RESEARCH ARTICLE

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A Novel Mutation (g.106737G>T) in Zone of Polarizing Activity Regulatory Sequence (ZRS) Causes Variable Limb Phenotypes in Werner Mesomelia

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Werner mesomelia is characterized by a sequence variation in the specific region (position 404) of the enhancer ZRS of *SHH*. The phenotype comprises variable mesomelia, abnormalities of the thumb and great toe and supernumerary digits. We describe extensive variation in limb phenotype in a large family and report on a novel sequence variation NG_009240.1: g.106737G>T (traditional nomenclature: ZRS404G>T) in the ZRS within the *LMBR1* gene. The newly recognized clinical features in this family include small thenar eminence, sandal gap, broad first metatarsals, mesoaxial polydactyly, and postaxial polydactyly. We provide information on 12 affected family members. We review the literature on how a sequence variation in ZRS may cause such diverse phenotypes. © 2014 Wiley Periodicals, Inc.

Key words: preaxial polydactyly; triphalangeal thumb; mesoaxial polydactyly; postaxial polydactyly; five fingered hand; mesomelia; tibial hemimelia; small thenar eminence

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INTRODUCTION

Mutations in regulatory regions of the genes are recognized to cause Mendelian phenotypes [Spielmann and Mundlos, 2013]. An enhancer of Sonic hedgehog (*SHH*, OMIM 600725) that is expressed in the zone of polarizing activity (ZPA) of the limb bud (designated as the ZPA regulatory sequence (ZRS)) lies in the intron 5 of *LMBR1* (OMIM 605522) [Lettice et al., 2003]. Several sequence variations (point mutations, duplications, and rarely a deletion) have been described in the ZRS associated with a variety of limb phenotypes confirming the role of ZRS in limb development by its effect on *SHH* [Wieczorek et al., 2010; Anderson et al., 2012]. These include Acheiropody (OMIM 200500), preaxial polydactyly type II (OMIM 174500), syndactyly type IV (OMIM 186200), triphalangeal thumb type II (OMIM 174500), triphalangeal thumb-polysyndactyly syndrome (OMIM 174500) and Werner mesomelic syndrome (OMIM 188770). Werner mesomelia is characterized by abnormalities of hands and feet with mesomelia and is caused by mutations in a specific location (at position 404; G>A and G>C) of the ZRS region [Lettice et al., 2003; Wieczorek et al., 2010; Cho et al., 2013]. Here we report on a large family demonstrating autosomal dominant Werner mesomelia syndrome with a novel sequence variation (G>T) in the same position of ZRS and describe the intra-familial variation in the limb phenotype.

MATERIAL AND METHODS

A family with 16 affected members demonstrating autosomal dominant inheritance of diverse limb defects was ascertained

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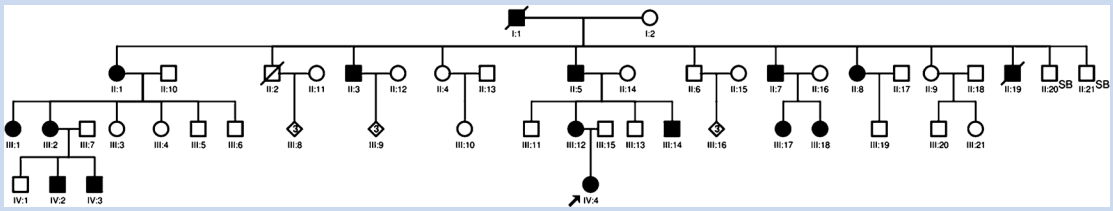

FIG. 1. Pedigree of the family.

(Fig. 1). The diagnosis of a ZRS-related phenotype was made. Clinical details were noted, and limb radiographs and blood samples were obtained from the consenting affected (Individuals IV:4, II:7, III:18, II:5, IV:3, III:12) and unaffected family members (III:11). Photographs of 12 affected individuals and radiographs of eight patients were evaluated (see Supplementary Figures in Supporting Information online). The mutation was tested in six affected members and one healthy family member. The study has the approval of the ethics committee of the institute.

DNA was extracted from 2 ml peripheral blood using DNeasy kit (Qiagen, Valencia, CA). Amplification of ZRS region [Lettice et al., 2003] was performed using the forward: 5′-CCAA-GATTTTTCTGGGAGTAAA-3′ and reverse: 5′-TGATCCATAAC-CATTTCTAAGG-3′ primers. Direct sequencing was carried out using BigDye Terminator v1.1 Cycle (Applied Biosystems, Foster City, CA). Sequences were obtained on ABI Prism 3130 Genetic Analyzer (Applied Biosystems) and were compared with the reference sequence (NG_009240.1).

MUTATION IN ZRS

Sequencing of a 930 bp region of the ZRS region showed a novel heterozygous mutation NG_009240.1: g.106737G>T (traditional nomenclature: ZRS404G>T) in the affected individuals of the family (Fig. 2) which was absent in the unaffected family members. The mutation segregated in an autosomal dominant fashion.

CLINICAL REPORTS

The proband (IV:4) was first seen at 2 months of age. She was born by normal delivery at full term. Her mother was also affected (Individual III:12). The prenatal period was unremarkable except for pregnancy-induced hypertension in the third trimester. The patient's birth weight was 3.2 kg (normal). At 6 months, she had age appropriate motor and social development. On examination, her length (67 cm) and weight (6.2 kg) were normal and OFC was 41 cm (−2 SD). Her facial features were non-dysmorphic. Her hands showed bilateral triphalangeal thumbs and small thenar eminences. There was no mesomelia of upper limbs. She had bilateral mesomelia of the legs and club feet. Her right leg was severely affected with absent tibia. She also had preaxial polydactyly in the feet with seven toes bilaterally. Clinical evaluation showed normal respiratory system, liver, spleen, and central nervous system. She had unremarkable blood count and serum biochemistry. Echocardiogram was normal.

Individual III:12 was a 30-year-old female with a normal height (152 cm). Her OFC was 52 cm (−2 SD) and weight was 46 kg (3rd–10th centile). Her history was notable for normal growth and

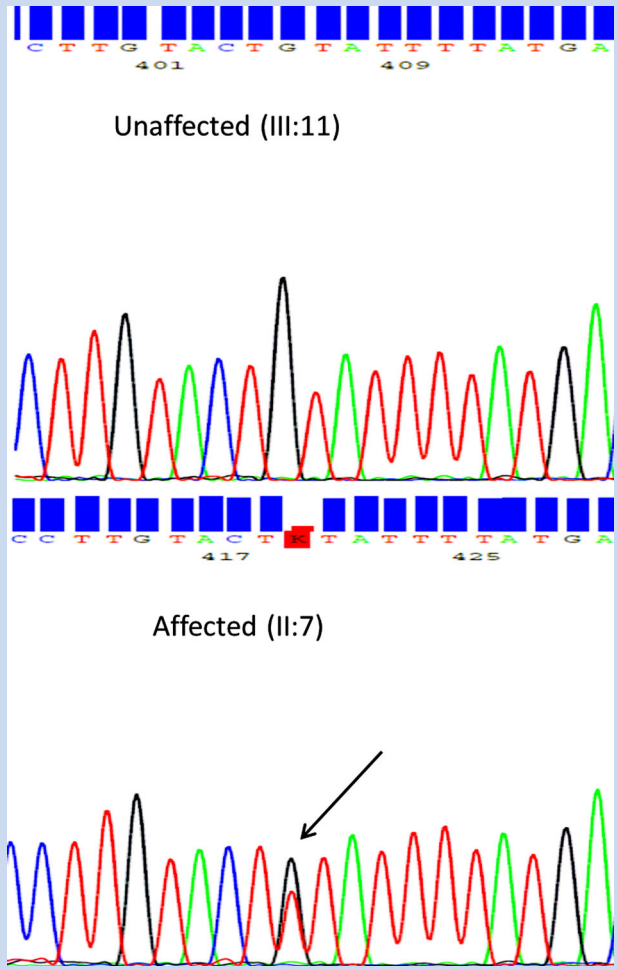

FIG. 2. Electropherogram showing the heterozygous mutation G>T in ZRS at position g.106737 in the affected Individual II:7 (black arrow, bottom) compared to unaffected Individual III:11 in the family (top).

development except for her limb anomalies. She had an apparently normal intellect. She showed bilateral complete cutaneous syndactyly of F1 and F2 resulting in a fused digit that also retained opposition. There was a wide gap between the fused digits and rest of the fingers. She had small thenar eminences and small wrists. A small radius was noted bilaterally. She had normal leg bones. Her feet showed bilateral pre-axial polydactyly with seven toes on right side and eight on left. The first four toes on the left and three on right had cutaneous syndactyly. Six metatarsals on either side with polydactylous first and second digital rays on the left foot and polydactylous second digital ray on the right foot were observed. Her examination was otherwise unremarkable.

Individual III:14 was seen at 26 years of age. He had unremarkable birth and development. His height was normal (165 cm), weight 52 kg (<3rd centile) and OFC 53 cm (−2 SD). He had bilateral mesomelia of upper limbs with small radius and ulna. The thenar eminences were small. There was cutaneous syndactyly of F1 and F2 and limited extension at interphalangeal joints (camptodacytly). He had bilateral broad great toes, sandal gap, and broad halluces on a radiograph.

Individual II:5 presented at 61 years of age. His height was normal (162.5 cm), OFC 54 cm (−2 SD) and weight 48.5 kg (<3rd centile). He had radial deviation of left hand with a small radius and bowing of the ulna. He had bilateral small first fingers with small thenar eminences and decreased flexion creases. He had bilateral broad great toes, sandal gap, and broad halluces on a radiograph. He did not have any other health problems.

Individual II:7 was 49 years old and had a height of 155.5 cm (−3 SD), weight 43.5 kg (<3rd centile), and OFC 51.5 cm (−3 SD). He presented with bilateral symmetrical triphalangeal thumbs. His forearms, legs, and feet were normal except for bilateral broad first metatarsals on radiograph. The rest of his clinical examination was unremarkable. He had two affected daughters aged 7 years (Individual III:17) and 4 years (Individual III:18).

Individual III:18 had a normal birth and development. Her length was 106 cm (normal for 5 years of age at which she was seen), weight 14.5 kg (25th centile) and OFC 48 cm (−3 SD). Echocardiogram showed an asymptomatic 4 mm perimembranous ventricular septal defect. She had non-opposable triphalangeal thumbs with preaxial polydactyly type B on right side. Forearm and leg bones were normal. Her feet showed broad halluces and a sandal gap bilaterally. A bifid left first toe nail was also noted.

Individual IV:2 was a 25-year-old male with a height of 158 cm (−2 SD). He weighed 51 kg (<3rd centile). His OFC was normal (56 cm). He had apparently normal intellectual functioning. His systemic examination did not disclose any abnormality. He demonstrated complex pre-axial, mesoaxial and postaxial polydactyly with 29 digits in hands and feet combined. He had a non-opposable triphalangeal thumb with mesoaxial and postaxial polydactyly of the left hand. Pre-axial polydactyly and cutaneous syndactyly F1-2 and F5-6 with mesoaxial polydactyly in right hand were observed. Bilateral small thenar eminences were also seen. His wrists showed small carpal bones on radiographs on either side. Bones of forearm and leg were normal. He showed phalangeal type of postaxial polydactyly with incomplete mesoaxial polydactyly on the left and phalangeal type of pre-axial polydactyly with incomplete central polydactyly on the right hand. He had seven digits on the left foot and eight digits on the right. Radiographs of the feet showed mesoaxial polydactyly in both feet. He also had postaxial polydactyly on the right foot.

Individual IV:3 was a 25-year-old male. He had bilateral non-opposable triphalangeal thumbs with postaxial polydactyly on the left and pre-axial polydactyly on the right. His height (173 cm) and OFC (54.5 cm) were normal. His weight was 57 kg (3rd–10th centile). He had preaxial polydactyly with a short, triplicated hallux on the left foot and mesoaxial polydactyly on the right foot. He did not have any other abnormality.

Clinical photographs and anthropometric data were provided by the family members for four other affected individuals (II:8, III:1, III:2, and III:17). Hence other clinical details were not available. Individual II:8 was a 43-year-old female. She weighed 45 kg (∼3rd centile). Her height was 132 cm (−5 SD) and OFC was 52 cm (−2 SD). Photographs of II:8 showed bilateral cutaneous syndactyly of small F1 and F2. She had small thenar eminences. Severe mesomelia of the right leg was seen. She also had bilateral clubfeet and pre-axial polydactyly of the feet.

Individual III:1 was noted to have bilateral pre-axial polydactyly with non-opposable triphalangeal thumbs and polydactyly in both feet.

Individual III:2 was 45 years of age with a height of 145 cm (−3 SD), weight 50 kg (10th–25th centile), and OFC of 52 cm (−2 SD). She had non-opposable triphalangeal thumb on the left hand (five fingers). Her right hand had preaxial syndactyly. Broad halluces with a fused first left toe nail and a bifid firth first toe nail were noted.

Individual III:17 was a 7-year-old girl with a height of 113 cm (−2 SD), weight 20 kg (∼10th centile) and a normal OFC (56 cm). She had bilateral symmetrical non-opposable triphalangeal thumbs. Broad halluces and sandal gaps were noted bilaterally on photographs. She had a fused left first toe nail.

SUMMARY OF FINDINGS

The affected individuals had phenotypes limited to the limbs. The axial skeleton was normal. All affected relatives had a normal face and apparently normal intellect, although formal testing was not performed. One individual (III:18) had a small ventricular septal defect. The rhizomelic segments of the limbs were normal. None of the patients had undergone surgical correction. The disorder showed complete penetrance in the family.

Forearms

Mesomelia in the upper limbs with varying degrees of small radius was observed in the family (Fig. 3). Shortening of the forearms was noted in four patients. It was severe and asymmetric in Individual II:5, mild in IV:3 and moderate in III:14 (see Supplementary Figures in Supporting Information online). Normal forearms were also observed. The radius showed mild to severe reduction, and the ulna showed bowing and shortening in severe cases. The wrists were small with small and disorganized carpal bones (II:5, III:12, III:14, and IV:2).

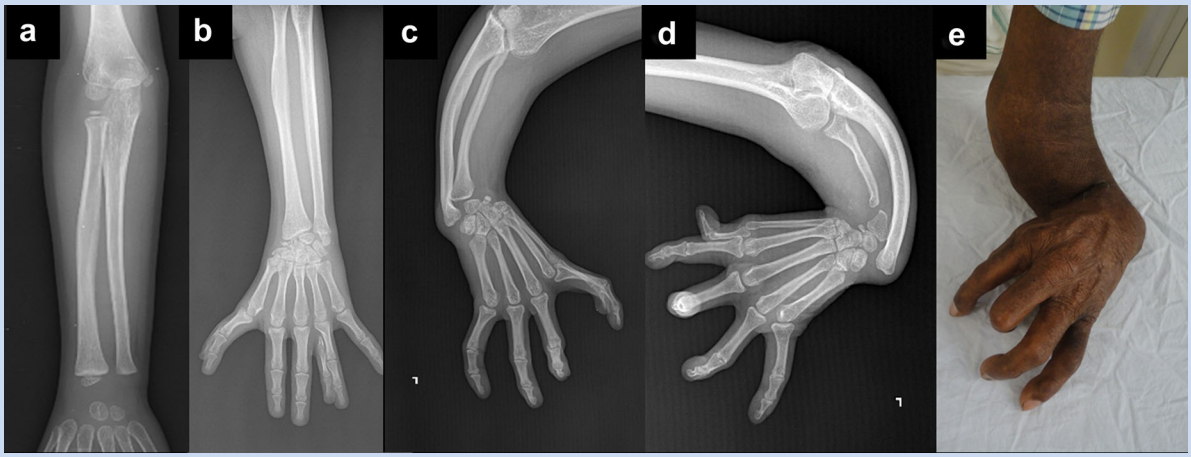

FIG. 3. Radiographs of the forearm showing normal forearm (Individual III:18) (a), mild radius bowing (Individual IV:2) (b), moderately bowed small radius and a small ulna (Individual III:14) (c), and a small radius with bowing of the ulna (Individual II:5) (d). Clinical photograph of radial deviation of hand (Individual II:5) (e).

Hands

The total number of fingers ranged from five to seven (including syndactylous digits). The thumbs were affected in all individuals (Figs. 4 and 5). We noted triphalangeal thumb (with or without preaxial polydactyly) as the most common feature in this family. This often gave rise to the phenotype of a five fingered hand as it was found together with loss of opposition and small thenar eminence (Fig. 4h). Occasionally the thumb had either two or three phalanges and was syndactylous with the second finger (Figs. 4g and 5e). Rarely, the thumbs were small with cutaneous syndactyly with the second digit in a hand with only five digits (Fig. 4k). A small thenar eminence was associated with loss of opposition in most of thumbs

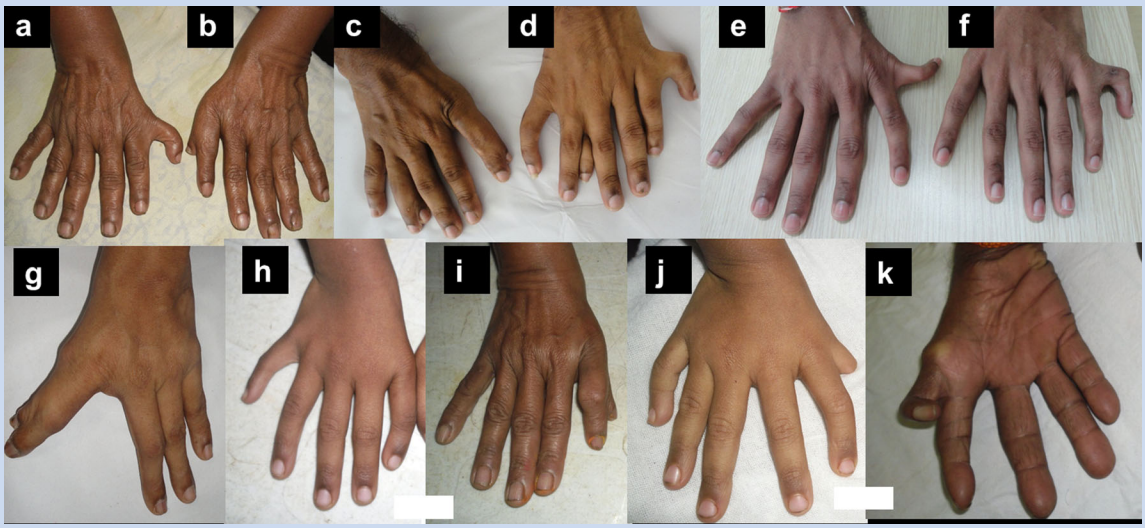

FIG. 4. Clinical photographs of hands displaying a wide spectrum of phenotypic variation: (a) triphalangeal thumb with preaxial polydactyly (Individual III:1), (b) preaxial polydactyly and cutaneous syndactyly with triphalangeal thumb (Individual III:1), (c) preaxial polydactyly and syndactyly (F1-2) with mesoaxial polydactyly (Individual IV:2), (d) triphalangeal thumb with mesoaxial and postaxial polydactyly (Individual IV:2), (e) triphalangeal thumb with preaxial polydactyly, (Individual IV:3), (f) triphalangeal thumb with postaxial polydactyly (Individual IV:3), (g) Complete syndactyly F1,F2 (Individual III:12), (h) triphalangeal thumb (non-opposable thumb) giving the appearance of a five fingered hand with no thumb (Individual III:17), (i,j) different phenotypes of preaxial polydactyly and cutaneous syndactyly with triphalangeal thumbs (Individuals III:2 and III:18) and (k) rudimentary F1 with small thenar eminence (Individual II:5).

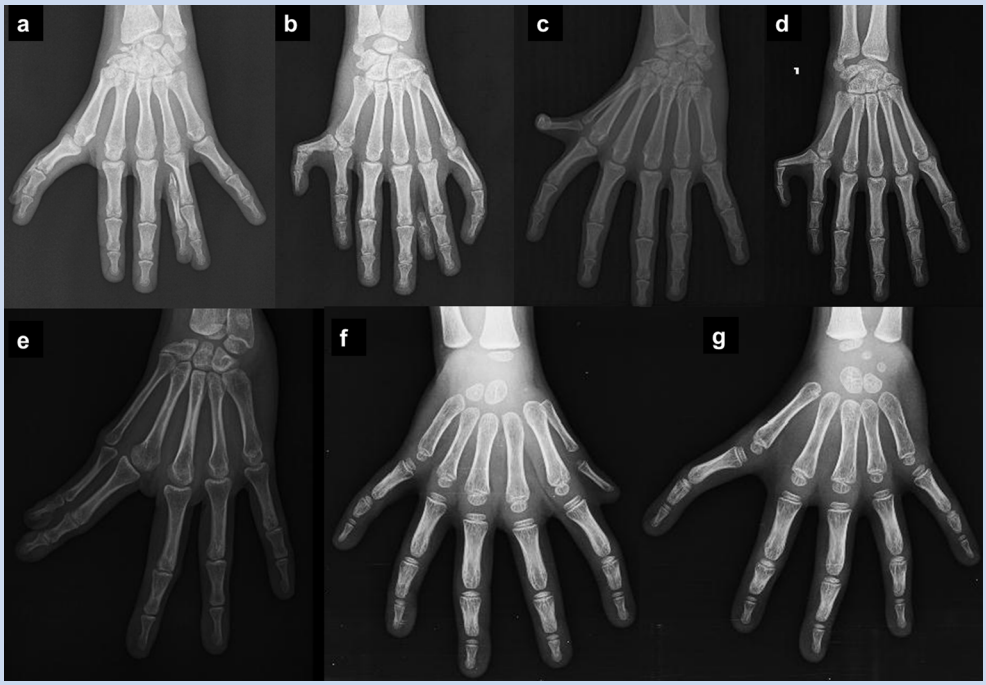

FIG. 5. Hand radiographs of various phenotypes: (a) preaxial and mesoaxial polysyndactyly involving the phalanges (Individual IV:2), (b) central polysyndactyly and postaxial polydactyly involving the phalanges (Individual IV:2), (c) preaxial polydactyly involving the metacarpals (Individual IV:3), (d) postaxial polydactyly involving the phalanges (Individual IV:4), (e) complete cutaneous syndactyly of F1,F2 (Individual III:12), (f) preaxial polydactyly type B (Individual III:18), (g) triphalangeal thumb (Individual III:18).

to the first digits except in Individual III:12 (Fig. 4g) where the first two digits of the hand with five digits still retained some opposition.

Preaxial polydactyly of the hands was a common feature (Fig. 4 a, b, e, f, i, and j). The size ranged from a small rudimentary digit to a fully identifiable digit-like structure on either side. Two individuals had postaxial polydactyly (Fig. 4d, f). Mesoaxial polydactyly was seen in Individual IV:2 (Fig. 4c, d).

Legs

Two of the patients had significant mesomelia of the lower limbs (see supplementary figures—in supporting information online). All except these two patients (II:8 and IV:4) had normally functioning legs (Fig. 6). These two individuals also had clubfeet. The mesomelia was asymmetric in both of them with varying degrees of tibial involvement. The complete absence of the right tibia and small left tibia were noted in IV:4. Individual II:8 had short right leg and slender left leg. One individual had obvious mesomelia with normal function (IV:2). However, exact limb measurements were not available. The fibula appeared normal in morphology in radiographed individuals, although we did not measure the length.

Feet

The great toes were either broad (with broad metatarsal) or affected with preaxial polydactyly and sandal gap (Figs. 7 and 8). Bifid and fused nails of the first toe were also noted in the family. Preaxial polydactyly was manifested as either an extra rudimentary digit or varying degree of duplication and even triplication of the left first ray (Fig. 8a, c, e, and f). Mesoaxial polydactyly was also noted in the feet (Fig. 8b, e). Postaxial polydactyly was found in one person (Fig. 8a).

The most complex phenotype of the hands and feet was seen in Individual IV:2 who had 29 digits, preaxial, postaxial, and mesoaxial polydactyly of hands and feet and complete syndactyly of first and second digits in hands and feet (see Supplementary Figures in Supporting Information online).

DISCUSSION

We report on a four-generation family with Werner mesomelic dysplasia due to a novel nucleotide change in ZRS region of *LMBR1*. The family exhibited wide intra-familial variability, but complete penetrance.

The affected individuals had varying degrees of mesomelia with mild to severe underdevelopment of the radius and ulna often with radial deviation of hand. We also noted small thenar eminences in the family. The wrists manifested disorganized, small carpal bones. Digital anomalies included preaxial polydactyly of the hands, triphalangeal thumb, mesoaxial polydactyly of fingers, postaxial polydactyly of hands, and preaxial, mesoaxial, or postaxial polydactyly in feet with number of digits varying between five and eight. The polydactyly was either a partial or complete digit, sometimes with duplication of metacarpal or metatarsal bones. Other man-

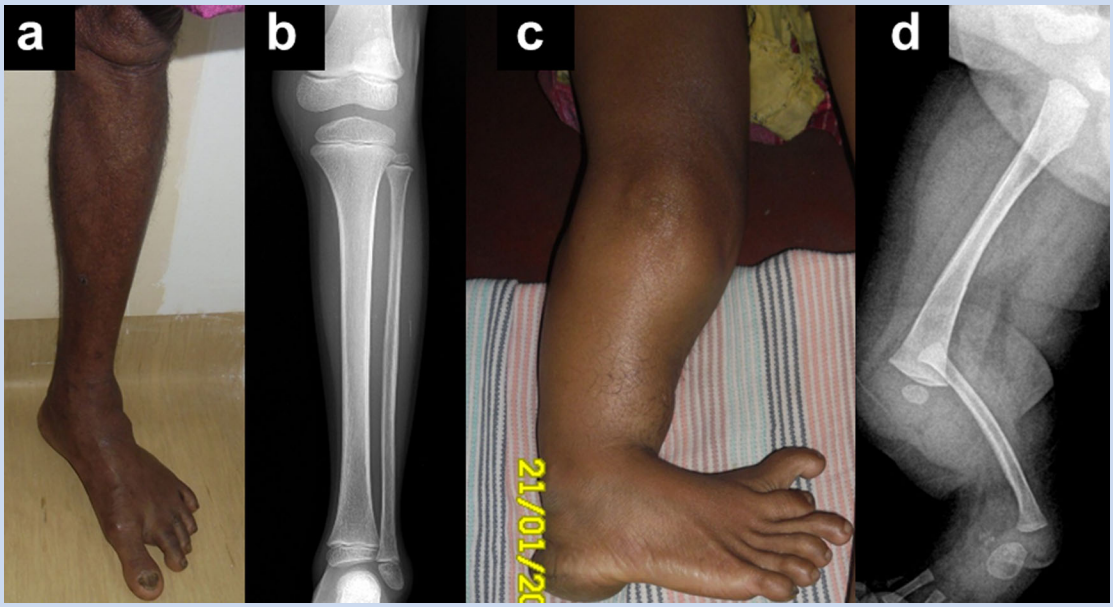

FIG. 6. Clinical photograph (a) and radiograph (b) of normal leg (Individual II:5), clinical photograph (Individual II:8) shows mesomelia and clubfoot (c) and radiograph (Individual IV:4) (d) demonstrating complete absence of tibia.

ifestations included varying degree of syndactyly of digits, broad halluces, fused nail, bifid nail, sandal gap, and congenital talipes equinovarus. It is interesting to note that the nucleotide change reported here (G>T) is the third possible mutation of the 404th nucleotide of ZRS region with the other two variations (G>A and G>C) being reported earlier in three reports on four patients [Lettice et al., 2003; Wieczorek et al., 2010; Cho et al., 2013]. This suggests that this position is likely to be a mutational hotspot of ZRS.

We compared phenotypes in different affected individuals in this family with the other three families reported to have a mutation in position 404 of the ZRS [Lettice et al., 2003; Wieczorek et al., 2010; Cho et al., 2013]. We observed small thenar eminences (II:5, II:8, III:12, III:14, and IV:4), wide sandal gap (II:5, III:17, and III:18), eight toes (III:12 and IV:2), broad first metatarsal (II:5, II:7, III:14, III:17, and III:18), syndactyly F1-2 with retained opposition (III:12), mesoaxial polydactyly of hands (IV:2), mesoaxial polydactyly of feet (III:12, IV:2, and IV:3), postaxial polydactyly of hands (IV:2 and IV:3) and postaxial polydactyly of foot (IV:2) as novel features associated with Werner mesomelia.

We considered Laurin–Sandrow syndrome (OMIM 135750) in the differential diagnosis. This condition is characterized by absent tibia and radius, mirror image polydactyly, and a characteristic abnormality of the nose. A definitive genetic cause for this condition is yet to be identified. The noses were unremarkable in this family. The phenotype of a 35-bp deletion in exon 31 of *PITX1* gene (OMIM 602149) shares a somewhat similar phenotype with deficiency of long bones and mirror image polydactyly (OMIM 119800)

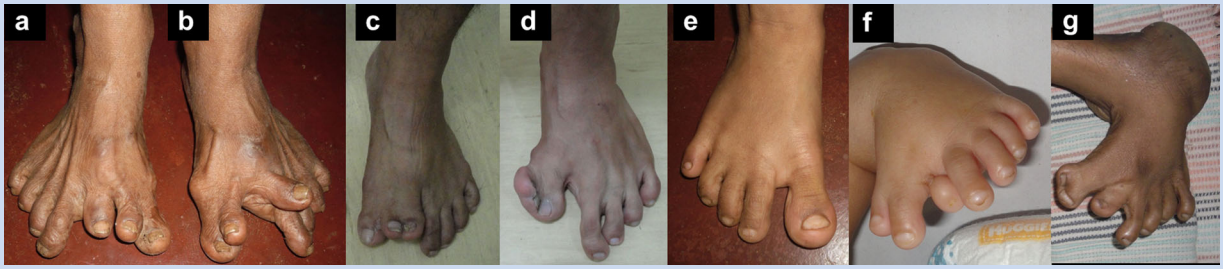

FIG. 7. Clinical photographs of feet: (a,b) mesoaxial polydactyly (Individual III:1), (c) mesoaxial polysyndactyly (Individual IV:2), (d) triplicate hallux (Individual IV:3), (e) broad hallux with increased sandal gap (Individual III:18), (f) preaxial polydactyly with seven toes (mimicking mirror-image foot) (Individual IV:4) and (g) preaxial polysyndactyly with fused nails and broad T1 (Individual II:8).

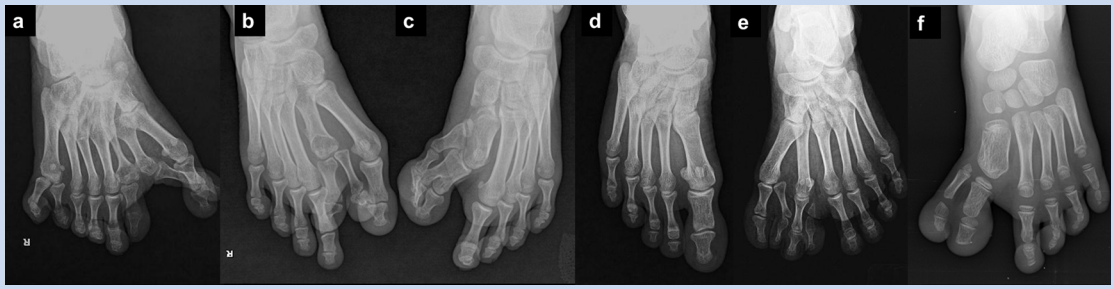

FIG. 8. Foot radiographs of several affected individuals: (a) metatarsal type of preaxial polydactyly and cutaneous syndactyly with phalangeal type postaxial polydactyly (Individual IV:2), (b) mesoaxial polydactyly and cutaneous syndactyly (Individual IV:3), (c) all the phalanges of the first toe are triplicated with short first metatarsal (Individual IV:4), (d) broad hallux (Individual III:14), (e) duplicated T1 and T2 with eight toes (Individual III:12) and (f) duplicated hallux with broad first metatarsal (Individual III:18).

[Klopocki et al., 2012]. However, a close evaluation of the hand phenotype in of the present patients did not indicate a typical mirror image polydactyly.

Several limb phenotypes have been ascribed to ZRS. Acheiropody (OMIM 200500) is caused by a deletion in this region [Ianakiev et al., 2001]. Heterogenous point mutations and duplications in this region result in overlapping phenotypes affecting the hands and feet [Lettice et al., 2003; Gurnett et al., 2007; Furniss et al., 2008; Klopocki et al., 2008; Sun et al., 2008; Semerci et al., 2009; Farooq et al., 2010; Wieczorek et al., 2010; Albuisson et al., 2011; Al-Qattan et al., 2012; Laurell et al., 2012; VanderMeer et al., 2012; Cho et al., 2013]. There are other reports on hand phenotypes (familial cutaneous syndactyly and preaxial polydactyly) that map to this region but no mutations have been reported [Li et al., 2009; Al-Qattan et al., 2013]. The variety of mutations and phenotypes probably highlight the complex interaction of ZRS and other regulatory elements in limb development through SHH [Lettice et al., 2008, 2012].

An effort has been made to correlate the genotype with the phenotype [Wieczorek et al., 2010; Anderson et al., 2012]. Point mutations (type Ia), other than those at position 404, of ZRS cause triphalangeal thumb and preaxial polydactyly often leading to the appearance of a five fingered hand. This phenotype is confined to the hand. Mutations at position 404 of ZRS define Werner mesomelia (type Ib), which includes small tibias, polydactyly and cutaneous syndactyly, and triphalangeal thumb and may have involvement of radius. The third phenotype (type II) is caused by duplications in the ZRS region with extensive cutaneous syndactyly and polydactyly [Sun et al., 2008; Wu et al., 2009]. Werner mesomelia probably demonstrates the widest and overlapping phenotypic spectrum when compared with other types. With additional features being described here, the spectrum of Werner mesomelia is now extended to comprise preaxial polydactyly, triphalangeal thumb, mesoaxial polydactyly, syndactyly, postaxial polydactyly, mesomelia of upper and lower limbs, and involvement of radius and tibia. It appears that point mutations at other locations and duplications are less likely to be associated with mesomelia. The severity of the syndactyly appears less with point mutations than duplications [Sun et al., 2008]. We agree that a genotype-phenotype correlation is not apparent and as suggested earlier, they may be collectively referred to as ZRS-associated syndromes [Wieczorek et al., 2010].

It is quite complex and not clearly understood how a sequence variation in the non-coding region (ZRS) causes the limb phenotypes in humans. The ZRS is a highly conserved extragenic sequence in lower animals and regulates the expression of SHH [Anderson et al., 2012; Hill and Lettice, 2013]. The *SHH* gene has several *cis* regulatory elements that determine temporal and spatial patterns of its expression [Jeong et al., 2006]. The ZRS is the farthest regulator that is located inside the intron 5 of *LMBR1*, 850 kb upstream from the *SHH* gene. The SHH protein acts as a morphogen and is found in high concentrations in ZPA of the developing limb bud. This pattern of expression appears to regulate the number of digits and their identity. Ectopic expression of SHH underlies the pathogenicity of a mutation in ZRS [Lettice et al., 2002, 2003, 2008; Furniss et al., 2008]. Given the current understanding of the SHH signaling pathway, it appears that disturbances in the amount and spatiotemporal of activity of SHH are likely to result in digits losing their identity (triphalangeal thumb), number (polydactyly), position (pre-, meso-, and post-axial polydactyly), and varying extents of cutaneous syndactyly.

The molecular mechanisms that cause limb anomalies due to a mutation in ZRS have been reviewed previously [Hill and Lettice, 2013]. It is postulated that mutations in ZRS would affect the binding of different transcription factors. Studies have shown that GABPα and ETS1 bind to the ZRS in the limb bud and play a role in establishing the expression boundaries of SHH in the posterior limb bud [Lettice et al., 2012]. The ectopic expression is probably repressed by two more transcription factors, ETV4 and ETV5. All four of these transcription factors are members of the ETS family and contribute to the spatial patterning of SHH expression [Hollenhorst et al., 2007; Mao et al., 2009]. In addition, HnRNP U is expected to affect the interaction between the ZRS and the promoter of *SHH* [Zhao et al., 2009].

Some insights are available for long bone (mesomelic) involvement in Werner mesomelia. The 404G>C mutation has been shown to produce a high concentration of SHH in the posterior limb bud interfering with the development of tibia in transgenic

experiments [Lettice et al., 2008]. A similar phenomenon may explain the involvement of the anterior element of upper limb (radius) by the same mutation. However, the factors that mediate this activity are unidentified. Cutaneous syndactyly may be partly attributed to the impaired apoptosis of inter-digital tissue by temporal mis-expression of SHH [Lettice et al., 2011].

Further studies are required in animal models to understand how a mutation is ZRS causes postaxial polydactyly. It is interesting to note that previous reports have described preaxial polydactyly and not postaxial polydactyly in patients with Werner mesomelia. *GLI3* (OMIM 165240) and *ALX4* (OMIM 605420) are also known to repress SHH expression [Qu et al., 1997]. Mutations in *GLI3* are known to cause postaxial polydactyly, syndactyly, preaxial polydactyly and broad thumbs and great toes (Greig cephalopolysyndactyly syndrome, OMIM 175700 and Pallister–Hall syndrome, OMIM 146510). Hence it is plausible that an increased expression of SHH in Werner mesomelia has a similar effect.

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REFERENCES

Albuisson J, Isidor B, Giraud M, Pichon O, Marsaud T, David A, Le Caignec C, Bezieau S. 2011. Identification of two novel mutations in Shh long-range regulator associated with familial pre-axial polydactyly. Clin Genet 79:371–377.

Al-Qattan MM, Al Abdulkareem I, Al Haidan Y, Al Balwi M. 2012. A novel mutation in the SHH long-range regulator (ZRS) is associated with preaxial polydactyly, triphalangeal thumb, and severe radial ray deficiency. Am J Med Genet Part A 158A:2610–2615.

Al-Qattan MM, Shamseldin HE, Al Mazyad M, Al Deghaither S, Alkuraya FS. 2013. Genetic heterogeneity in type III familial cutaneous syndactyly and linkage to chromosome 7q36. Am J Med Genet Part A 161:1579–1584.

Anderson E, Peluso S, Lettice LA, Hill RE. 2012. Human limb abnormalities caused by disruption of hedgehog signaling. Trends Genet 28:364–373.

Cho TJ, Baek GH, Lee HR, Moon HJ, Yoo WJ, Choi IH. 2013. Tibial hemimelia-polydactyly-five-fingered hand syndrome associated with a 404 G>A mutation in a distant sonic hedgehog cis-regulator (ZRS): A case report. J Pediatr Orthop B 22:219–221.

Farooq M, Troelsen JT, Boyd M, Eiberg H, Hansen L, Hussain MS, Rehman S, Azhar A, Ali A, Bakhtiar SM, Tommerup N, Baig SM, Kjaer KW. 2010. Preaxial polydactyly/triphalangeal thumb is associated with changed transcription factor-binding affinity in a family with a novel point mutation in the long-range cis-regulatory element ZRS. Eur J Hum Genet 18:733–736.

Furniss D, Lettice LA, Taylor IB, Critchley PS, Giele H, Hill RE, Wilkie AO. 2008. A variant in the sonic hedgehog regulatory sequence (ZRS) is associated with triphalangeal thumb and deregulates expression in the developing limb. Hum Mol Genet 17:2417–2423.

Gurnett CA, Bowcock AM, Dietz FR, Morcuende JA, Murray JC, Dobbs MB. 2007. Two novel point mutations in the long-range SHH enhancer in three families with triphalangeal thumb and preaxial polydactyly. Am J Med Genet Part A 143:27–32.

Hill RE, Lettice LA. 2013. Alterations to the remote control of Shh gene expression cause congenital abnormalities. Philos Trans R Soc Lond B Biol Sci 368:20120357.

Hollenhorst PC, Shah AA, Hopkins C, Graves BJ. 2007. Genome-wide analyses reveal properties of redundant and specific promoter occupancy within the ETS gene family. Genes Dev 21:1882–1894.

Ianakiev P, van Baren MJ, Daly MJ, Toledo SP, Cavalcanti MG, Neto JC, Silveira EL, Freire-Maia A, Heutink P, Kilpatrick MW, Tsipouras P. 2001. Acheiropodia is caused by a genomic deletion in C7orf2, the human orthologue of the Lmbr1 gene. Am J Hum Genet 68:38–45.

Jeong Y, El-Jaick K, Roessler E, Muenke M, Epstein DJ. 2006. A functional screen for sonic hedgehog regulatory elements across a 1 Mb interval identifies long-range ventral forebrain enhancers. Development 133:761–772.

Klopocki E, Ott CE, Benatar N, Ullmann R, Mundlos S, Lehmann K. 2008. A microduplication of the long range SHH limb regulator (ZRS) is associated with triphalangeal thumb-polysyndactyly syndrome. J Med Genet 45:370–375.

Klopocki E, Kahler C, Foulds N, Shah H, Joseph B, Vogel H, Luttgen S, Bald R, Besoke R, Held K, Mundlos S, Kurth I. 2012. Deletions in PITX1 cause a spectrum of lower-limb malformations including mirror-image polydactyly. Eur J Hum Genet 20:705–708.

Laurell T, Vandermeer JE, Wenger AM, Grigelioniene G, Nordenskjold A, Arner M, Ekblom AG, Bejerano G, Ahituv N, Nordgren A. 2012. A novel 13 base pair insertion in the sonic hedgehog ZRS limb enhancer (ZRS/LMBR1) causes preaxial polydactyly with triphalangeal thumb. Hum Mutat 33:1063–1066.

Lettice LA, Horikoshi T, Heaney SJ, van Baren MJ, van der Linde HC, Breedveld GJ, Joosse M, Akarsu N, Oostra BA, Endo N, Shibata M, Suzuki M, Takahashi E, Shinka T, Nakahori Y, Ayusawa D, Nakabayashi K, Scherer SW, Heutink P, Hill RE, Noji S. 2002. Disruption of a long-range cis-acting regulator for Shh causes preaxial polydactyly. Proc Natl Acad Sci USA 99:7548–7553.

Lettice LA, Heaney SJ, Purdie LA, Li L, de Beer P, Oostra BA, Goode D, Elgar G, Hill RE, de Graaff E. 2003. A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. Hum Mol Genet 12:1725–1735.

Lettice LA, Hill AE, Devenney PS, Hill RE. 2008. Point mutations in a distant sonic hedgehog cis-regulator generate a variable regulatory output responsible for preaxial polydactyly. Hum Mol Genet 17:978–985.

Lettice LA, Daniels S, Sweeney E, Venkataraman S, Devenney PS, Gautier P, Morrison H, Fantes J, Hill RE, FitzPatrick DR. 2011. Enhancer-adoption as a mechanism of human developmental disease. Hum Mutat 32:1492–1497.

Lettice LA, Williamson I, Wiltshire JH, Peluso S, Devenney PS, Hill AE, Essafi A, Hagman J, Mort R, Grimes G, DeAngelis CL, Hill RE. 2012. Opposing functions of the ETS factor family define Shh spatial expression in limb buds and underlie polydactyly. Dev Cell 22:459–467.

Li H, Wang CY, Wang JX, Wu GS, Yu P, Yan XY, Chen YG, Zhao LH, Zhang YP. 2009. Mutation analysis of a large Chinese pedigree with congenital preaxial polydactyly. Eur J Hum Genet 17:604–610.

Mao J, McGlinn E, Huang P, Tabin CJ, McMahon AP. 2009. Fgf-dependent Etv4/5 activity is required for posterior restriction of Sonic Hedgehog and promoting outgrowth of the vertebrate limb. Dev Cell 16:600–606.

Qu S, Niswender KD, Ji Q, van der Meer R, Keeney D, Magnuson MA, Wisdom R. 1997. Polydactyly and ectopic ZPA formation in Alx-4 mutant mice. Development 124:3999–4008.

Semerci CN, Demirkan F, Ozdemir M, Biskin E, Akin B, Bagci H, Akarsu NA. 2009. Homozygous feature of isolated triphalangeal thumb-preaxial polydactyly linked to 7q36: No phenotypic difference between homozygotes and heterozygotes. Clin Genet 76:85–90.

Spielmann M, Mundlos S. 2013. Structural variations, the regulatory landscape of the genome and their alteration in human disease. Bioessays 35:533–543.

Sun M, Ma F, Zeng X, Liu Q, Zhao XL, Wu FX, Wu GP, Zhang ZF, Gu B, Zhao YF, Tian SH, Lin B, Kong XY, Zhang XL, Yang W, Lo WH, Zhang X. 2008. Triphalangeal thumb-polysyndactyly syndrome and syndactyly type IV are caused by genomic duplications involving the long range, limb-specific SHH enhancer. J Med Genet 45:589–595.

VanderMeer JE, Afzal M, Alyas S, Haque S, Ahituv N, Malik S. 2012. A novel ZRS mutation in a Balochi tribal family with triphalangeal thumb, preaxial polydactyly, post-axial polydactyly, and syndactyly. Am J Med Genet Part A 158A:2031–2035.

Wieczorek D, Pawlik B, Li Y, Akarsu NA, Caliebe A, May KJ, Schweiger B, Vargas FR, Balci S, Gillessen-Kaesbach G, Wollnik B. 2010. A specific mutation in the distant sonic hedgehog (SHH) cis-regulator (ZRS) causes Werner mesomelic syndrome (WMS) while complete ZRS duplications underlie Haas type polysyndactyly and preaxial polydactyly (PPD) with or without triphalangeal thumb. Hum Mutat 31:81–89.

Wu L, Liang D, Niikawa N, Ma F, Sun M, Pan Q, Long Z, Zhou Z, Yoshiura K, Wang H, Sato D, Nishimura G, Dai H, Zhang X, Xia J. 2009. A ZRS duplication causes syndactyly type IV with tibial hypoplasia. Am J Med Genet Part A 149A:816–818.

Zhao J, Ding J, Li Y, Ren K, Sha J, Zhu M, Gao X. 2009. HnRNP U mediates the long-range regulation of Shh expression during limb development. Hum Mol Genet 18:3090–3097.

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