Review Article

Advances in understanding the genetics of syndromes involving congenital upper limb anomalies

Liying Sun[1#], Yingzhao Huang[2,3,4#], Sen Zhao[2,3,4], Wenyao Zhong[1], Mao Lin[2,3,4], Yang Guo[1], Yuehan Yin[1], Nan Wu[2,3,4], Zhihong Wu[2,3,5], Wen Tian[1]

[1]Hand Surgery Department, Beijing Jishuitan Hospital, Beijing 100035, China; [2]Beijing Key Laboratory for Genetic Research of Skeletal Deformity, Beijing 100730, China; [3]Medical Research Center of Orthopedics, Chinese Academy of Medical Sciences, Beijing 100730, China; [4]Department of Orthopedic Surgery, [5]Department of Central Laboratory, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China

Contributions: (I) Conception and design: W Tian, N Wu, Z Wu, S Zhong; (II) Administrative support: All authors; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: Y Huang; (V) Data analysis and interpretation: L Sun; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Wen Tian. Hand Surgery Department, Beijing Jishuitan Hospital, Beijing 100035, China. Email: wentiansyz@hotmail.com.

Abstract: Congenital upper limb anomalies (CULA) are a common birth defect and a significant portion of complicated syndromic anomalies have upper limb involvement. Mostly the mortality of babies with CULA can be attributed to associated anomalies. The cause of the majority of syndromic CULA was unknown until recently. Advances in genetic and genomic technologies have unraveled the genetic basis of many syndromes-associated CULA, while at the same time highlighting the extreme heterogeneity in CULA genetics. Discoveries regarding biological pathways and syndromic CULA provide insights into the limb development and bring a better understanding of the pathogenesis of CULA. The aim of this review is to provide an overview of the genetic basis of syndromic CULA and discuss the role of biological pathways in syndromic CULA.

Keywords: Congenital upper limb anomalies (CULA); genetics; genome; biological pathway

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Introduction

Congenital upper limb anomalies (CULA) include a wide spectrum of structural abnormalities caused by perturbation in the morphogenesis of the upper limb, which affect approximately 1 per 500 live births (1,2). About 20% to 30% of babies with CULA has at least one associated non-limb anomaly (2), and approximately 10% to 20% of all congenital anomalies has upper limb involvement (3). This condition exhibits an extreme heterogeneity of presentations with different grades of severity. Although some of them result in mild functional consequences, they can also bring psychological problems for children (4).

So far, there are two commonly used classification schemes in clinical settings, i.e., the Swanson Classification and the Oberg-Manske-Tonkin (OMT) Classification. The Swanson Classification was proposed in 1964 and utilized the anatomical understanding and the surgical perspective on management (5). Although the Swanson Classification is satisfactory in several criteria, it does not include the knowledge acquired in the last 50 years (2). The OMT Classification combines more recent knowledge about limb development and the pathogenesis of limb anomalies using dysmorphology terminology (6) and provides a more practical and easily utilized scheme.

The pathogenesis of CULA remains poorly understood. A limited number of studies provide evidence in linking exposure to environmental chemicals and CULA, including persistent organic and air pollution (7). Interestingly, chorionic villus sampling has been identified as a risk factor

of syndromic and non-syndromic CULA (8). In contrast, the observation of familial CULA cases highlighted the contribution of genetic factors in CULA. Novel approaches such as single nucleotide polymorphism (SNP) array, comparative genomic hybridization-array (array-CGH) and next generation sequencing (NGS) enable the identification of numerous causative genes of CULA. Unravelling the genetic background of syndromic CULA helps to broaden the spectrum of causative genes, identify the pathways required for normal limb organogenesis and explain the mechanism underlying the cooccurrence of CULA and non-limb anomalies. In this review, we first introduce the developmental process of the upper limbs and the involved signaling pathways. Then, we provide an overview of the genetic basis of syndromic CULA according to the related pathways of the causal genes.

Development of the upper limbs

The development of vertebrate limbs during embryonic life is a multi-stage process, which is orchestrated by complex interactions between signaling centers. In humans, the development of the limb bud initiates at around the fourth week of gestation with the appearance of a small bud from the lateral body wall. The limb bud consists of an inner mesenchymal core and an outer ectodermal cap (9). Following the limb bud emergence is the formation of three axes: the proximal-distal (PD), the anteroposterior (AP) and the dorso-ventral (DV) axes. Mesenchymal fibroblast growth factor 10 (FGF10), bone morphogenetic proteins (BMP) signaling induce the formation of the apical ectodermal ridge (AER), which is located at the DV interface. AER mainly mediates PD patterning through the regulation of FGF and WNT signals (10). Besides FGF from AER, DV axis is also defined by retinoic acid (RA) signaling from the lateral walls (9). Zone of polarizing activity (ZPA) locates in the posterior limb bud mesenchyme and is controlled by Sonic Hedgehog (SHH) signaling. SHH secreted by ZPA diffuse along the limb bud to form a gradient, which control the formation of AP axis. Multiple signals including TBX, HAND2, RA, HOXD and GLI3R are involved in the regulation of SHH expression and further influence AP patterning. SHH from ZPA and FGF from AER form an epithelial-mesenchymal feedback loop that governs the formation of PD and AP axis. WNT family member 7A (WNT7A), produced by the presumptive dorsal limb ectoderm, is considered to be the organizer of DV axis. The specific expression of LIM-homeodomain transcription factor 1 beta (LMX1B) in the dorsal mesenchyme of the limb bud is induced by WNT7A (6). LMX1B is a necessary and sufficient factor in guidance of the dorsal limb patterning (11). The signal transduction of SHH, WNT and BMP is critically coordinated by the primary cilium, which is a microtubule-based organelle that emerge from the cell surface of most vertebrate cell types (10).

Genetic advances in syndromic CULA

Aat)Non-limb anomalies associated with syndromic CULA include a broad range of presentations, e.g., genitourinary, cardiovascular, nervous, facial, etc. (11). Syndromic CULA contribute to 20% to 30% of all CULA (1,2), and about 10% to 20% of all congenital anomalies have upper limb involvement (3). Associated non-limb anomalies can be life threatening and thus require more attention from clinicians and parents.

Both genetic mutations and genomic rearrangements can lead to syndromic CULA (summarized in *Table 1*). Many genes involved in the pathogenesis of syndromic CULA can be attributed to several biological pathways. Here, we introduce roles of Cohesin proteins, WNT signaling, Hedgehog signaling, FGFs, FGFRs and PI3K-AKT1-mTOR signaling in the pathogenesis of syndromic CULA.

Cohesin proteins and Cornelia de Lange syndrome

Cohesin proteins are important Structural Maintenance of Chromosomes (SMC) protein containing complexes. By mediating the chromatin organization and long-distance chromatin interaction, the cohesion proteins can affect gene expression (12). Several disorders originate from mutations in cohesin subunits and its key regulators are termed as cohesinopathies. Cornelia de Lange syndrome (CdLS; MIM: 122470), a prominent member of cohesinopathies, is a dominantly inherited disorder characterized by dysmorphic features, CULA, global developmental retardation and gastrointestinal system involvement. NIPBL load the cohesin complex onto DNA and is required for topological movements of DNA (13). Mutations in *NIPBL* are responsible for a significant proportion of CdLS (12). Currently there are six cohesins-related genes have been implicated in CdLS, i.e., *HDAC8* (MIM: 300882), *RAD21* (MIM: 614701), *NIPBL* (MIM: 122470), *SMC1A* (MIM: 300590), *BRD4* and *SMC3* (MIM: 610759) (14). Among them, HDAC8, BRD4 and NIPBL are key regulators of

Table 1 Known syndromes associated with CULA

Syndrome	Locus	Inheritance mode	MIM ID	Main limb presentations	Related pathway
Robinow syndrome, autosomal dominant 2	DVL1	AD	616331	Brachydatyly	WNT signaling
Cardiospondylocarpofacial syndrome	MAP3K7	AD	157800	Syndactyly, polydactyly	WNT signaling
Focal dermal hypoplasia	PORCN	XLD	305600	Syndactyly, polydactyly	WNT signaling
Robinow syndrome, autosomal dominant 1	WNT5A	AD	180700	Brachydatyly	WNT signaling
Fuhrmann syndrome	WNT7A	AR	228930	Polydactyly, syndactyly, oligodactyly	WNT signaling
Schinzel phocomelia syndrome	WNT7A	AR	276820	Hypoplasia of the ulna and fibula	WNT signaling
Proteus syndrome	AKT1	S	176920	Macrodactyly	PI3K-mTOR-AKT
CLAPO syndrome	PIK3CA	S	613089	Macrodactyly	PI3K-mTOR-AKT
CLOVE syndrome,	PIK3CA	S	612918	Macrodactyly	PI3K-mTOR-AKT
Greig cephalopolysyndactyly syndrome	GLI3	AD	175700	Polydactyly, syndactyly	Hedgehog signaling
Pallister-hall syndrome	GLI3	AD	146510	Polydactyly	Hedgehog signaling
Laurin-Sandrow syndrome	LMBR1	AD	135750	Syndactyly, polydactyly	Hedgehog signaling
Nail-patella syndrome	LMX1B	AD	161200	Limited elbow movements	Hedgehog signaling
Basal cell nevus syndrome	PTCH2	AD	109400	Polydactyly	Hedgehog signaling
Basal cell nevus syndrome	PTCH1	AD	109400	Polydactyly	Hedgehog signaling
Robinow syndrome, autosomal recessive	ROR2	AR	602337	Brachydatyly	Hedgehog signaling
Basal cell nevus syndrome	SUFU	AD	109400	Polydactyly	Hedgehog signaling
Joubert syndrome 32	SUFU	AR	617757	Polydactyly	Hedgehog signaling
LADD syndrome	FGF10	AD	149730	Clinodactyly, polydactyly	FGF-FGFR
Multiple synostoses syndrome 3	FGF9	AD	612961	Synostoses	FGF-FGFR
Pfeiffer syndrome	FGFR1	AD	101600	Broad thumb, syndactyly	FGF-FGFR
Apert syndrome	FGFR2	AD	101200	Syndactyly	FGF-FGFR
LADD syndrome	FGFR2	AD	149730	Clinodactyly, polydactyly	FGF-FGFR
Pfeiffer syndrome	FGFR2	AD	101600	Broad thumb, syndactyly	FGF-FGFR
Saethre-chotzen syndrome	FGFR2	AD	101400	Syndactyly	FGF-FGFR
LADD syndrome	FGFR3	AD	149730	Clinodactyly, polydactyly	FGF-FGFR
Cornelia de Lange syndrome 5	HDAC8	XLD	300882	Clinodactyly, oligodactyly	Cohesin protein
Cornelia de Lange syndrome 1	NIPBL	AD	122470	Clinodactyly, oligodactyly	Cohesin protein
Cornelia de Lange syndrome 4	RAD21	AD	614701	Clinodactyly, oligodactyly	Cohesin protein
Cornelia de Lange syndrome 2	SMC1A	XLD	300590	Clinodactyly, oligodactyly	Cohesin protein
Cornelia de Lange syndrome 3	SMC3	AD	610759	Clinodactyly, oligodactyly	Cohesin protein
Thrombocytopenia-absent radius syndrome	1q21.1	AR	274000	Radial aplasia	–
2p15-p16.1 deletion syndrome	2p15-p16.1	AD	612513	Camptodactyly	–

Table 1 (*continued*)

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Syndrome	Locus	Inheritance mode	MIM ID	Main limb presentations	Related pathway
2q37 deletion syndrome	2q37	AD	600430	Brachydactyly	–
EEC syndrome	7q11.2-q21.3	AD	129900	Ectrodactyly	–
Trichorhinophalangeal syndrome, type II	8q24.11-q24.13	AD	150230	Syndactyly	–
Smith-Magenis syndrome	17p11.2	AD	182290	Brachydatyly	–
Rubinstein-Taybi syndrome 1	16p13.3	AD	610543	Broad thumb	–
Acrofacialdysostosis1,Nagertype	SF3B4	AD	154400	Thumb hypoplasia/aplasia, triphalangeal thumb	–
Baller-Gerold syndrome	RECQL4	AR	218600	Radial aplasia	–
Bardet-Biedl syndrome 1**	BBS1	AR	209900	Polydactyly	–
Bardet-Biedl syndrome 10	BBS10	AR	615981	Polydactyly	–
Beals syndrome	FBN2	AD	121050	Arachnodactyly, camptodactyly	–
Catel-Manzke syndrome	TGDS	AR	616145	Hyperphalangy	–
Holt-Oram syndrome	TBX5	AD	142900	Triphalangeal thumb	–
Larsen syndrome	FLNB	AD	150250	Spatulate thumb	–
Leri-Weilldyschondrosteosis	SHOX/SHOY	PAD	127300	Madelung deformity	–
Multiplesynostoses syndrome 1	NOG	AD	186500	Synostoses	–
Multiplesynostoses syndrome 2	GDF5	AD	610017	Synostoses	–
Multiplesynostoses syndrome 4	GDF6	AD	617898	Synostoses	–
Otopalatodigital syndrome, type I	FLNA	XLD	311300	Short hallux, short thumb	–
Otopalatodigital syndrome, type II	FLNA	XLD	304120	Short hallux, short thumb	–
Roberts syndrome	ESCO2	AR	268300	Tetraphocomelia	–
Saethre-chotzen syndrome	TWIST1	AD	101400	Syndactyly	–
Townes-Brocks syndrome 1	SALL1	AD	107480	Triphalangeal thumb, polydactyly	–
Ulnar-mammary syndrome	TBX3	AD	181450	Ulna hypoplasia/aplasia	–

*, these syndromes are considered to be most relevant with CULA; **, there are more than 10 subtypes of Bardet-Biedl syndrome have been discovered. Only two utmost mutated genes are listed here. AD, autosomal dominant; AR, autosomal recessive; XLD, X-linked dominant; XLR, X-linked recessive; S, somatic; NA, not available.

cohesin proteins while SMC1A. SMC3 and RAD21 are structural components of the cohesin ring (13,15).

NIPBL induce histone deacetylation through recruiting histone deacetylase (HDAC) and regulate gene expression. In a zebrafish model with *Nipbl* knockdown, the size reductions and patterning defects of pectoral fin (forelimb) were observed along with impaired expression of several key limb development genes including *fgfs*, *hand2* and multiple *hox* genes (16), which suggests that the limb malformations in CdLS can be attributed to the impact of Cohesin proteins upon the expression of critically limb development genes.

WNT signaling

WNT signals comprise a diverse group of secreted proteins that mediates crucial developmental processes

including embryonic axis patterning, cell fate specification, proliferation and migration (17). Genes coding for a variety of signaling molecules involved in Wnt signaling pathway have been identified in Mendelian inherited syndromic CULA. Recessively inherited *WNT7A* mutations were described in individuals with Schinzel phocomelia syndrome (MIM: 276820) and Fuhrmann syndrome (MIM: 228930) (18). Both syndromes have limb deformities, but Schinzel phocomelia syndrome comprise a broader set of phenotypes including severe anomalies of upper and lower limbs with hypoplastic pelvis and genitalia. There are many other genes in Wnt signaling pathway which have been associated with syndromic CULA. Cardiospondylocarpofacial syndrome (CSCF; MIM: e157800) is characterized by cardiac septal defects, vertebral synostosis, brachydactyly with carpal-tarsal fusion and dysmorphic facial features. CSCF is caused by heterozygous mutation in *MAP3K7* gene. MAP3K7 act as an upstream kinase regulating several pathways including WNT, NF-κB and p38 MAPK (19). Focal dermal hypoplasia (MIM: 305600), caused by mutations in *PORCN*, is a X-linked dominant syndrome with in utero lethality in males. Common features of this syndrome include distinct skin manifestations, digits malformations (mainly comprise syndactyly and polydactyly), oral and ocular anomalies. PORCN is essential for the acetylation and secretion of WNT ligands in mice and humans (20). Given the crucial role of Wnt signaling in human development, it is not surprised to find out these Wnt signaling associated syndromic CULA display complex phenotypes commonly affecting multiple organs and systems.

Robinow syndrome is a genetically heterogeneous severe skeletal dysplasia characterized by mesomelic limb shortening, dysmorphic features including midface hypoplasia, hypoplastic genitalia and vertebral anomalies (21,22). Mutations of several genes involved in the WNT signaling pathway have been implicated in different subtypes of Robinow syndrome. *WNT5A* is involved in both the canonical and noncanonical Wnt signaling pathway (23). Dominantly inherited *WNT5A* mutations have been associated with autosomal dominant Robinow syndrome-1 (DRS; MIM: 180700) and homozygous *Wnt5a* null mice presented with anatomical defects resembling individuals with autosomal DRS (24). ROR2, a tyrosine kinase-like orphan receptor, interacts with WNT5A both functionally and physically (23). The binding of WNT5A with ROR2 lead to phosphorylation of ROR2, resulting in Rho GTPase dependent activation of the WNT-JNK and WNT calcium pathway, i.e., the noncanonical WNT pathway. Recessive and dominant mutations of *ROR2* have also been implicated in autosomal recessive Robinow syndrome (RRS; MIM: 268310) and brachydactyly type B1 (BDB1; MIM: 113000), respectively. Interestingly, not all patients carrying heterozygous null mutations in *ROR2* exhibited brachydactyly. By measuring steady-state protein model levels as well as surface location in *ROR2* variant-expressing cell lines, Schwarzer *et al.* presented a hypothesis that the phenotypic outcome can be explained by the equilibrium between intracellular retention and cell surface expression rather than a simple loss-or gain-of-function model (25). Dishevelled (DVL): 13 is intracellular protein Tinvolved in the canonical and non-canonical WNT signaling (26). The binding of DVL to the cell membrane facilitates the binding of Axin and GSK3β, which subsequently phosphorylate LRP5/6 and thereby preventing degradation of β-catenin. The accumulation of β-catenin in the nucleus activates WNT responsive genes (26). In the non-canonical WNT pathway, DVL plays key role in regulating polarity and cytoskeletal determination by acting as a branchpoint for activation of Rho, Rac and Cdc42 (26). Heterozygous *DVL1* and *DVL3* mutations have been shown to lead to autosomal dominant Robinow syndrome-2 (MIM: 616331) and autosomal dominant Robinow syndrome-3 (MIM: 616894) (27). Together, these evidences suggest that WNT signaling pathway play an important role in the pathogenesis of a wide spectrum of hypoplastic disorders including CULA.

Hedgehog signaling

The Hedgehog (HH) signaling pathway has many roles in controlling of the cell proliferation, embryonic patterning and limb development (9). Gli-kruppel (GLI) genes encode transcription factors involved in SHH signaling pathway. *GLI1*, *GLI2 and GLI3*, members of GLI family, have been associated with multiple human diseases including both syndromic and non-syndromic CULA. GLI3 is a transcription factor and act as a modulator for SHH pathway with either facilitative and suppressive function (28). SHH pathway inactivation constitutively transforms GLI3 to GLI3R isoform, which is translocated into the nucleus and negatively regulates SHH target genes (29). Activated SHH inhibits GLI3R isoform formation and induces the formation of GLI2A and GLI3A (GLI activators). Subsequently, GLI activators promotes the expression of SHH targeted genes. Besides *ROR2*, *GLI3* is another gene that have been implicated in both syndromic and non-syndromic CULA. Heterozygous mutations in *GLI3*

gene can lead to Greig cephalopolysyndactyly syndrome (GCPS; MIM: 175700), Pallister-Hall syndrome (PHS; MIM: 146510), postaxial polydactyly type A1 and B (PAP; MIM:174200) and preaxial polydactyly type IV (PPD; MIM: 174700) (28). GCPAS and PHS are allelic syndromes with distinct clinical entities (30). GCPS is mainly characterized by polysyndactyly, macrocephaly with frontal bossing and hypertelorism (31). Preaxial of the feet and postaxial in the hands are most commonly identified forms of polydactyly (31). Typical phenotypes of PHS include central polydactyly, pituitary malfunction, hypothalamic hamartoma and visceral malformations (30). The genotype-phenotype correlations of *GLI3* associated disorders are well characterized (28). The N-terminal part of the GLI3 protein contains the zinc finger domain. Mutations in the zinc finger domain mostly result in GCPS through a haploinsufficiency mechanism. The middle part of the GLI3 protein encompasses the protein cleavage site. Patients with PHS usually carry mutations in the middle part. The C-terminal of contains the transactivating domains 1 and 2. Mutations in the C-terminal part lead to distinct phenotypes including GCPS, PAP and PPD (28). Nevertheless, the mechanism underlying the distinct phenotype spectrum needs further studies.

SUFU, acronym for Suppressor of Fused, plays a critical role in primary cilia. SUFU acts as the main negative regulator of the SHH pathway through forming a complex with GLI3 and GLI2 in primary cilia (32). Transportation of SUFU to primary cilia is potentially governed by GLI activators but not suppressors (33). Recessive and dominant mutations of *SUFU* were known to cause Joubert syndrome 32 (MIM: 617757) and basal cell nevus syndrome (BCNS; MIM: 109400), respectively (32). Joubert syndrome 32 is characterized by congenital ataxia, postaxial polydactyly, cerebellar vermis hypoplasia and craniofacial dysmorphisms. While postaxial syndactyly is relatively rare in BCNS (32). Heterozygous mutations in *PTCH1* and *PTHC2* can also lead to basal cell nevus syndrome. Basal cell nevus syndrome is a familial cancer predisposing syndrome which comprises a broad spectrum of systemic manifestations, the most characteristic symptoms of which are basal cell carcinomas, jaw keratocytes and cerebral calcifications (34). *PTCH1* and *PTCH2*, encoding members of the patched family, suppress SHH signaling through inhibition of SMO. Loss-of-function of PTCH1 and PTCH2 result in aberrant increase in SHH signaling (34).

The ZPA regulatory sequence (*ZRS*), a long-range limb-specific enhancer of the SHH, is located in intron 5 of *LMBR1* (35). Recently, a missense mutation in p-*ZRS* (pre-*ZRS*), a noncoding sequence 500 bp upstream of the *ZRS*, have been associated with triphalangeal thumb-polysyndactyly syndrome (MIM: 174500) in a multigenerational family (36). Transgenic mice carrying this mutation showed ectopic SHH expression (36). Although regulatory effects of *ZRS* upon Shh have been well characterized (37), further study is still needed to determine the role of pZRS in Shh limb expression.

Fibroblast growth factors (FGFs) and FGFRs

FGFs are a family of proteins that regulates many developmental processes in the early stage of embryonic development. FGFs carry out their functions through binding to the fibroblast growth factor receptors (FGFRs). Currently there are four FGFRs (FGFR1 to FGFR4) known to interact with FGFs in an HSGAG-dependent manner (38). Either increasing and decreasing of FGF signaling can cause human diseases. For example, gain-of-function mutations of *FGFR2* lead to Apert syndrome (MIM: 101200) (39), a developmental disorder characterized by craniosynostosis, midface hypoplasia and severe syndactyly with fusion tendency. Studies have revealed that about two-thirds of Apert syndrome is caused by the S252W mutation in *FGFR2*, and the rest one third is caused by the P253R mutation in *FGFR2* (40). Syndactyly in hands and feet was likely to be more severe in patients carry the P253R mutation, while cleft palate was more frequently observed in patients carrying the P252W mutation (40). Prolyl isomerase peptidyl-prolyl cis–trans isomerase interacting 1 (*PIN1*) is a crucial regulator of FGFR signaling and $Pin1^{+/-}$ mice displayed delayed closure of cranial sutures (41). By crossing $Pin1^{+/-}$ mice with $Fgfr2^{S252W/+}$ mice, a mouse model of Apert syndrome, the downregulation of *Pin1* function attenuated premature cranial and frontal-nasal suture fusion. Although other phenotypes like syndactyly was not rescued by the reduced dosage of *Pin1*, this approach do provide novel therapeutic insights in alleviating other phenotypes in Apert syndrome. Gain-of-function mutations of *FGF10* is responsible for Lacrimoauriculodentodigital syndrome (LADD syndrome; MIM: 149730) (42), a congenital disorder mainly involving lacrimal glands and duct, ears, teeth and digits. Activating mutations in *FGFR2* and *FGFR3* can also lead to LADD syndrome (43). FGFR2 interacts with FGF10 and conditional knockout of *Fgfr2* in mice leads to limb and digit malformations (43). Mutations in *FGFR3* gene are now associated with at least10 human

disorders, involving skeletal dysplasia, skin malformation and cancer (44). Attenuation of the chondrocytes are responsible for the *FGFR3*-related skeletal dysplasia (44). Recently, Bosakova *et al.*, found that the sustained and transient activation of FGF signaling result in shortening and elongation of primary cilia, respectively (45). The SHH signaling is subsequently influenced by the impaired cilia function (45), providing insights into the pathogenic mechanism underlying the limb phenotypes of FGFR-related disorders.

PI3K-AKT1-mTOR

PI3K-AKT-mTOR signaling mediates cell proliferation, survival and metabolism, activating of this pathway is commonly involved in tumorigenesis. *PIK3CA* and *AKT1* are frequently mutated gene in human tumors (46,47). Somatic mosaicism of *PIK3CA* and *AKT1* can both lead to overgrowth syndromes. Genetic mosaicism refers to a process occurring postzygotically that result in the presence of genetically distinct cells within one individual. Advent of the deep NGS facilitated the discovery of developmental disorders caused by mosaic mutations. Proteus syndrome (MIM: 176920), caused by mutations in *AKT1*, is characterized by cerebriform connective tissue nevi and patchy overgrowth most commonly involving limbs. Most patients with Proteus syndrome carried a hotspot gain of function mutation c.G49A in *AKT1* (47). Somatic mosaicism of *PIK3CA* is responsible for a wide spectrum of disorders named as *PIK3CA* related overgrowth syndrome, including congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal/scoliosis/spinal abnormalities (CLOVE syndrome; MIM: 612918), capillary malformation of the lower lip, lymphatic malformation of the face and neck, asymmetry and partial/generalized overgrowth (CLAPO syndrome; MIM: 613089), megalencephaly-capillary malformation (MCAP, MIM: 602501). Focal forms of *PIK3CA* related overgrowth include macrodactyly, epidermal nevi, infiltrating lipomatosis lymphatic malformations and venous malformations (48). The most frequently observed limb component of these disorders is macrodactyly and the degree of overgrowth is variable.

CNVs

Several well-characterized CNVs underlying syndromes with limb involvement have been identified. Typically, thrombocytopenia-absent radius syndrome (TAR syndrome; MIM: 274000), a syndrome with multiple anomalies affecting the blood circulation of the upper limb, is caused by compound heterozygosity for a 1q21.1 deletion involving the *RBM8A* gene and a noncoding SNP in *RBM8A* (49). The impact of a rare null variant and a noncoding common SNP was also observed in *TBX6*-associated congenital scoliosis (TACS) (50,51). A common *TBX6* hypomorphic allele *in trans* with a rare 16p11.2 deletion or *TBX6* loss-of-function variant lead to TACS (52). Mouse models of *TBX6* compound heterozygosity display vertebral malformations and provide further evidence supporting the gene dosage model (51,52). Blood system anomalies encompass reduction in the number of platelets in blood and reduced number of megakaryocytes and platelet precursor cells in bone marrow. The severity of the upper limb involvement ranges from the absence of the radius to the absence of the most part of upper limbs, but thumbs are always well-preserved. Malformations in spine, lower limbs are less common.

Another well-characterized CULA associated syndrome is 2p15-p16.1 microdeletion syndrome (MIM: 612513) (53). This syndrome has a wide spectrum of phenotypes and about 80% patients present with hand anomalies, mainly consisting of camptodactyly (54). The size of the deletions (ranging from 0.1 to 9.5 Mb) and breakpoints vary dramatically. Recently, *XPO1*, *REL* and *BCL11A* have been identified as candidate genes for 2p15-p16.1 microdeletion syndrome (54).

There are other recurrent CNVs associated with syndromic CULA, e.g., including 2q37 (MIM: 600430), 7q11.2-q21.3 (MIM: 129900), 16p13.3 (MIM: 613458) and 17p11.2 (MIM: 182290). In a large cohort of individuals with non-syndromic congenital limb malformation, high resolution CNV analysis identified that 10% individuals harbored disease relevant CNVs (55). Among CNVs identified in that study, 57% affect the noncoding *cis*-regulatory genome, suggesting the contribution of non-coding regions to non-syndromic CULA.

Conclusions

The last decade has witnessed dramatic progress in the genetic and genomic sequencing technologies. With the improvements in molecular genetics in past few years, the field of CULA genetics is progressing very rapidly. These improvements have allowed a detailed genotype-phenotype and genotype-prognosis correlation for CULA-associated syndromes. More precise diagnosis and managements

can be adopted utilizing this knowledge. Moreover, the association between limb phenotypes and specific genetic/genomic variants has led to the identification of genes indispensable in the limb development and subsequently to a better understanding of the mechanisms regulating limb development. This knowledge gained from syndromes with CULA will help us decipher the genetic basis of CULA also in non-syndromic individuals.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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