Clinica Chimica Acta 433 (2014) 195–199

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Novel frame-shift mutations of *GLI3* gene in non-syndromic postaxial polydactyly patients

Zhigang Wang [a,1], Jian Wang [b,c,d,1], Yuchan Li [a], Juan Geng [b,c], Qihua Fu [b,c], Yunlan Xu [a,*], Yiping Shen [b,d,**]

[a] Department of Pediatric Orthopedic, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, Shanghai 200127, PR China
[b] Department of Laboratory Medicine, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, Shanghai 200127, PR China
[c] Institute of Pediatric Translational Medicine, Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine, Shanghai 200127, PR China
[d] Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 27 February 2014
Accepted 13 March 2014
Available online 22 March 2014

Keywords:
Postaxial polydactyly
GLI3 gene
Frame-shift mutations
Non-syndromic polydactyly

ABSTRACT

Polydactyly is a common congenital limb deformity. This anomaly may occur in isolation (non-syndromic) or as part of a syndrome. The glioma-associated oncogene family zinc finger 3 (*GLI3*) is known to be associated with both syndromic and non-syndromic polydactyly. *GLI3* plays a predominant role in the pathogenesis of syndromic polydactyly: mutations have been identified in 68% of patients with Greig cephalopolysyndactyly syndrome and 91% of patients with Pallister–Hall syndrome. The knowledge regarding the contribution of *GLI3* in non-syndromic polydactyly is currently very limited. In this study, we assembled a cohort of individuals of Chinese ethnicity with non-syndromic postaxial polydactyly. We presented the clinical features and molecular evaluations of 19 probands. *GLI3* mutations were identified in 15.8% of probands (3/19) including two novel frame-shift mutations c.3855dupC (p.Met1286HisfsTer18) and c.4141delA (p.Arg1381GlyfsTer38) detected in sporadic cases and one previously reported nonsense mutation (c.1927C > T/p.Arg643Ter) in a familial case. Of note, *GLI3* mutations were exclusively detected in patients with bilateral polydactyly affecting both hands and feet. Three out of five (60%) probands with bilateral polydactyly on both hands and feet carried pathogenic mutations in *GLI3*. Our study demonstrated the role of *GLI3* in a significant fraction of patients with non-syndromic bilateral polydactyly affecting both hands and feet.

1. Introduction

Polydactyly is a very common form of congenital limb deformity noticeable prenatally or immediately after birth. It has an incidence of approximately 1 in 1000 live births [1]. There are many different schemes for polydactyly classification. The Temtamy–McKusick scheme is the simplest and is commonly used by geneticists and dysmorphologists [2,3]. Based on the Temtamy–McKusick scheme, polydactyly can be classified as preaxial in which the extra digit is located outside the thumb or great toe; postaxial in which the extra digit is located outside the fifth digit, and complex types include mesoaxial polydactyly in which the extra digit is located among three central digits [2,3]. Postaxial polydactyly consists of two subtypes, type A and type B. In type A, the extra digit is well-formed and articulates with the fifth or an extra meta-carpal/-tarsal. In type B, the extra digit is rudimentary; it appears as a small protuberance or pedunculated nubbin. Type B is more common than type A. Newer and more sophisticated classification schemes are also available [3]. Polydactyly may occur as an isolated event (non-syndromic) or in association with other anomalies (syndromic) (for reviews, see [3,4]). Most of the polydactyly cases are sporadic, which usually have unilateral presentation, whereas familial cases are often bilateral [5].

Polydactyly is caused by aberrant anterior–posterior patterning of the limb during development. A network of genes is known to be involved with this patterning process [6] and about 100 genes are known to be associated with human polydactyly [4]. The glioma-associated oncogene family zinc finger 3 transcription factor *GLI3* gene (MIM#165240) has been shown to be associated with five distinct polydactyly disorders: three are syndromes with polydactyly as a significant feature: Greig cephalopolysyndactyly syndrome (GCPS) [7], Pallister–Hall syndrome (PHS) [8], and Acrocallosal syndrome (ACLS) [9]; two are non-syndromic polydactyly: postaxial polydactyly type A/B (PAP-A/B) [10] and preaxial polydactyly type-IV (PPD-IV) [11]. A large cohort mutation screening study revealed that *GLI3* played a predominant role in the

Abbreviations: *GLI3*, glioma-associated oncogene family zinc finger 3; GCPS, Greig cephalopolysyndactyly syndrome; PHS, Pallister–Hall syndrome; ACLS, Acrocallosal syndrome; PAP-A/B, postaxial polydactyly type A/B; PPD-IV, preaxial polydactyly type-IV; SAM, Syndactylism, Axis deviation, Metatarsal extension.

* Correspondence to: Y. Xu, Department of Pediatric Orthopedic, Shanghai Children's Medical Center, 1678 Dongfang Road, Shanghai 200127, PR China. Fax: +86 21 58756923.
** Correspondence to: Y. Shen, Department of Laboratory Medicine, Boston Children's Hospital, 300 Longwood Ave., Boston, MA 02115, USA. Tel.: +1 617 553 5832.
E-mail addresses: xu.yunlan@hotmail.com (Y. Xu), yiping.shen@childrens.harvard.edu (Y. Shen).

[1] These authors contributed equally to this work, and should be considered as co-first authors.

http://dx.doi.org/10.1016/j.cca.2014.03.012
0009-8981/

pathogenesis of syndromic polydactyly. Mutations were identified in 68% of patients with GCPS and 91% of patients with PHS [12]. The knowledge regarding the contribution of *GLI3* in non-syndromic polydactyly is currently very limited. Fujioka et al. reported a nonsense mutation in the *GLI3* gene in a Japanese family presented with familial non-syndromic bilateral preaxial polydactyly associated with variable syndactyly features. They did not detect any *GLI3* mutation in four sporadic cases with non-syndromic preaxial polydactyly only affecting the hands (4 unilateral and 1 bilateral) [13]. Johnston et al. reported one individual with *GLI3* mutation among five non-syndromic polydactyly individuals [12]. Jamsheer et al. detected *GLI3* mutations in 3 out of 10 cases of non-syndromic bilateral preaxial polydactyly affecting both hands and feet [14]. In addition, frame-shift mutations in the *GLI3* gene were detected in families with various digit abnormalities including polydactyly without other syndromic features [15–17]. The degree of *GLI3* involvement in the pathogenesis of non-syndromic postaxial polydactyly has not been evaluated.

In this study, we assembled a cohort of individuals of Chinese ethnicity with non-syndromic postaxial polydactyly and screened for GLI3 mutations. We aimed to determine the percentage of *GLI3* mutations found in this population and what genotype–phenotype correlations exist between *GLI3* mutations and polydactyly phenotypes.

2. Materials and methods

2.1. Patients

Nineteen unrelated probands with postaxial polydactyly were recruited at Shanghai Children's Medical Center (SCMC), China. A personal medical history and physical examination were conducted. Patient's hands, feet, and head were radiographed. Measurements included occipitofrontal (head) circumference, head shape, interpupillary distance, inner canthal distance, height, hand length, foot length, and finger length. The genitalia, anus, and throat (epiglottis) were also examined in order to exclude other congenital malformations.

On the basis of clinical findings and the radiographs, all participating probands were diagnosed with isolated postaxial polydactyly except one case with a complex type with mesoaxial polydactyly in the left foot in addition to postaxial polydactyly in the other three limbs (Case SCMC-359) (Table 1). The classification was done based on the Temtamy–McKusick scheme [2]. In addition, they were further classified according to the modified Rayan–Frey classification for hand [18,19] and SAM (Syndactylism, Axis deviation, Metatarsal extension) classification for foot [20]. There were nine individuals who presented with bilateral polydactyly, five of them with both hands and feet affected (including two with a family history) and four with only the feet affected. There are ten individuals with unilateral polydactyly, eight with affected foot and two with an affected hand (none had a family history).

2.2. Genetic analysis

The genomic DNA of the probands and their parents was extracted from peripheral blood samples using a QIAamp Blood DNA Mini kit® (Qiagen GMBH, Hilden, Germany). The primers (Supplementary Table 1) for amplification of the *GLI3* (GenBank accession number: NM_000168.5) gene were designed using Primer3 online software (http://frodo.wi.mit.edu/). All of the 15 exons and exon-intron boundaries were amplified by polymerase chain reaction (Takara Bio, Dalian, China). The amplified products were purified from agarose gel using a QIAquick Gel Extraction Kit (Qiagen GMBH, Hilden, Germany) and sequenced via the ABI3730XL sequencer (Applied Biosystems, Foster City, CA, U.S.).

An additional group of 105 Chinese subjects without skeletal deformities were recruited for examining the allele frequencies in ethnicity

Table 1
The clinical feature and genetic analysis of nineteen Chinese probands with isolated postaxial polydactyly.

Proband	Gender	Age	Clinical feature	Classification-1[a]	Classification-2[b]	Family history	*GLI3* Mutation
SCMC-001	Girl	1Y	Hand bilateral	Postaxial-A	Type V (left)	Yes	c.1927C > T/p.Arg643X
				Postaxial-A	Type IIIA (right)		
			Foot bilateral	Postaxial-B	$S_1A_1M_1$ (left)		
				Postaxial-A	$S_0A_2M_2$ (right)		
SCMC-024	Girl	5Y	Foot right	Postaxial-B	$S_1A_0M_0$		
SCMC-025	Boy	8Y	Foot bilateral	Postaxial-A	$S_0A_1M_1$ (bilateral)		
SCMC-033	Boy	2Y	Foot right	Postaxial-B	$S_2A_0M_1$		
SCMC-048	Girl	2 M	Foot bilateral	Postaxial-A	$S_0A_2M_2$ (left)		
				Postaxial-A	$S_0A_2M_1$ (right)		
SCMC-097	Girl	2Y	Foot right	Postaxial-B	$S_1A_1M_0$		
SCMC-101	Girl	2Y	Foot left	Postaxial-B	$S_0A_1M_0$		
SCMC-130	Boy	3Y	Hand right	Postaxial-A	Type V		
SCMC-192	Boy	5M	Foot left	Postaxial-A	$S_0A_0M_2$		
SCMC-196	Girl	5M	Hand bilateral	Postaxial-B	Type IIB (bilateral)	Yes	
			Foot bilateral	Postaxial-A	$S_0A_2M_2$ (bilateral)		
SCMC-206	Girl	8M	Foot bilateral	Postaxial-A	$S_0A_1M_1$ (left)		
				Postaxial-B	$S_1A_0M_1$ (right)		
SCMC-254	Boy	2M	Hand left	Postaxial-B	Type IIA		
SCMC-310	Boy	8M	Foot left	Postaxial-B	$S_2A_0M_1$		
SCMC-311	Boy	1Y	Hand bilateral	Postaxial-B	Type IIB (bilateral)		
			Foot bilateral	Postaxial-A	$S_0A_1M_2$ (bilateral)		
SCMC-327	Girl	2Y	Foot bilateral	Postaxial-A	$S_0A_1M_1$ (left)		
				Postaxial-B	$S_1A_0M_0$ (right)		
SCMC-331	Boy	5M	Hand bilateral	Postaxial-A	Type IIIA (left)		c.4141delA/p.Arg1381GlyfsX38
				Postaxial-B	Type IIB (right)		
			Foot bilateral	Postaxial-A	$S_0A_2M_2$ (bilateral)		
SCMC-344	Boy	1Y	Foot left	Postaxial-B	$S_2A_1M_0$		
SCMC-348	Boy	1Y	Foot left	Postaxial-A	$S_0A_1M_1$		
SCMC-359	Boy	2Y	Hand bilateral	Postaxial-A	Type V (left)		c.3855dupC/p.Met1286HisfsX18
				Postaxial-A	Type IIIA (right)		
			Foot bilateral	Mesoaxial	$S_0A_1M_1$ (left)		
				Postaxial-A	$S_0A_0M_2$ (right)		

[a] Based on the Temtamy–McKusick classification.
[b] Modified Rayan–Frey classification for hand, SAM (Syndactylism, Axis deviation, metatarsal extension) classification for foot.