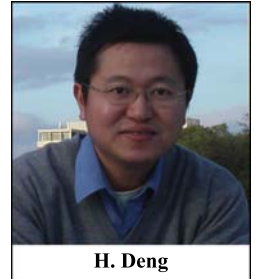# Advances in the Molecular Genetics of Non-syndromic Syndactyly

Hao Deng[1,2,]* and Ting Tan[1]

[1]*Center for Experimental Medicine;* [2]*Department of Neurology, the Third Xiangya Hospital, Central South University, Changsha, China*

H. Deng

Abstract: Syndactyly, webbing of adjacent digits with or without bony fusion, is one of the most common hereditary limb malformations. It occurs either as an isolated abnormality or as a component of more than 300 syndromic anomalies. There are currently nine types of phenotypically diverse non-syndromic syndactyly. Non-syndromic syndactyly is usually inherited as an autosomal dominant trait, although the more severe presenting types and subtypes may show autosomal recessive or X-linked pattern of inheritance. The phenotype appears to be not only caused by a main gene, but also dependant on genetic background and subsequent signaling pathways involved in limb formation. So far, the principal genes identified to be involved in congenital syndactyly are mainly involved in the zone of polarizing activity and sonic hedgehog pathway. This review summarizes the recent progress made in the molecular genetics, including known genes and loci responsible for non-syndromic syndactyly, and the signaling pathways those genetic factors involved in, as well as clinical features and animal models. We hope our review will contribute to the understanding of underlying pathogenesis of this complicated disorder and have implication on genetic counseling.

Keywords: Heterogeneity, Limb malformation, Molecular genetics, Mutation, Syndactyly, Webbed digits.

1. INTRODUCTION

Syndactyly, coming from the Greek syn (meaning together) and dactyly (meaning digits), is a digital malformation in which adjacent fingers and/or toes are webbed due to failure to separate during embryological limb development [1-4]. Simple syndactyly refers to only soft tissue involvement, while complex syndactyly refers to bony or cartilaginous fusions of digits, and musculotendinous and/or neurovascular abnormalities [5]. Complete syndactyly refers to web involvement of nail folds, while incomplete or partial syndactyly refers that the nail folds are not involved, but the web depth is distal to its normal position [6]. Syndactyly may be unilateral or bilateral, and may be symmetrical or asymmetrical in the upper/lower and right/left limbs [1]. It can also be categorized into cutaneous or bony types, involving phalanges, metacarpal/metatarsal or carpal/tarsal levels, even approximating the distal end of forearm/foreleg, though a milder phenotype may only alter the interphalangeal creases and peculiarities in dermatoglyphics [1]. The approximate prevalence of syndactyly is 0.03%-0.1% at birth and it is twice as common in males [1, 4, 7]. It can be an isolated exhibit or associated with other anomalies, such as acrosyndactyly, clindodactyly, synostosis, cleft hand, polydactyly, or as a feature in several syndromes, including Apert's syndrome, Poland's syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome, and Holt-Oram syndrome [4]. Syndactyly and polydactyly are the two most frequent congenital limb abnormalities [6].

*Address correspondence to this author at the Center for Experimental Medicine and Department of Neurology, the Third Xiangya Hospital, Central South University, 138 Tongzipo Road, Changsha, Hunan 410013, China; Tel: 011-86-731-88618372; Fax: 011-86-731-88618339; E-mail: hdeng008@yahoo.com

Syndactyly is genetic in origin [8]. Up to date, at least nine types of phenotypically diverse non-syndromic syndactyly have been reported [4]. Types I to V follow autosomal dominant mode of inheritance with variable expressivity and incomplete penetrance [9]. The classification was extended to four additional variants (Type VI-IX), including two autosomal recessive variants, one X-linked recessive variant and one autosomal dominant variant. The recent progresses of molecular genetics have led to identification of a growing number of genes whose mutations are implicated in the pathogenesis of syndactyly [10]. The increasing knowledge of embryonic development has significantly enhanced our understanding of congenital limb malformations [11]. In this review, we discuss the epidemiology, clinical features (Fig. **1**), molecular genetics, signaling pathway (Fig. **2**), and animal models of non-syndromic syndactyly.

2. CLINICAL CLASSIFICATION OF NON-SYNDROMIC SYNDACTYLY

Although most of the syndactyly types are inherited as autosomal dominant inheritance, the more severe presenting types and subtypes appear to have autosomal recessive and in some cases X-linked pattern of inheritance [4]. Up to date, at least 11 loci and 8 disease-causing genes, including the homeobox D13 gene (*HOXD13*), the fibulin-1 gene (*FBLN1*), the gap junction protein alpha 1 gene (*GJA1*), the limb development membrane protein 1 gene (*LMBR1*), the low density lipoprotein receptor-related protein 4 gene (*LRP4*), the gremlin 1 gene (*GREM1*), the formin 1 gene (*FMN1*) and the fibroblast growth factor 16 gene (*FGF16*), have been identified. Though the disease-causing genes for syndactyly types I-c, II-a, II-b, III, IV, V, VII and VIII-a have been found, the causative genes for other types including
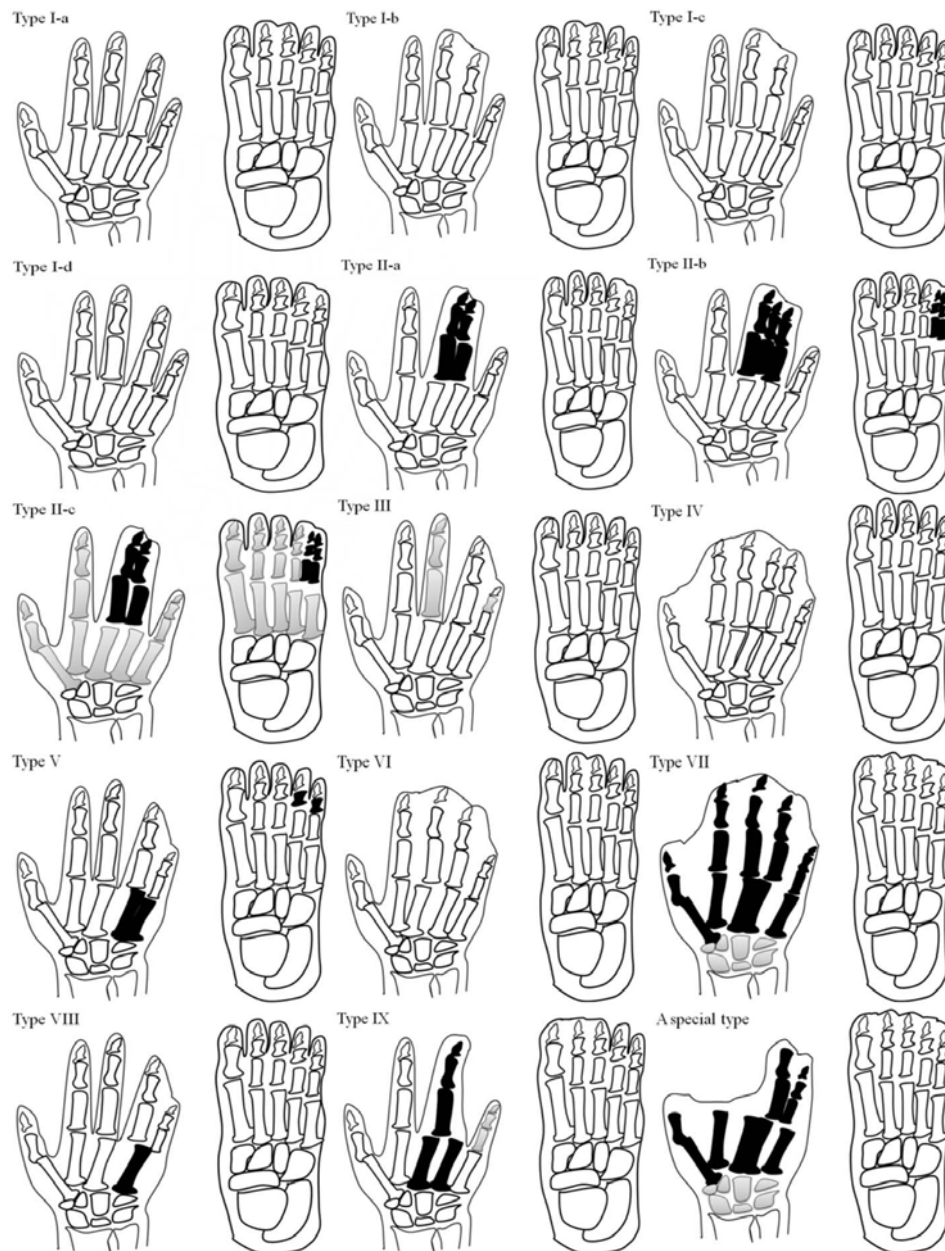
Fig. (1). Schematic drawing of non-syndromic syndactyly types. Black digital symbols mean synostosis, grey digital symbols mean sympha-langism, clinodactyly, hypoplastic terminal phalanx or other complex irregular abnormalities.

I-a, I-b, I-d, II-c, VI, VIII-b and IX, are still unknown (Table **1**).

3. SYNDACTYLY TYPE I (SD1)

SD1 is the most common form of non-syndromic syndactyly. It is a clinically heterogeneous condition with complete or partial webbing between the 3rd and 4th fingers, and/or the 2nd and 3rd toes [12, 13]. It has been proposed to further divide the condition into four subtypes, I-a, I-b, I-c and I-d, all of which are autosomal dominant inherited [12].

3.1. Syndactyly Type I-a (OMIM 609815) and Chromosome 3p21.31

Syndactyly type I-a is also known as weidenreich type, zygodactyly, or 2/3 toes syndactyly [1]. It is characterized by cu-

taneous webbing of 2nd and 3rd toes without hand involvement. It is the most common and mildest form of syndactyly type I [12], and it affects more males than females [7]. The phenotype varies from unilateral minor impression of webbing to bilateral complete webbing of the 2nd and 3rd toes, including fusion of nails, without the involvement of bone [12]. Type I-a disease gene locus was mapped to chromosome 3p21.31 by linkage and haplotype analysis of a large Pakistani family. The highest LOD score was 3.38 at D3S2409, and the disease interval was flanked by D3S4919 and D3S4940 encompassing about 0.2 Mb [12]. However, the disease-causing gene has not been identified.

3.2. Syndactyly Type I-b (OMIM 185900) and Chromosome 2q34-q36

Syndactyly type I-b, also named Lueken type, or 3/4 fingers and 2/3 toes syndactyly [1], is characterized by bilateral
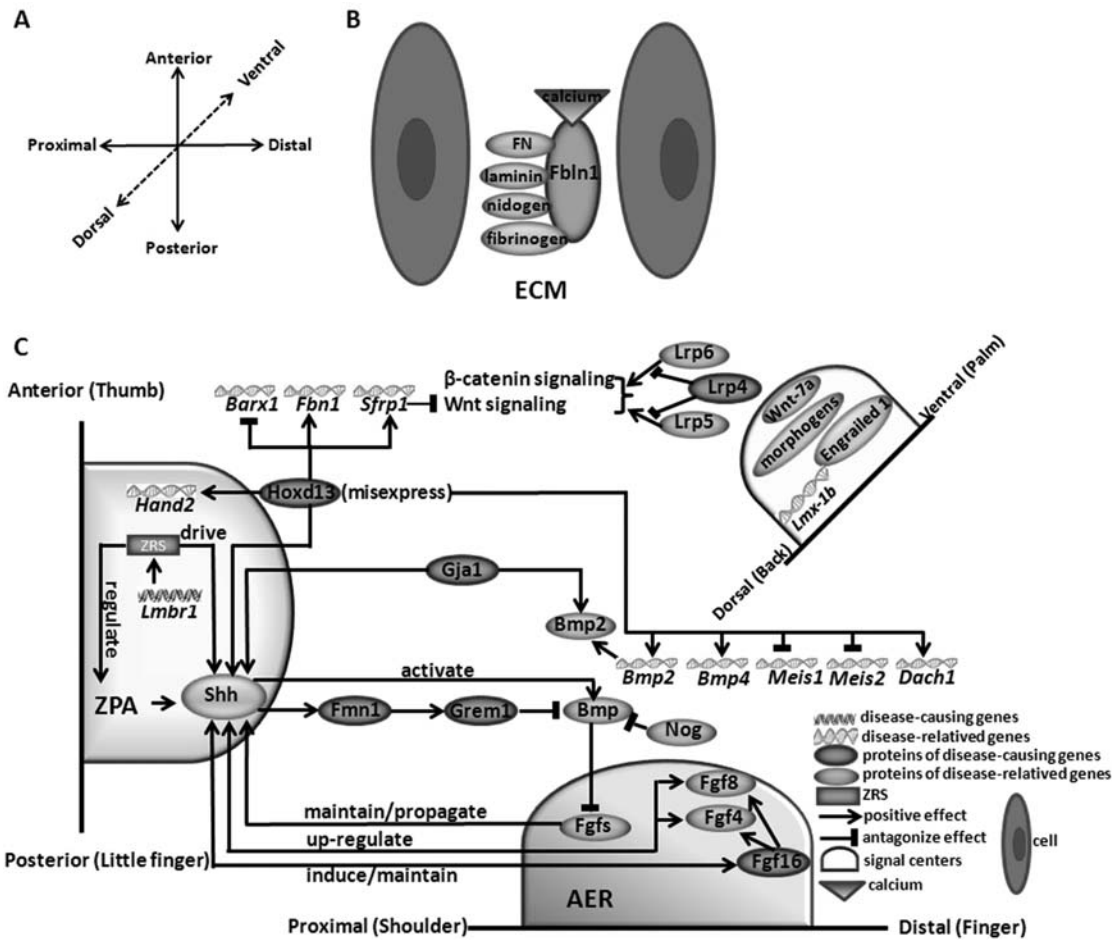
Fig. (2). The disease-causing genes and proteins associated with syndactyly. **A** Three axis of limb development. **B** Fbln 1 is expressed in ECM, and self-associates as well as binds calcium, FN, laminnin, nidogen, and fibrinogen. **C** The signal pathways of syndactyly disease-causing genes and their products, and the related genes and proteins involved in. Abbreviations: ECM: the extracellular matrix, Nog: noggin, FN: fibronectin, *Bmp2*: the bone morphogenetic protein 2 gene, *Bmp4*: the bone morphogenetic protein 4 gene, *Hand2*: the heart and neural crest derivatives-expressed 2 gene, *Meis1*: the meis homeobox 1 gene, *Meis2*: the meis homeobox 2 gene, *Dach1*: the dachshund 1 gene, *Barx1*: the BarH-like homeobox 1 gene, *Fbn1*: the fibrillin 1 gene, *Sfrp1*: the secreted frizzled-related protein 1 gene, *Lmx-1b*: the LIM homeobox transcription factor 1 beta gene.

Table 1.   Gene loci and disease-causing genes of non-syndromic syndactyly.

Types	Clinical Phenotype of Fingers/toes	Phenotype MIM Number	Inheritance	Location	Gene/ Locus	Gene/Locus MIM Number	References
I-a	Cutaneous webbing of 2/3 toes including a fusion of nails, with normal hands	609815	AD	3p21.31	-	-	[12]
I-b	Bilateral cutaneous/bony webbing of 3/4 fingers, and 2/3 toes	185900	AD	2q34-q36	-	-	[12, 13]
I-c	Bilateral cutaneous/bony webbing of 3/4 fingers and with normal feet	-	AD	2q31-q32	*HOXD13*	142989	[12, 15]
I-d	Bilateral cutaneous webbing of 4/5 toes	-	AD?	-	-	-	[1, 12]
II-a	Typical features: syndactyly between the 3/4 fingers, occasional duplication of the 3/4 fingers in the web, syndactyly of the 4/5 toes, and duplication of the 5th toe	186000	AD	2q31	*HOXD13*	142989	[20, 22, 23]

(Table 1) contd….

Types	Clinical Phenotype of Fingers/toes	Phenotype MIM Number	Inheritance	Location	Gene/ Locus	Gene/Locus MIM Number	References
II-b	Minor features: mesoaxial synpolydatyly in hand, postaxial synpolydatyly in foot	608180	AD	22q13.3	*FBLN1*	135820	[20, 35]
II-c	Atypical features: severe manifestation of syn-polydactyly	610234	AD	14q11.2-q12	-	-	[3]
III	Bilateral complete syndactyly of the 4/5 fingers, missing/rudimentary middle phalanges of the 5th finger, and the 3rd finger may camptodac-tyly, with normal foot	186100	AD	6q22-q24	*GJA1*	121014	[40, 43]
IV	Complete cutaneous syndactyly of all fingers, often accompanied by polydactyly, cup-shaped hand, sometimes with variable polydactyly of toes	186200	AD	7q36	*LMBR1*	605522	[50, 51]
V	Synostotic fusion of 4/5 metacarpal and meta-tarsal synostosis	186300	AD	2q31	*HOXD13*	142989	[58-60]
VI	Fusion of 2nd to 5th fingers, and 2/3 toes	-	AD	-	-	-	[63]
VII	Total synostotic syndactyly with metacarpals fusion, spoon-head shape	212780	AR	11p11.2-q13.1	*LRP4*	604270	[64, 65]
VIII-a	4/5 metacarpal fusion	309630	XR	Xq21	*FGF16*	300827	[72, 73]
VIII -b	4/5 metacarpal fusion	-	AD	-	-	-	[72, 73]
IX	Mesoaxial synostotic syndactyly with phlanag-eal reduction of 3/4 fingers and clinodactyly of 5th finger, preaxial webbing and distal pha-langeal hypoplasia of toes	609432	AR	17p13.3	-	-	[80, 81]
A special type	Oligodactyly and partial syndactyly	-	AD	15q13.3	*GREM1*; *FMN1*	603054; 136535	[83]

AD: Autosomal dominant inheritance, AR: Autosomal recessive inheritance, XR: X-linked recessive inheritance.

cutaneous and/or bony webbing of the 3rd and 4th fingers, and the 2nd and 3rd toes [12]. It is the second most frequent type of syndactyly type I [7]. In 2000, the type I-b disease gene locus was mapped to chromosomal 2q34-q36 by genome-wide linkage and haplotype analysis of an 8-generation German family [13]. The maximum LOD score was 12.4 for D2S301, and the disease gene interval was flanked by D2S2319 and D2S344 encompassing about 9.4 cM region [13]. The locus was subsequently confirmed in an Iranian family. However, the disease-causing gene has not been identified [12, 14].

3.3. Syndactyly Type I-c (2q31-q32) and the *HOXD13* Gene

Syndactyly type I-c, also named Montagu type or 3/4 fingers syndactyly [1], is characterized by bilateral cutaneous or bony webbing of 3rd/4th finger and occasionally of 3rd/5th finger, with normal feet [12]. It is a rare autosomal dominant type [12]. A large Chinese family of 23 patients presented with variable degree of bilateral osseous fusion of the 3rd and 4th or the 3rd, 4th and 5th fingers, and one patient also had partial fusion of the 3rd to 5th toes [1]. In 2014, the type I-c disease gene locus was mapped to chro-

mosome 2q31-2q32, and two mutations (p.R306Q and p.R306G) in the homeodomain of *HOXD13* were identified by analysis of two Chinese families [15].

3.4. Syndactyly Type I-d

Syndactyly type I-d, also named Castilla type, 4/5 toes syndactyly [1], is characterized by bilateral cutaneous webbing of the 4th and 5th toes [12]. It is the third most common type of syndactyly type I with a rare incidence [7, 12]. Occasionally, the 5th toe is tucked inside the fibular aspect of the 4th toe, and thus any minor form of webbing could be easily overlooked [1]. The inheritance pattern and penetrance, disease gene locus and causative gene for this subtype have not been worked out [1, 12].

4. SYNDACTYLY TYPE II (SD2)

SD2, also named vordingborg type, or 3/4 fingers and 2/3 toes synpolydactyly (SPD) [1], is characterized by a distinctive combination of syndactyly and polydactyly with an autosomal dominant inheritance [3]. It is the second most frequent syndactyly type [3]. The cardinal features are syndactyly between the 3rd and 4th fingers, occasional duplica-

tion of the 3rd or 4th finger in the web, syndactyly of the 4th and 5th toes, and duplication of the 5th toe [2]. In addition, SPD may exhibit as comorbidity with brachydactyly, camptodactyly, or clinodactyly of the 5th finger, and variable syndactyly of the 3nd to 5th toe with middle phalanx hypoplasia [16]. SPD is clinically and genetically the most heterogeneous abnormality type among non-syndromic syndactyly [17, 18]. It presents with incomplete penetrance and high inter- and intra-familial phenotypic variability [3, 19]. A homozygous mutation was identified in a large Turkish family with very severe phenotype [3, 19].

Though a characteristic clinical delineation among these entities has not been appreciated, SD2 families may be divided into three categories: (i) typical SPD features (SPD1, type II-a), (ii) minor variants (SPD2, type II-b), and (iii) unusual phenotypes (SPD3, type II-c) [20]. Gene mutations have been identified in only two types, SPD1 and SPD2 [21]. Polyalanine-expansion mutations in *HOXD13* cause typical SPD, whereas deletions and missense mutations in *HOXD13* lead to atypical SPD [18].

4.1. SPD1 (2q31, OMIM 186000) and the *HOXD13* Gene

SPD1 is most commonly caused by an addition of seven or more alanine residues to the polyalanine repeat in the coding region of the *HOXD13* gene located in 2q31, showing an autosomal dominant fashion and causing a dominant-negative effect [17, 21]. In 1995, the disease gene locus for SPD1 was mapped to 2q31 with an LOD score of 12.96 by linkage analysis of a family with 425 members [22]. In 1996, expansions of a polyalanine stretch in the amino-terminal region of *HOXD13* have been indentified in patients with SPD, suggesting the polyalanine stretch outside of the DNA binding domain of this gene is a region necessary for proper protein function [23]. Subsequently, duplication of 9 residues in the *HOXD13* gene was observed in 2 unrelated Turkish families with SPD [24]. In 2002, p.R31W, the first missense mutation in the homeodomain of *HOXD13*, was identified in a 4-generation SPD family. The mutation probably destabilizes the homeodomain-DNA complex, closely resembling those produced by frame shifting deletions in *HOXD13*, and results in functional haploinsufficiency [25]. In 2009, p.G220V mutation within the N-terminal transcription regulating domain was identified in a Greek family of a variant form of SPD [26]. In 2011, p.Q248X was identified to be the genetic cause of a large Pakistani family with incomplete penetrance, and a severe form of SPD was evidenced in the homozygous state, while a milder form of SPD with less than 50% of penetrance was observed in the heterozygous state [21]. A heterozygous 27-bp expansion, c.184_210dup, in exon 1, resulting in a gain of 9 alanine residues, was reported in a large Chinese kindred [27]. The p.R298Q mutation, affecting the transcriptional activation ability of *HOXD13*, was identified in a 3-generation Chinese family with six patients [18]. *HOXD13* is a member of the *HOX* gene family, encoding regulatory transcription factors belonging to the vast family of homeodomain-containing proteins, which control cell fates and regional identities along the primary body and limb axes during the development process [28]. The *HOX* genes are clustered in four genomic loci, including *HOXA*, *B*, *C* and *D* members [29], and *HOXD13* is the most 5'-located *HOXD* gene [21], with ge-

nomic structure comprised of a 1032-bp exonic sequence encoding 343 amino acids. The *HOXD13* gene plays an important role in patterning the most distal limb region, and the protein regulates the expression of genes acting in key pathways for early limb and skeletal patterning [28].

It was reported that three most informative mouse models of SPD were constructed, including the synpolydactyly homolog (*spdh*) mouse, the *Hoxd13* knockout mouse and the *Hoxd11-13* null mouse [16]. The *spdh* mouse is a spontaneous mouse model of human polyalanine tract expansion mutations in the 5' region of the *Hoxd13* gene due to a 21-bp in-frame duplication [30]. *Hoxd13$^{spdh/spdh}$* mice showed malformations, including polydactyl and lack of joint formation, and severe defects in chondrogenesis and osteogenesis. These abnormalities are similar to those of the human SPD [30-32]. *Hoxd13$^{spdh/+}$* mice caused more subtle phenotypes than their human counterparts [30]. *Hoxd13$^{-/-}$* mice had brachydactyly, joint fusions, delayed chondrification and ossification, and partial postaxial polydactyly, while *Hoxd13$^{+/-}$* mice occasionally displayed mild limb abnormalities, including minor metacarpal and carpal defects, or a rudimentary extra postaxial digit in the forelimb [16]. The *Hoxd11-13$^{-/-}$* mice can mimic human SPD features, such as shortening, webbing, fusion and duplication of the digits [16], though phenotype of homozygous *Hoxd13* mutant mice is more severe than that of *Hoxd11* or *Hoxd12* mutant mice [33]. Intriguingly, the *Hoxd13$^{spdh/spdh}$* mice may exhibit polydactyly in SPD by decreasing retinoic acid synthesis [34]. The *spdh* probably acts in a dominant-negative effect and plays an important role in examining interactions with other *Hox* genes, such as *Hoxa*, *Hoxb*, and *Hoxc* [29, 31].

4.2. SPD2 (22q13.3, OMIM 608180) and the *FBLN1* Gene

In 1998, co-segregation of an apparently balanced reciprocal translocation, t(12;22)(p11.2;q13.3), was identified in a Belgian family in which three individuals have a complex type of SPD associated with metacarpal and metatarsal synostoses, and the disease-associated breakpoint was mapped to a 1.5 Mb region on 12p11.2 [35]. The *FBLN1* gene was disrupted and haploinsufficiency of the *FBLN1-D* variant led to the complex type of SPD [36]. The extracellular matrix protein, FBLN1, can be expressed in most tissues, including the perichondrium and calcifying regions of developing bones, the gut subepithelium and epithelial basement membranes of the skin, in the early human embryo [37].

The exon-intron organization of the human *FBLN1* gene is similar to that of the mouse gene [38]. *Fbln1*-deficient mice cause perinatal lethality and endothelial cell abnormalities in several vessel compartments [39].

4.3. SPD3 (OMIM 610234) and Chromosome 14q11.2-q12

In 2006, SPD3 disease gene locus was mapped to chromosome 14q11.2-q12 by linkage and haplotype analysis of a 5-genreation Pakistani kindred with osseous fusion of the 3rd and 4th fingers and postaxial SPD of toes, and variable other features, including cutaneous webbing, symphalangism, abnormal metacarpals, clinodactyly, and camptodactyly [3]. However, no gene has yet been implicated [21].

5. SYNDACTYLY TYPE III (SD3, OMIM 186100)

SD3, also named Johnston-Kirby type, 4/5 or 3/4/5 fingers fusion, is inherited in autosomal dominant pattern with incomplete penetrance [1]. It is characterized by bilateral complete syndactyly of the 4th and 5th fingers, occasional involvement of the 3rd finger, and associated camptodactyly [40]. SD3 has been reported to occur as an isolated entity or as a part of oculodentodigital dysplasia (ODDD, OMIM 164200), which commonly involves digit as well as phalangeal abnormalities, diffuse skeletal dysplasia, enamel dysplasia, craniofacial dysmorphia, neurological degeneration and hypotrichosis [40, 41].

5.1. Chromosome 6q22-q24 and the *GJA1* Gene

In 1997, isolated type III syndactyly and ODDD were located on chromosome 6q22-q24 by analysis of a large family with atypical facial features of SD3 and six families with ODDD [40]. In 1999, the disease gene locus of ODDD was mapped to an interval of 1.01 cM (male) to 2.87 cM (female) flanked between D6S266/D6S261 (centromeric) and D6S1639 (telomeric) by two-point linkage analysis with seven ODDD families [42]. In 2003, mutations of *GJA1*/connexin43 *(Cx43)*, probably causing misassembly of channels or altering of channel conduction properties, were found to be responsible for the pleiotropic phenotype of ODDD with syndactyly [43]. Subsequently, *GJA1* mutations were also found in craniometaphyseal dysplasia (OMIM 218400), hypoplastic left heart syndrome 1 (OMIM 241550), and atrioventricular septal defect 3 (OMIM 600309) [44, 45]. In 2012, the *GJA1* p.I130T mutation was observed in an autosomal dominant Pakistani family showing typical form of ODDD with SD3 [41], and heterozygous p.H95Y mutation was found in a 44-year woman with ODDD and SD3 [46]. Cx43, encoded by *GJA1*, plays a key role in normal facial and limb development. Mutation of *Gja1* can result in craniofacial anomalies and limb abnormalities through modifying sonic hedgehog (Shh) and bone morphogenic protein 2 (Bmp2) [47, 48].

Cx43-deficient mice showed decreased apoptosis in embryonic limbs, resulting in syndactyly in ODDD [48]. *Gja1$^{Jrt/+}$* mice harboring p.G60S mutation had similar syndactyly, enamel hypoplasia, cataract and iris abnormalities, and craniofacial dysplasia as patients with ODDD [49]. Heterozygous *Gja1$^{+/-}$* mice showed no ODDD-like phenotypes [49], while homozygous *Gja1$^{-/-}$* or *Gja1$^{G138R/-}$* mice exhibited syndactylies and other morphologic alterations in ODDD [48].

6. SYNDACTYLY TYPE IV (SD4, OMIM 186200)

SD4, also named Haas type polysyndactyly as described by Haas, in 1940 [4], is characterized by complete cutaneous syndactyly of all fingers, and is often accompanied by polydactyly [50]. The disorder is inherited in autosomal dominant pattern with variable expressivity [50]. SD4 is extremely rare and it is divided into at least two subtypes: (i) typical Haas type without involvement of feet, and (ii) complete fusion of all fingers with variable fusion of all digits in feet [1, 50].

6.1. Chromosome 7q36 and the *LMBR1* Gene

SD4 and triphalangeal thumb-polysyndactyly syndrome (TPTPS) are genetically homogeneous and are considered as a continuum of phenotypes [50]. Both entities are autosomal dominant and are caused by mutations in the zone of polarizing activity regulatory sequence (ZRS) located in chromosome 7q36 within intron 5 of the *LMBR1* gene [51]. In 2007, SD4 disease gene locus was suggested to be on chromosome 7q36 by linkage and haplotype analysis of a Chinese family, with the maximum two-point LOD score of 1.613 [52]. In 2008, the duplications involving the limb specific SHH enhancer, ZRS, were found to be the cause of SD4 and TPTPS by analysis of 6 Chinese families [53]. In 2009, ZRS duplication was suggested to cause SD4 with tibial hypoplasia [54]. In 2013, 115.3 kb duplication of the ZRS was detected in a family with SD4 and TPTPS [50]. The ZRS locates approximately 1 Mb away from *SHH* [50], and is conserved among mammals and fish [51].

The mouse with *Shh* turned-off in the distal mesenchyme of the limb at later stages of limb bud development showed syndactyly [55]. The Hammertoe (*Hm*) mutant mouse has a mutation in the *Lmbr1* gene [51]. The *Lmbr1$^{Hm/+}$* and *Lmbr1$^{Hm/Hm}$* mice displayed syndactyly involving digits II to V [51]. The syndactyly extends to the distal phalanx and the markedly flexed digits form a shape resembling a hammer in *Lmbr1$^{Hm/Hm}$* mice, while the phenotype of *Lmbr1$^{Hm/+}$* mice is milder than that of *Lmbr1$^{Hm/Hm}$* mice [56]. There is a deletion of proximal mouse chromosome 5 named *Hdh^{df4J}*, and *Hdh$^{df4J/+}$* mice showed syndactyly of the central to distal carpals 2 or 3 [57].

7. SYNDACTYLY TYPE V (SD5, OMIM 186300)

SD5, also named Dowd type or fusion of 4/5 metacarpals [1], is characterized by synostotic fusion of metacarpals 4 and 5 [58]. SD5 shows an autosomal dominant trait and represents one of the rarest types of non-syndromic syndactyly [58]. Other hand anomalies include abnormal origin of the 5th fingers, anomalies of digits 4 and 5, brachydactyly, syndactyly, camptodactyly, absence of distal interphalangeal creases and unusual palmar dermatoglyphics [59]. In the feet, the anomalies, including hyperplasia of the 1st metatarsal and shortening of the 2nd to the 5th metatarsals, lead to varus deviation of metatarsals and valgus deviation of toes/phalanges [1, 59].

7.1. Chromosome 2q31 and the *HOXD13* Gene

In 2005, expansion of the polyalanine repeats in the *HOXD13* gene on chromosome 2q31 was suggested to be responsible for SD5 [60]. In 2007, p.Q317R mutation was identified in a large Han Chinese family with SD5 [58]. Mutations in the *HOXD13* gene also lead to syndactyly type I-c [15], SPD1 [23], brachydactyly type A4 (BDA4, OMIM 112800) [58], brachydactyly type D (BDD, OMIM 113200) [61], brachydactyly type E1 (BDE1, OMIM 113300) [61], and VACTERL association (OMIM 192350) [62]. VACTERL association is comprised of vertebral defects (V), anal atresia (A), cardiac anomaly (C), tracheoesophageal fistula with esophageal atresia (TE), renal dysplasia (R) and limb lesions (L) [62]. Given mutations of *HOXD13* gene can cause a series of phenotypes involving the limb abnormality, these disorders may be considered as *HOXD13*-associated limb disorders with genetic classification.

Hoxd13$^{-/-}$ mice showed extensive limb defects, including a strong diminution of the metacarpal length, reduction or

absence of phalange 2 on the 2nd and the 5th digits, shortening of phalangeal bones on other digits, sometimes associated with syndactyly, extra wrist bone with a rudimentary digit VI [33]. Intriguingly, trans-heterozygotes for the *Hoxd13*^{+/-} mice showed more severe autopodal phenotypes than heterozygotes, demonstrating a potential genetic interaction [33].

8. SYNDACTYLY TYPE VI (SD6)

SD6, also named Mitten type or fusion of 2/5 fingers and 2/3 toes, is characterized by fusion of fingers from the 2nd to the 5th in right hand, amalgamation of distal and terminal phalanges in a knot-like structure and involvement of the 2nd and the 3rd toes, or only webbing between the 2nd and the 3rd toes without the involvement of fingers [63]. SD6 is rare and is autosomal dominant in inheritance with reduced penetrance and variable expressivity [63]. Up to now, no additional cases of this type have been described, and no disease-causing locus or gene has been found.

9. SYNDACTYLY TYPE VII (SD7, OMIM 212780)

SD7, also named Cenani-Lenz syndactyly (CLS), is characterized by complete syndactyly with metacarpal/phalanges fusions or oligodactyly sometimes accompanied by radius and ulna synostosis [64]. The frequent features companied with SD7 include kidney agenesis/hypoplasia, craniofacial dysmorphism and teeth abnormalities [64, 65].

9.1. Chromosome 11p11.2-q13.1 and the *LRP4* Gene

In 2010, recessive mutations in the *LRP4* gene on chromosome 11p11.2-q13.1 were identified by homozygosity mapping approach and sequencing candidate genes of 12 families with CLS [65]. In 2013, a homozygous *LRP4* p.L953P mutation was identified in a large consanguineous Pakistani pedigree with Cenani-Lenz syndrome restricted to limb and kidney anomalies [64]. In 2013, homozygous mutation p.E97X in the *LRP4* gene, resulting in absence of residual *LRP4* function, was identified in a child with severe Cenani-Lenz syndrome [66]. In 2014, truncating mutations in the *LRP4* gene were found to lead to a prenatal lethal form of Cenani-Lenz syndrome [67]. *LRP4* is a multifunctional member of the low-density lipoprotein receptors (LDLRs) gene family, which are essential for various developmental processes [65]. The LRP4 protein is a modulator of extracellular cell signaling pathways during development [65]. LRP4 antagonizes LRP5 and LRP6 activation of Wnt/β-catenin signaling, which is an essential developmental process during organogenesis and tissue regeneration [65-67]. Loss of LRP4 function by the identified mutations leads to excessive Wnt/β-catenin signaling in the limb bud and causes abnormal limb development [67], such as syndactyly, synostosis, and renal agenesis in Cenani-Lenz syndrome [65].

Lrp4^{-/-} mice are growth-retarded, with autosomal recessive phenotype, including a full penetrance of polysyndactyly, sometimes in combination with a mild and partially penetrance of craniofacial abnormalities resembling humans [68]. *Lrp4*^{-/-} mice can also show a delay in ureteric bud formation resulting in unilateral or bilateral kidney agenesis like humans [69]. Two recessive mutations (*dan* and *mdig*) of the *Lrp4* gene in mice have been identified to cause polysyndac-

tyly [70]. Defective splicing of *Lrp4* can cause autosomal recessive mulefoot disease (MFD) in bovines. A 2-bp substitution, c.4863_4864delCGinsAT, in exon 33 of the *Lrp4* gene was identified as a causal mutation for syndactyly in Holstein cattle. Four *Lrp4* non-synonymous mutations (p.P1647K, p.G81S, p.G1199S and p.G907R) were found to cause congenital syndactyly in cows and co-segregate in Holstein, German Simmental and Simmental-Charolais families [71].

10. SYNDACTYLY TYPE VIII (SD8, OMIM 309630)

SD8, also named Orel-Holmes type, metacarpals 4/5 fusion, or MF4, is X-linked recessive [1]. It is mainly characterized by partial or complete fusion of the 4th and 5th metacarpal bones [72], lateral deviation of the 5th finger, shortness of metacarpals 4 and 5, excessive separation between their distal ends, and normal flexion of the affected fingers [73]. The isolated SD8 is a rare distinctive congenital malformation of the hand showing X-linked recessive or autosomal dominant inheritance [72, 73].

10.1. Chromosome Xq21 and the *FGF16* Gene

In 2013, two nonsense mutations p.R179X and p.S157X in exon 3 of the *FGF16* gene on chromosome Xq21.1 were suggested to cause SD8 by exome sequencing and mutational screening of a Polish family and a German pedigree [72]. In 2014, a truncating mutation p.E158DfsX25 in exon 3 of the *FGF16* gene was confirmed in a sporadic male patient with SD8 [74]. *FGF16* belongs to the *FGF* gene family, which consists of 22 proteins in humans and mice [75, 76]. *FGF16* is a member of the *FGF* gene subfamily E (orthologs of *FGF9*, *FGF16*, and *FGF20* but also of *LET-756* from nematodes) and has an open reading frame of 624 bp encoding a protein comprised of 207 amino acids [72, 76, 77]. FGF16 functions as a key intercellular signaling molecule in limb formation through controlling outgrowth and patterning of the established limb bud during embryogenesis [78].

Fgf16 knockout mice exhibited severe cardiac defects and craniofacial defects, which led to embryonic death because *Fgf16* was required for embryonic heart development [79].

11. SYNDACTYLY TYPE IX (SD9, OMIM 609432) AND CHROMOSOME 17P13.3

SD9, also named mesoaxial synostotic syndactyly with phalangeal reduction (MSSD, Malik-Percin type), segregating in an autosomal recessive manner, is characterized by mesoaxial reduction of the fingers, synostoses of the 3rd and 4th metacarpals with associated single phalanges, clinodactyly of the 5th finger, hypoplasia of the thumbs/halluces and the middle phalanges of the 2nd and 5th fingers, and complete or partial soft tissue syndactyly of the toes with hypoplastic terminal phalanx [80, 81]. In 1998, *HOXD13* and other relevant genes on 2q31 were excluded as candidates for SD9 in a Turkish kindred [80]. In 2004, an autosomal recessive Pakistani family with a novel non-syndromic osseous syndactyly was excluded from linkage to 2q34-q36, 2q31, and 6q22-q23, encompassing loci for syndactyly types I, II, and III [82]. In 2005, merging of the two families revealed that the SD9 was mapped to chromosome 17p13.3 with mul-

tipoint LOD score of 5.1 [81]. No causative gene for this type has been identified.

12. A SPECIAL TYPE OF NON-SYNDROMIC SYN-DACTYLY

In clinical practice, oligosyndactyly type has been divided into two entities: (i) an autosomal recessive oligosyndactyly, radio-ulnar synostosis, hearing loss and renal defect syndrome, and (ii) an autosomal dominant CenanieLenz-like non-syndromic oligosyndactyly [83]. However, given that oligosyndactyly type belongs to SD7 with the number of 212780 in OMIM database, indicating an autosomal recessive inheritance, the later subtype, CenanieLenz-like non-syndromic oligosyndactyly, which is caused by duplication of *GREM1-FMN1* locus and is transmitted in an autosomal dominant manner [83], should be classified into another type of non-syndromic syndactyly. *Grem1* is a BMP antagonist. Like Shh and limb specific Fgfs, Grem1 is a key component of the feedback loop between the zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER), which is crucial in the complex process of limb formation [84].

Over-expression of *Grem1* in developing chick limbs repressed the programmed cell death in the interdigital mesenchyme, resulting in interdigital webbing and truncation of distal cartilage elements [83]. Mutation of *Grem1* or loss of a transcriptional global control region located in the neighboring *Fmn1* gene caused the mice limb deformity (*ld*) phenotype with digit reduction, syndactyly, radio-ulnar synostosis, variable renal defects [84, 85]. The mice *Fmn1* gene is composed of at least 24 exons and spans 400 kb, and the protein is the founding member of the formin family, which are predominantly nuclear proteins controlling polarizing activity in limb buds through establishment of the Shh/Fgf-4 feedback loop [86]. Mutations in the mouse *Fmn1* gene, formerly known as the *ld* gene, led to recessively inherited limb deformities and renal malformations or aplasia [87]. Knockout of *Fmn1* in mice increased Bmp activity and exhibited a reduction of digit number to four, a deformed posterior metatarsal, phalangeal soft tissue fusion, and kidney aplasia [86].

13. DIAGNOSIS, TREATMENT AND PROGNOSIS

Accurate diagnosis of syndactyly with combination of clinical features, family history, X-ray and ultrasound detection, and molecular genetic tools may help to guide medical care and rehabilitation [27, 88]. The goal of surgical treatment for syndactyly is to separate the fingers, reconstruct a webspace, and obtain a functional hand with the least surgical procedures and complications possible [89]. Syndactyly should be corrected early in life, with optimal surgical time ranging from 6 months to 18 months of age in infants with simple syndactyly, and before 6 months for complex syndactyly [89]. Most of conventional surgical techniques use skin grafts [90]. However, open treatment is better than skin grafting because of better match of texture without a patchwork-like scar [91]. Combined use of "Z"-method incision with full-thickness free skin grafts is the most common treatment. Such surgical method can minimize final scar formation and thus obtain good functional and cosmetic result [92]. Additionally, methotrexate medication is helpful for keloid excision after syndactyly division [93], and

hyaluronic acid scaffold is a promising alternative to skin grafting in syndactyly surgery [94]. It is difficult to accurately predicate outcomes of these treatments because of large variations in the extent of syndactyly. The primary prognostic factor is whether the patient has simple or complex syndactyly [95]. In simple syndactyly, functional and cosmetic results are usually excellent with less than 10% risk of complications. While in complex syndactyly, postoperative prognosis depends on the severity of bone, joint and tendons abnormalities. Patients with complex syndactyly usually have poorer functional outcomes and higher risk of complications, such as rotational/angular deformity, nails deformity and web creep [95-97]. Patients should be examined periodically after reconstruction until they have achieved skeletal maturity because late complications may occur [89].

14. CONCLUSION AND PERSPECTIVES

Syndactyly is a failure in the separation of developing digits during organogenesis, which may occur as an isolated entity or a component of more than 300 syndromic anomalies [1]. The identification of novel disease causative genes for syndactyly could not only utilize gene diagnosis and genetic counseling, but also help to elucidate the limb patterning and digit specification mechanisms [98]. Additionally, in some cases, multiple genes with different variants, epigenetics or other unknown factors may participate in the development of bone and attachments, which determines the diversity of the syndactyly clinical phenotype. With the advent of next-generation sequencing (NGS) technology, including exome sequencing, whole-genome sequencing and RNA-sequence analysis, more novel gene mutations or variants resulting in non-syndromic syndactyly will be discovered. Construction of various genetic-deficient syndactyly animal models could contribute substantially to the understanding of limb developmental pathways, help to clarify the pathogenesis of syndactyly and carry on experimental treatment study. The unveiling of the pathways involved in the development of non-syndromic syndactyly will help to explain diverse clinical phenotypes, strong genetic heterogeneity, and may contribute to the target therapy of this condition.

ABBREVIATIONS

All abbreviations have been defined in the text where first used.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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