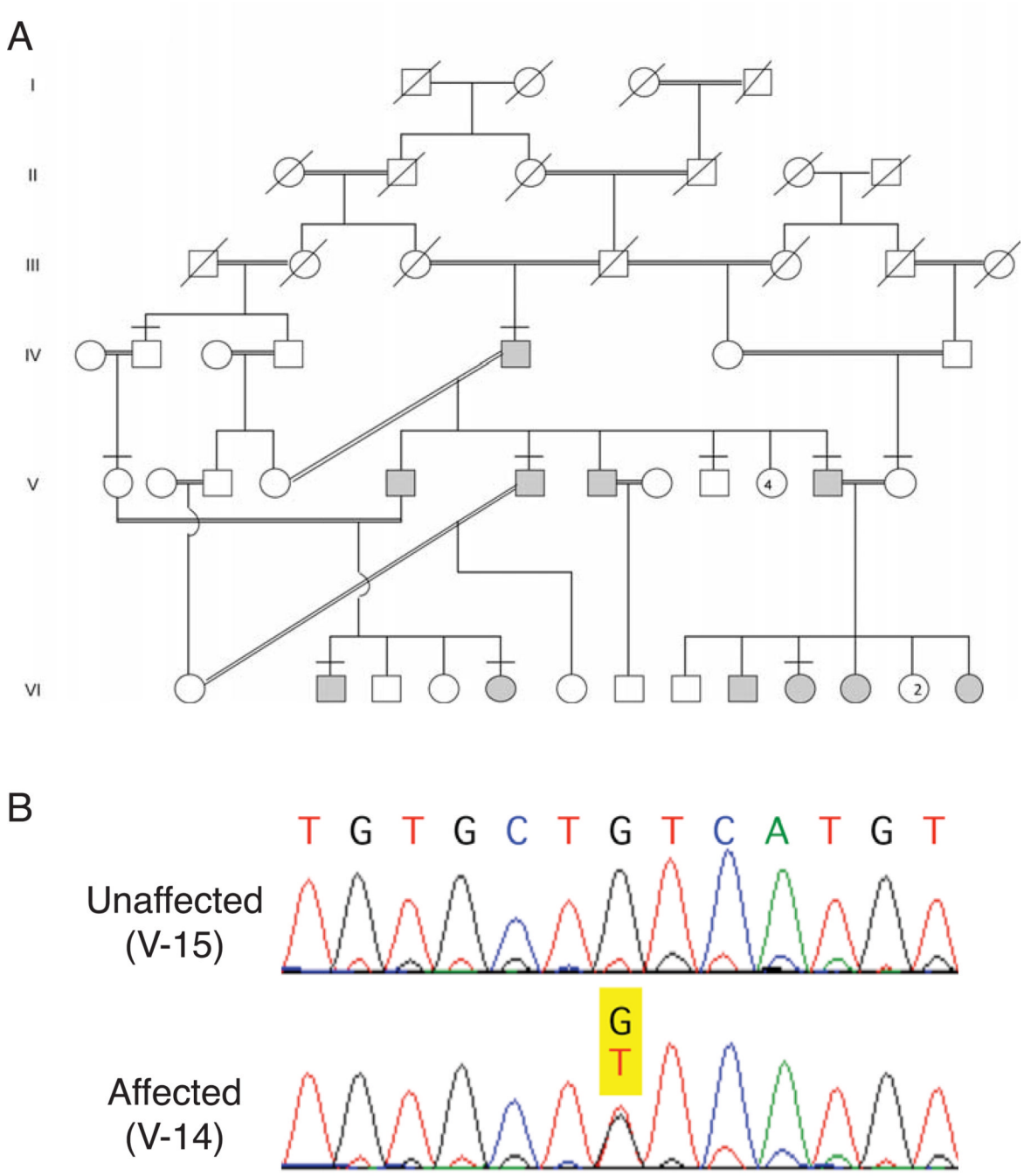

Figure 1.

A: Pedigree of the family. Shaded symbols denote patients with hand malformations, details of phenotypes can be found in Table I. Bars above symbols denote family members who were sequenced for ZRS mutations. B: Representative chromatogram from an affected subject V-14 showing the heterozygous mutation at position ZRS 287 (bottom) compared to an unaffected subject V-15 wildtype sequence (top). The mutation name was designated in the opposite strand as C>A in accordance with previously published ZRS mutations.

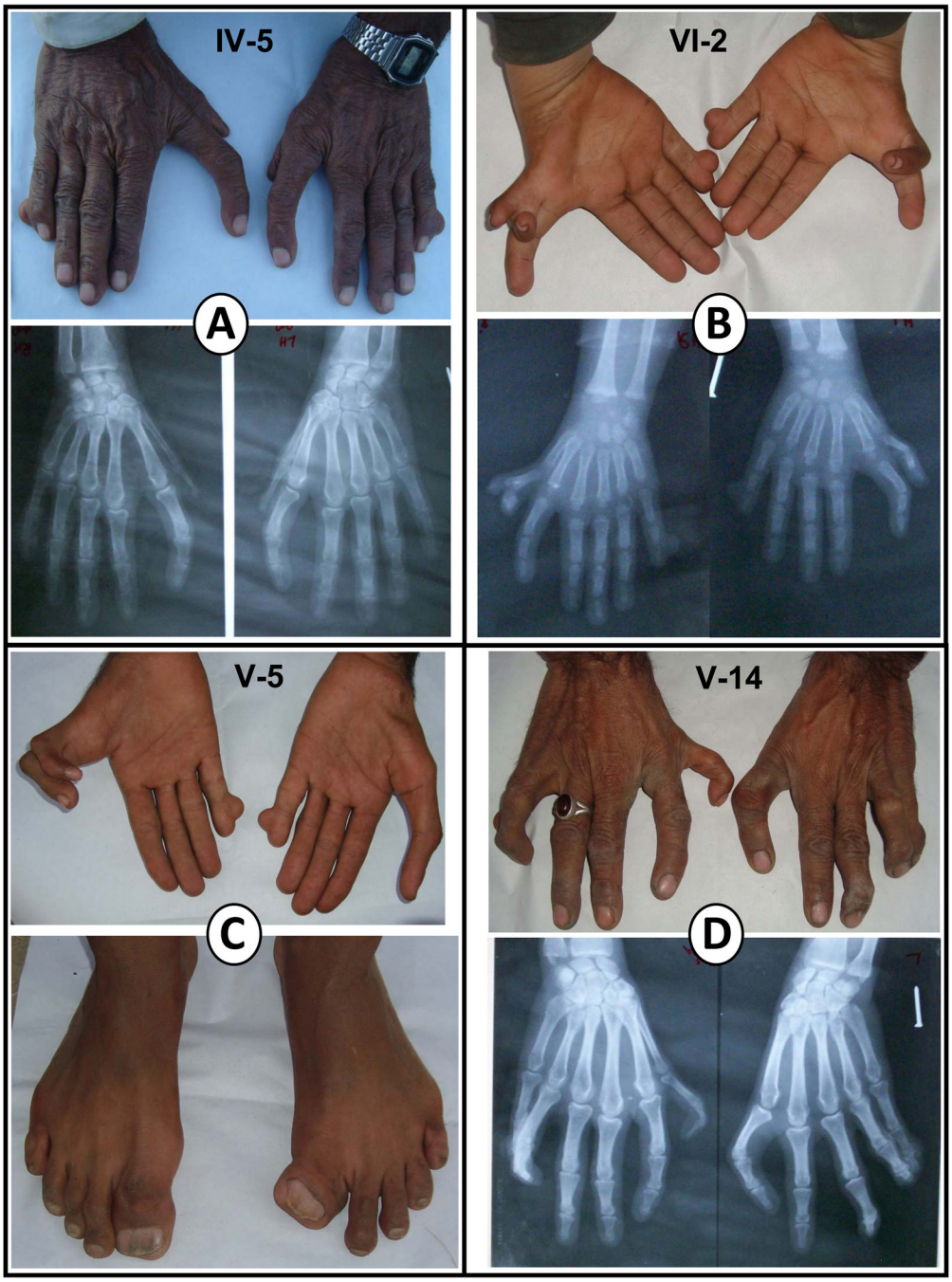

Figure 2.
Phenotypic presentation in the affected family subjects. Triphalangeal thumb of essentially non-opposable nature is associated with duplication or triplication of preaxial digital ray (A, B, D). In addition to the pre- or post-axial polydactyly phenotypes, this family displays a cutaneous, knob-like tag on the 5th finger that contains no nail or bony element (A, B, C). Malformation in the feet was represented only by bifid halluces (C).

Table I

Phenotypic spectrum in the affected subjects

Clinical variant	IV-5	V-5	V-6	V-7	VI-2	VI-5	VI-9	VI-10	V-14
Triphalangeal thumb	+	+	+	+	+	+	+	+	+
Varus curvature of 1st digit	+	+	+	+	+	+	+	+	+
Preaxial polydactyly	+	R	R		+, 1	+			R
Extra digit	NF	NF	NF		NF	NF			F
Nail on extra digit		+	+		+	+			+
Postaxial polydactyly	+	+	+	+, 2	+	+	+	L	+
Clinodactyly of 5th digit	+					+		+	
Distal phalangeal hypoplasia of mesoaxial fingers		+			+				+
Syndactyly/osseous fusion of fingers 4–5			+	+, 4			+		+, 3
Bifid halluces		+							

(F = functional; L = in left hand only; NF = non-functional; R = in right hand only; 1 = seven digits in right hand; 2 = complete postaxial digit fused with digits 4–5; 3 = nails of syndactylous digits fused; 4 = fusion of digits 4–5–6)