IFSSH Scientific Committee on Genetics and Hand Surgery

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Report submitted November 2012

The genetic basis of failure of axis formation/differentiation of upper limb development

Recent progress in both clinical genetics and developmental biology has refined our understanding of how limbs develop and malformations occur[1]. This brief update highlights our current understanding regarding the genetic basis of congenital anomalies of the upper extremity and hand. This update focuses on upper limb malformations that occur from failed formation or differentiation along known axis-related pathways and utilizes the terminology of a recently proposed classification scheme that integrates clinical genetics, developmental biology and recognized upper limb abnormalities[2]. According to this classification, failure of axis formation/differentiation may be sub-classified into two groups: failure of the axis along the entire upper limb and failure of the axis within the hand-plate only as shown in Table 1.

Table 1: Selected upper limb malformations related to failure of axis formation/differentiation which will be discussed in the current report

Axis Failure	Axis Involved	Malformations
Axis failure along the entire upper limb	Proximo-distal	Transverse deficiency, Amelia, Intersegmental deficiency
	Antero-posterior	Radial ray deficiency, ulnar ray deficiency, mirror hand
	Dorso-ventral	Nail patella syndrome, *WNT7A* mutation syndromes
Axis failure within the hand-plate only	Antero-posterior	Radial polydactyly, triphalangeal thumb, ulnar polydactyly
	Dorso-ventral	Dorsal dimelia

The limb can be described in terms of three developmental axes – the proximo-distal axis, the anterior-posterior (or radial-unlar) axis and the dorsal-ventral (or dorsal-volar) axis as depicted in figures 1 and 2. Each of these axes is controlled by signaling centers that initiate a cascade of axes-related pathways.

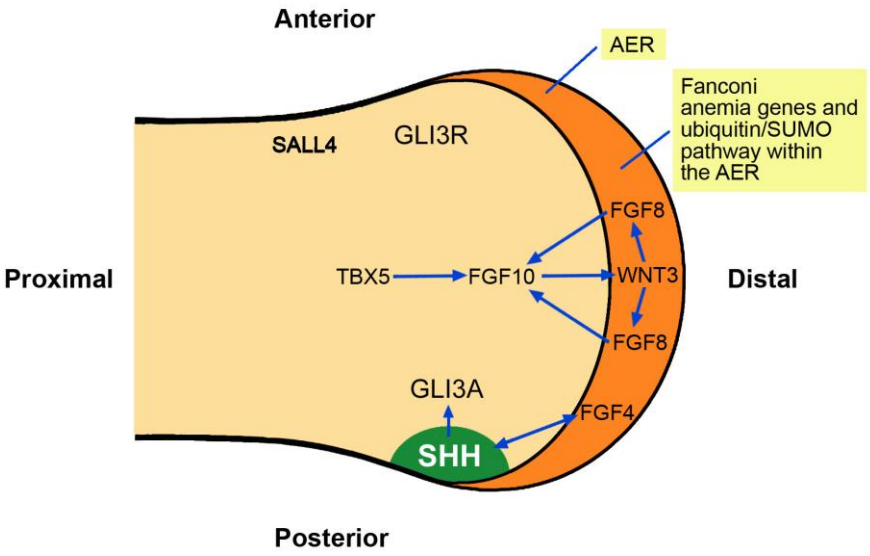

Figure 1: The proximo-distal axis: Mesodermal TBX5 induces FGF10 in the mesoderm. FGF10 induces WNT3 in the AER. WNT3 will then induce FGF8 in the AER which will help maintain FGF10 ; and the antero-posterior axis: SHH is normally loacted posteriorly and controls the development of the ulna and fingers. SHH induces FGF4 in the posterior aspect of AER. FGF4 will also help maintian SHH activity posteriorly. The thumb and radius develop normally under thhe influence ofGLI3R and SALL4 (in the mesoderm) as well as FGF8 in the overlying anterior ectoderm. Note that Fanconi anemia genes which interact with ubiquitin/SUMO pathway are highly expressed in the AER.

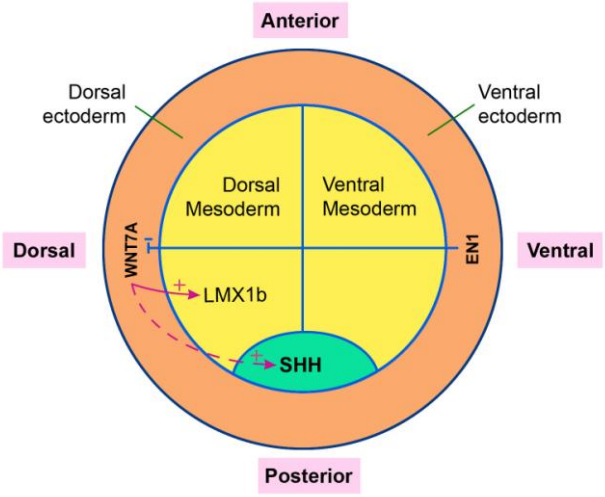

Figure 2: The dorso-ventral axis: EN-1 is in the ventral ectoderm. It supresses WNT7A preventing its expression ventrally. WNT7A of the dorsal ectoderm induces LMX1B in the dorsal mesoderm and helps to maintain SHH activity.

Failure of the proximo-distal axis (Fig 1): Transverse arrest, amelia, and segmental deficiencies (Table 2):

Limb positioning along the vertebral axis is controlled by Homeobox (HOX) transcription factors upregulating TBX5 in the presumptive upper limb which activates fibroblast growth factor (FGF) 10 in the mesoderm[3]. Haploinsufficiency or single allele disruption of *TBX5* causes Holt-Oram syndrome with a broad range of upper limb abnormalities, although radial defects appear to predominate[4]. In mice, a conditional knockout of *TBX5* in the limb stops limb bud initiation and outgrowth. However, the heart is also affected by TBX5 deficiency. Thus, the complete loss of TBX5 function in humans is likely a lethal mutation and no cases have been reported. Mesodermal FGF10 induces Wingless-type MMTV integration site family (WNT) 3 in the apical ectodermal ridge (AER) which upregulates several AER-related FGFs. These AER-related FGFs, particularly FGF8, are the primary signaling molecules of the proximo-distal axis and they maintain FGF10 secretion in the mesoderm. This reciprocal FGF10-FGF8 loop is critical for forelimb outgrowth[5]. Disruption of limb induction or the initiation of AER-related FGFs disrupts the proximo-distal axis and causes transverse deficiencies the most severe form being Amelia, the most severe form of transverse deficiency.

Interrupting the reciprocal FGF loop is also associated with failure or interruption of limb formation. Complete loss of WNT3 function (*OMIM* 273395) causes tetra-amelia.[6] In animals loss of Fgf10 function or ectodermal FGFs or transcription factors associated with AER formation (such as p63) has also been shown to abate limb outgrowth and lead to a range of defects from tetra-amelia to terminal truncations.[7-10] More recently, Al-Qattan's group showed that some patients with complete loss of WNT7A function (associated with dorsal-ventral patterning) will also have upper limb amelia suggesting a role for this factor in upper limb induction or maintenance.[11] Transverse deficiencies are uncommon, and in animal models these malformations can be mimicked by AER removal or functional ablation of FGF signaling[12;13]

Segmental disruption of the limb has been linked to SHOX and SHOX2 genes. The names of these structurally similar paralogs are derived from the initial recognition that the SHOX (short stature homeobox) gene was associated with the mesomelic short stature of Turner syndrome.[14] Disruption of Shox2 disrupts upper arm and thigh

development in animal models, but has not yet been reported in humans.[15] Both Roberts (pseudothalidomide) syndrome (*MIM* 268300) and SC phocomelia syndrome (*MIM* 26900) are caused by mutations of the *ESCO2*.[16] Upper limb malformations vary from radial ray deficiency to phocomelia. The *ESCO2* genes code for an acetyltransferase which is involved in the regulation of sister chromatid cohesion during the S phase of cell division. Newborns with thalidomide teratogenicity show phocomelia. Ito et al. recently identified CEREBLON (CRBN) as a thalidomide-binding protein. CRBN forms an E3 ubiquitin ligase complex with damaged DNA-binding protein 1 (DDB1) which is important for limb outgrowth and expression of FGF8.[17]

Table 2: The genetic basis of malformations secondary to failure of the proximo-distal axis

The Malformation	Genetic Basis
Amelia	Disruption of limb induction: - Conditional knockout of *TBX5* in mice (Homozygous *TBX5* mutations in humans are probably lethal because of cardiac defects) - Complete loss of *WNT3* - Complete loss of *FGF10* - Complete loss of *p63* - Some cases of complete loss of function of *WNT7A*
Transverse deficiencies	From animal models, disruption of the Apical ectodermal ridge (*AER*) or *AER*-related fibroblast growth factor signaling after initial limb bud formation (level of transverse deficiency is dependent on the time of disruption)

Intersegmental deficiencies	- Disruption of Shox2 (in animals) - Roberts-SC phocomelia spectrum in humans (*ESCO2* gene) - Thalidomide-induced tetartogenicity (possibly acts via CRBN and *FGF8* expression)

Failure of the antero-posterio axis (Fig 1): Longitudinal deficiencies, Radial polydactyly, Mirror hand, and ulnar polydactyly (Table 3):

HOX transcription factors also establish posterior polarity along the radial-ulnar axis which initiates sonic hedgehog (*SHH*) production from cells that become the zone of polarizing activity (ZPA).[18] SHH, the primary signaling molecule of the radial-ulnar axis, emanates from the ZPA in the distal ulnar aspect of the developing limb. SHH induces proliferation and ulnarizes nearby associated tissues.[19;20] As shown in Fig 1, the posteriorly located SHH controls the development of the ulna and all fingers except the radial aspect of the index finger. Disruption of SHH or its pathway leads to defects akin to ulnar longitudinal deficiency in animal models.[21-23]

Normal thumb and radius development only occurs if there is no SHH expression anteriorly. Instead, the development of thumb/radius is under the control of mesodermal TBX5, SALL4, GLI3R, HOXA13, HOXD13 and FGFs in the anterior ectoderm [24;25]. SALL4 is associated with TBX5 in limb/heart development; and with SALL1 in limb/kidney development. Hence, patients with Duane radial ray syndrome (*SALL4* mutation, *MIM* 607323), Holt-Oram syndrome (TBX5 mutation, *MIM* 142900), and Townes-Brocks syndrome (*SALL1* mutation, *MIM* 107480) have overlapping features of radial ray deficiency, cardiac and renal defects.[26] Radial ray deficiency can also be seen with *RECQL4* mutations (*RECQL4* codes a DNA helicase involved in DNA unwinding) and also with mutations in Fanconi anemia genes[27] (See Table 3). Al-Qattan[28] noted that both genes were involved in ubiquitin-DNA processing and this is interesting because Fanconi anemia genes are highly expressed in the apical ectodermal ridge in which FGF8 directly affects radial ray development[29] (Fig 1). Klopocki et al.[30] identified a microdeletion of 1q21.1 in a group of 30 patients with *TAR* (Thrombocytopenia-absent radius syndrome, *MIM* 274000). In 75% of these patients, the deletion was inherited from an unaffected parent. Klopocki et al. concluded that although the deletion is required, it is not sufficient for displaying the *TAR* phenotype. The phenotype develops only in the presence of an additional as-yet-unknown modifier (named *mTAR*). Al-Qattan[28] speculated that this *mTAR* may be related to the ubiquitin pathway. Finally, most cases of *VACTERL* have been sporadic, but Solomon et al.[31] recently reported an increased prevalence of isolated *VACTERL* clinical features in first-degree relatives.

Ectopic radial expression of SHH has been associated with triphalangeal thumb and preaxial polydactyly.[32;33] In animal models, elevated levels of ectopic radial SHH will generate mirror hand or ulnar dimelia.[34;35] In humans, most cases of familial radial polydactyly are related to mutations of the *ZRS* (zone of polarizing activity regulatory sequence).[32;33;36-39] The *ZRS* is a long-range limb specific *SHH* enhancer on chromosome 7q36, approximately 1Mb telomeric of *SHH*. The *ZRS* regulates the expression of *SHH* and point mutations in the *ZRS* results in enhanced *SHH* activity and ectopic anterior expression of *SHH* causing a variable phenotype of preaxial polydactyly and triphalangeal thumb. The racial distribution of the types of thumb polydactyly may also be explained genetically.[40] For example; thumb triplications and triphalangeal thumbs are known to be more prevalent in populations with genetic isolates with mutations located at chromosome 7q36. The best example is the South-Western region of the Netherlands where triplication of the thumb and thumb duplication with triphalangism are seen in 8% and 25% of all cases of thumb polydactyly; respectively.[41]

Table 3: The genetic basis of malformations secondary to failure of the antero-posterior axis

The Malformation	Genetic Basis
Ulnar ray deficiency	Deficiency in the normally located *SHH* (posteriorly)
Radial polydactyly-triphalangial thumb-mirror hand spectrum	Ectopic anterior expression of *SHH* (in humans, commonly due to *ZRS* mutations)
Ulnar polydactyly	*GLI3* mutations (Greig cephalopolysyndactyly, Pallister Hall syndrome, postaxial polydactyly types A/B)
Radial ray deficiency	- *RECQL4* mutations (Rothmund-Thomson syndrome type II, RAPADILINO syndrome, and Baller-Gerold syndrome) - Fanconi anemia genes (Fanconi anemia

	and *VACTREL* with hydrocephalus) -*TBX5* (Holt Oram syndrome) - *SALL4* (Duane radial ray syndrome) - *SALL1* (Townes-Brocks syndrome) - Microdeletion of 1q21.1 (Thrombocytopenia-absent radius syndrome)

Failures of dorsal-ventral axis formation/differentiation axis (Fig 2): Nail-patella syndrome, *WNT7A* mutation syndromes and dorsal dimelia (Table 4):

As seen in Figure 2, WNT7A is expressed in the dorsal ectoderm and is the key player of the dorsal-ventral axis, responsible for development of dorsal structures in the hand (such as the nails, the extensor tendons and the thin hairy skin).[42] EN-1expressed in the ventral ectoderm restricts WNT7A to the dorsal ectoderm. WNT7A induces the expression of LMX1B in the dorsal mesoderm and it is also important in maintaining SHH activity in the posterior mesoderm. Loss of EN-1 in animals leads to over-expression of WNT7A ventrally and hence all digits show dorsal dimelia with palmar nails as well as dorsalization of the palmar skin/structures.[43] Dorsal dimelia in humans does not involve all digits and probably represents stochastic developmental errors.[44] Human dorsal dimelia of the ulnar digits is commonly associated with ulnar-sided malformations such as 'ulnar cleft hand'.[45] Similarly, human dorsal dimelia of the radial digits is usually associated with radial-sided malformations.[27;46;47] More recently, Al-Qattan et al.[48] identified a different pattern of familial dorsal dimelia which only involved the skin of the proximal palm (all digits were normal in all affected family members). Linkage analysis and exome sequencing in that family identified a heterozygous *GLE1* mutation.

Mutations of *LMX1B* cause nail-patella syndrome with partial loss of dorsalization evidenced as hypoplastic nails and hypoplastic/absent patellae.[49] Finally, WNT7A loss-of-function mutations lead to ventral dimelia (appearance of palmar structures on the dorsum of the hand with absent/hypoplastic nails) as well as ulnar ray deficiency (secondary to loss of the effect of WNT7A on SHH). Humans with *WNT7A* mutations show a variable phenotype regarding the degree of ventralization of the dorsum of the hand and also regarding the severity of ulnar ray deficiency. Al-Qattan prefers to call these cases congenital duplication of the palm syndrome[50-52] In the genetics literature, the phenotype is known as Fuhrmann syndrome when the defects are mild and as Al-Awadi syndrome when the defects are severe.[53]

Table 4: The genetic basis of malformations secondary to failure of the dorso-ventral axis

The Malformation	Genetic Basis
Dorsal dimelia	- *EN-1* mutations in animals - Dorsal dimelia in humans are associated with other radial/ulnar malformations - One family with dorsal dimelia confined to the proximal part of palm was linked to *GLE1*
Nail-patella syndrome	*LMX1B* haplo-insufficiency
Ventral dimelia syndromes (Palmar duplication syndrome, Fuhrmann syndrome, Al-Awadi syndrome)	*WNT7A* mutations

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