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Pathogenesis of split-hand/split-foot malformation

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Split-hand/split-foot malformation (SHFM), also known as ectrodactyly, is a congenital limb malformation, characterized by a deep median cleft of the hand and/or foot due to the absence of the central rays. SHFM may occur as an isolated entity or as part of a syndrome. Both forms are frequently found in association with chromosomal rearrangements such as deletions or translocations. Detailed studies of a number of mouse models for ectrodactyly have revealed that a failure to maintain median apical ectodermal ridge (AER) signalling is the main pathogenic mechanism. A number of factors complicate the identification of the genetic defects underlying human ectrodactyly: the limited number of families linked to each SHFM locus, the large number of morphogens involved in limb development, the complex interactions between these morphogens, the involvement of modifier genes, and the presumed involvement of multiple genes or long-range regulatory elements in some cases of ectrodactyly. So far, the only mutations known to underlie SHFM in humans have been found in the *TP63* gene. The identification of novel human and mouse mutations for ectrodactyly will enhance our understanding of AER functions and the pathogenesis of ectrodactyly.

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CLINICAL FEATURES OF SHFM

SHFM is a limb malformation involving the central rays of the autopod (hand/foot). Although some authors use ectrodactyly to denote any absence deformity of the distal limbs and reserve SHFM for the typical malformation, others use it synonymously with SHFM. We shall here use the terms interchangeably. SHFM may present with syndactyly, median clefts of the hands and feet, and aplasia and/or hypoplasia of the phalanges, metacarpals and metatarsals. In severe cases, the hands and feet have a lobster claw-like appearance. However, the severity of SHFM is highly variable (Fig. 1A). In mildly affected patients, SHFM may be limited to syndactyly and several instances of non-penetrance have been documented. Clinical variability not only exists between patients, but also between limbs of a single individual (Fig. 1A).

A large number of human gene defects can cause SHFM. The most common mode of inheritance is autosomal-dominant with reduced penetrance. Anticipation has been suggested in some families (1). Autosomal-recessive (2) and X-linked forms (3) occur more rarely and other cases of SHFM are caused by chromosomal deletions and duplications. In addition to the EEC syndrome and related disorders, many syndromes comprising SHFM have been reported, but their status as independent entities is often uncertain (4).

Ectrodactyly has been observed in other species, including dogs (5), cats (6), cows (7), chickens (8), frogs and toads (9), mice (10), rabbits (11), marmosets (12) and West Indian manatees (13). Several mouse mutants have ectrodactyly, and some of these mutations may also be involved in human ectrodactyly (Tables 1 and 2, and text below).

THE ROLE OF THE APICAL ECTODERMAL RIDGE IN LIMB DEVELOPMENT

The developmental patterning of the limbs results from gradients of signalling molecules in three spatial dimensions: proximo-distal (shoulder-finger direction), antero-posterior (thumb-little finger direction), and dorso-ventral (back-palm direction) (Fig. 1B). For correct development, three specialized cell clusters are of primary importance: the apical ectodermal ridge (AER), the progress zone (PZ), and the zone of polarizing activity (ZPA). These groups of cells produce signalling molecules that determine the fate of neighbouring cells by instructing them to remain undifferentiated, to proliferate, or to differentiate into a particular cell type.

Failure to initiate the AER leads to truncations of all skeletal elements of the limb (stylopod, zeugopod, autopod). This was first demonstrated by studies in which the AER was surgically excised (14,15). Since SHFM only affects the autopod, this probably reflects a failure to maintain the normal function of the AER (Fig. 1B). Genetic defects, as well as environmental factors, may cause ectrodactyly by interfering with AER

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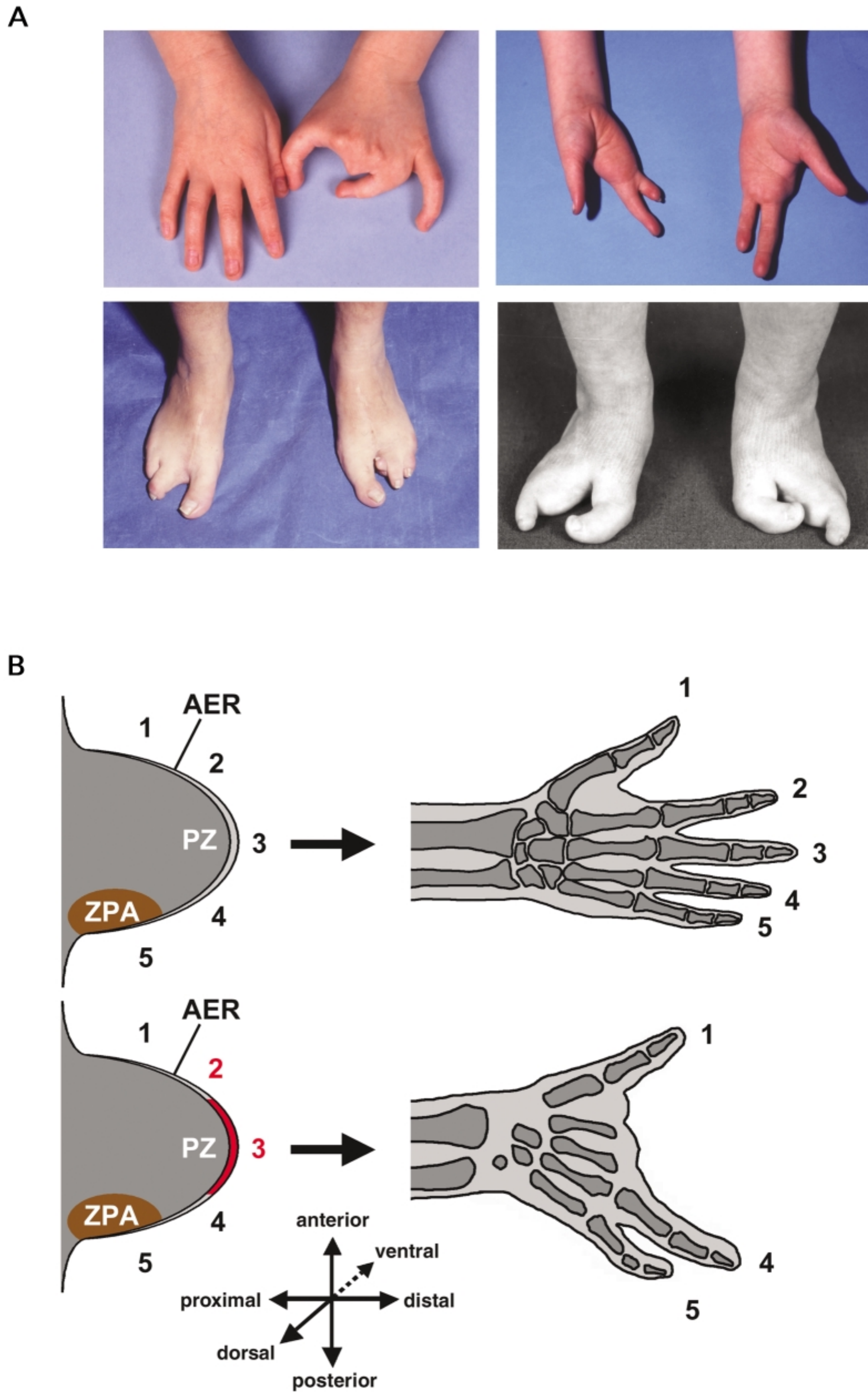

Figure 1. The ectrodactyly phenotype and underlying AER defect. (**A**) Clinical variability of ectrodactyly. (**B**) Normal development of the autopod (top) and ectrodactyly malformation (bottom). Ectrodactyly is caused by a failure to maintain median AER activity (red) in the developing limb bud (left), leading to the absence of the central rays (right). (Future) positions of digits 1–5 are indicated. AER, apical ectodermal ridge; PZ, progress zone; ZPA, zone of polarizing activity.

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Table 1. Human genetic disorders with ectrodactyly[a]

Disorder	Chromosomal location	Candidate gene(s)[b]	MIM
Isolated SHFM			
SHFM1	7q21	*DLX5, DLX6, DSS1*	183600
SHFM2	Xq26	*FGF13, TONDU*	313350
SHFM3	10q24	*Dactylin, SUFU, BTRC*	600095
SHFM4	3q27	***TP63***	605289
SHFM5	2q31	*DLX1, DLX2*	606708
EEC and related syndromes[c]			
Acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome	3q27	***TP63***	103285
Ectrodactyly-ectodermal dysplasia-cleft lip/palate (EEC) syndrome	3q27	***TP63***	604292
Limb-mammary syndrome (LMS)	3q27	***TP63***	603543
Other selected SHFM syndromes[d]			
Acro-renal-mandibular syndrome			200980
Ectrodactyly-cleft palate (ECP) syndrome		*TP63*	129830
Ectrodactyly-ectodermal dysplasia-macular dystrophy (EEM) syndrome			225280
Ectrodactyly-fibular aplasia/hypoplasia (EFA) syndrome			113310
Ectrodactyly-polydactyly			225290
Ectrodactyly-sensorineural hearing loss	7q21	*DLX5, DLX6, DSS1*	605617
Gollop–Wolfgang complex (GWC)/monodactylous ectrodactyly-split femur			228250
Goltz syndrome/focal dermal hypoplasia (FDH) syndrome			305600
Karsch–Neugebauer syndrome (KNS)			183800
Limb/pelvis hypoplasia/aplasia syndrome			276820
Microcephaly-microphthalmia-ectrodactyly-prognathism (MMEP)	6q21	*SNX3*	601349
Myelinated nerve fibres-vitreoretinopathy-split hand/foot			
Pfeiffer–Tietze–Welte/sagittal craniostenosis-mental retardation-split hand			
Recessive Robinow syndrome	9q22	***ROR2***, *GAS1*, *PTCH*[e]	268310
Split hand/foot malformation-long bone deficiency (SHFLD)			119100
Split hand-urinary anomalies-spina bifida/diaphragm defects			183802
Triphalangeal thumbs-brachyectrodactyly			190680
Ulnar aplasia-lobster claw deformity of feet			314360
Van Allen–Myhre/ectopia cordis-split hand/foot-skin defects			
Van den Ende/ectrodactyly-congenital heart disease-characteristic facies			601348
Verloes-Koulischer/oral-acral syndrome			603446

[a]This list is not complete.
[b]Causative genes in bold.
[c]Hay–Wells syndrome is not listed because it does not comprise ectrodactyly.
[d]Data from the London Dysmorphology Database (4).
[e]Recessive Robinow syndrome is caused by *ROR2* mutations, but it is not clear whether these mutations are also responsible for SHFM in some of these patients.

function or maintenance. For instance, treatment of pregnant rats with retinoic acid induces limb malformations, including ectrodactyly, by inducing AER cell death (16). Other environmental factors that are known to induce ectrodactyly in rodents include cadmium, hydroxyurea, cytarabine, methotrexate, ethanol, caffeine, cocaine, valproic acid, acetazolamide and methoxyacetic acid (17).

The AER, which is located at the distal rim of the developing limb bud, is crucial for the formation and identity of digits. Signals from the AER allow the underlying mesenchymal cells of the PZ to maintain their proliferative activity (18–22). A number of key players in the AER are known. These include fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), WNT signalling molecules, and homeobox-containing proteins, such as MSX1 and MSX2 (Fig. 2). AER formation is induced by mesodermal signalling to the overlying ectoderm. Molecules involved in this process include FGF10, its receptor FGFR2 (23), and BMPs, which control the ectodermal expression of *MSX* transcription factor genes (24). FGFs fulfil two major functions. They maintain limb outgrowth by inducing proliferation of mesenchymal cells in the PZ and they maintain Sonic Hedgehog (SHH) expression in the ZPA. Important signalling molecules involved in the latter are BMPs, whose activity is modulated by SHH signalling from the ZPA, through Formin (FMN) and Gremlin (GRE) (25–27). Several FGFs are restricted to the AER: FGF4, FGF8, FGF9 and FGF17. These AER-FGFs are crucial for limb development. In mice, simultaneous conditional ablation of Fgf4 and Fgf8 is compatible with normal AER initiation, but defective gene expression in the underlying mesenchyme. The AER itself is maintained until embryonic day E11.25, when it begins to degenerate. The *Fgf4* and *Fgf8* double knockouts have aplasia of both proximal and distal limb elements, which may be explained by a reduction of mesenchymal cells in the limb bud (19).

MOUSE MUTANTS WITH AER DISRUPTION

The *Dlx5/6* knockout mouse

The tandem *Dlx5* and *Dlx6* genes are homologues of the *Drosophila Distal-less* (*Dll*) gene. They encode homeobox-containing proteins involved in head and limb development and both are expressed in the AER (28,29). Targeted disruption of either *Dlx5* or *Dlx6* does not cause a limb phenotype, whereas simultaneous disruption of these genes results in axial skeletal,

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Table 2. Mouse mutants with AER defects

Mouse genotype	Site of limb expression	Limb phenotype	AER defect	References
Dlx5$^{-/-}$*Dlx6*$^{-/-}$	AER	Ectrodactyly	From day E11.5 onward median AER expression declines and the median AER becomes thinner	(30)
mdac/mdac Dac/+	Mesenchyme?	Ectrodactyly	Failure of median AER maintenance either due to increased AER cell death or reduced AER cell proliferation	(17,31)
mdac/mdac Dac/Dac	Mesenchyme?	Monodactyly	Failure of median and anterior AER maintenance either due to increased AER cell death or reduced AER cell proliferation	(17,31)
Tp63$^{-/-}$	AER	Forelimbs: monodactyly; hindlimbs: absent	Failure of AER maintenance due to loss of regenerative activity in ectodermal cells	(34,35)
N-Shh/–[a]	ZPA; posterior mesenchyme	Ectrodactyly, lacking digits 2 and 3	Absence of *Fgf4* expression in anterior AER due to loss of long-range Shh signalling, which normally activates *Fgf4*	(44)
Lrp6$^{-/-}$	N/D	Ectrodactyly (variable)	Failure of AER maintenance due to loss of *Fgf8* expression in median AER	(46)

[a]In contrast to wild-type Shh, N-Shh cannot be cholesterol-modified.
N/D, not determined.

craniofacial and inner ear defects. The limbs show a typical SHFM phenotype with absence of the central rays and a deep cleft between the remaining digits. Adjacent digits are often misshapen or fused to phalanges or metacarpals/metatarsals. These abnormalities have reduced penetrance in the forelimbs and complete penetrance in the hindlimbs (30,31).

In *Dlx5/6* double mutant mice, a progressive loss of median AER cells starts prior to E11.5. This is accompanied by normal *Shh* expression, but severely reduced *Fgf8* levels. No increase in apoptosis is observed, suggesting a principal role of Dlx5 and Dlx6 in promoting proliferation in the median AER (30).

The *Dactylaplasia* mouse

Increased cell death in the AER underlies the absence of the central digits in the *Dactylaplasia* (*Dac*) genetic mouse mutant (31). *Dac* is a semi-dominant mutant which displays missing central digits in the fore- and hindlimbs of heterozygous animals and monodactyly in homozygous animals. In *Dac*/+ mice, only the central portion of the AER is degenerated at day E10.5, while *Dac*/*Dac* mice have a disruption of both the central and the anterior part of the AER (17,32). The loss of the AER leads to a reduction in cell proliferation in the sub-ridge mesenchyme at E11.5. This constitutes direct genetic evidence for the existence of an AER maintenance activity that is distinct from AER induction and differentiation.

Early speculations marked *Fgf8* as a strong positional candidate gene for the *Dactylaplasia* phenotype, but mutations could not be detected in this gene (17). In a subsequent study, two independent *Dac* mutations were fine-mapped and eventually cloned (33). One mutation is an insertion in the *dactylin* gene, which encodes a member of the F-box/WD40-repeat protein family. Owing to this mutation, transcripts are produced with a frameshift in the coding region of the fifth WD40 repeat, suggesting that disruption of the *dactylin* gene causes SHFM in mice. However, the other mutation is also an insertion, which neither affects the amount nor the integrity of the *dactylin* transcript. Therefore, it cannot be excluded that these *Dac* alleles disrupt long-range regulatory sequences from one or more other genes. The latter has been reported for *SHH* in another limb malformation, preaxial polydactyly (34).

The *Tp63* knockout mouse

Mice deficient in functional p63 exhibit striking limb, craniofacial and ectodermal abnormalities, including the absence of skin and its derivatives (35,36). The forelimbs of *Tp63* knockout mice are severely malformed, lacking the radius and the complete autopod, while the hindlimbs do not develop at all. As AER remnants are observed at E11, the origin of these limb defects is not a failure to initiate the AER. Therefore, p63 is thought to function as an AER maintenance factor, or more generally speaking, as a factor that preserves the proliferative activity in specialized ectodermal cells. In line with this, the absence of skin in these *Tp63*-deficient mice is caused by a lack of regenerative proliferation of the basal stem cells of the epidermis (36,37). The precise mechanisms and pathways by which p63 executes this function remain to be established. Although p63 is able to transactivate many of the same target genes as p53, it is not known whether these genes are of any relevance to normal limb development. A few AER-specific target genes of p63 were recently identified: *Jag1*, *Jag2*, and *REDD1* (38,39). Interestingly, *Jag2* mutations give rise to a limb phenotype in the *syndactylism* mouse (40,41). Even less is known about the factors that control expression of p63 and the various p63-isoforms that exist. Recent studies in zebrafish have established that one of the two alternative p63 promoters, the ΔN-p63 promoter, is a direct target of Bmp signalling during neural development (42). Perhaps *Tp63* is also a target of BMP-signalling during control of the integrity of the AER in limb bud outgrowth.

Mice deficient in cholesterol-modified Shh

Shh is highly expressed in the ZPA. Shh signalling from the ZPA is the primary determinant of anterior–posterior polarity. The long-range signalling effects of Shh (over a few 100 microns) are brought about by the addition of a cholesterol group to an N-terminal cleavage product of Shh (43). Shh signalling is crucial in maintaining the integrity and function of the AER, which is underscored by the effects of mutations in genes from the Shh pathway, such as *Formin*, *Twist* and *Gli3*. Mutation of these genes gives rise to morphogenetic changes of

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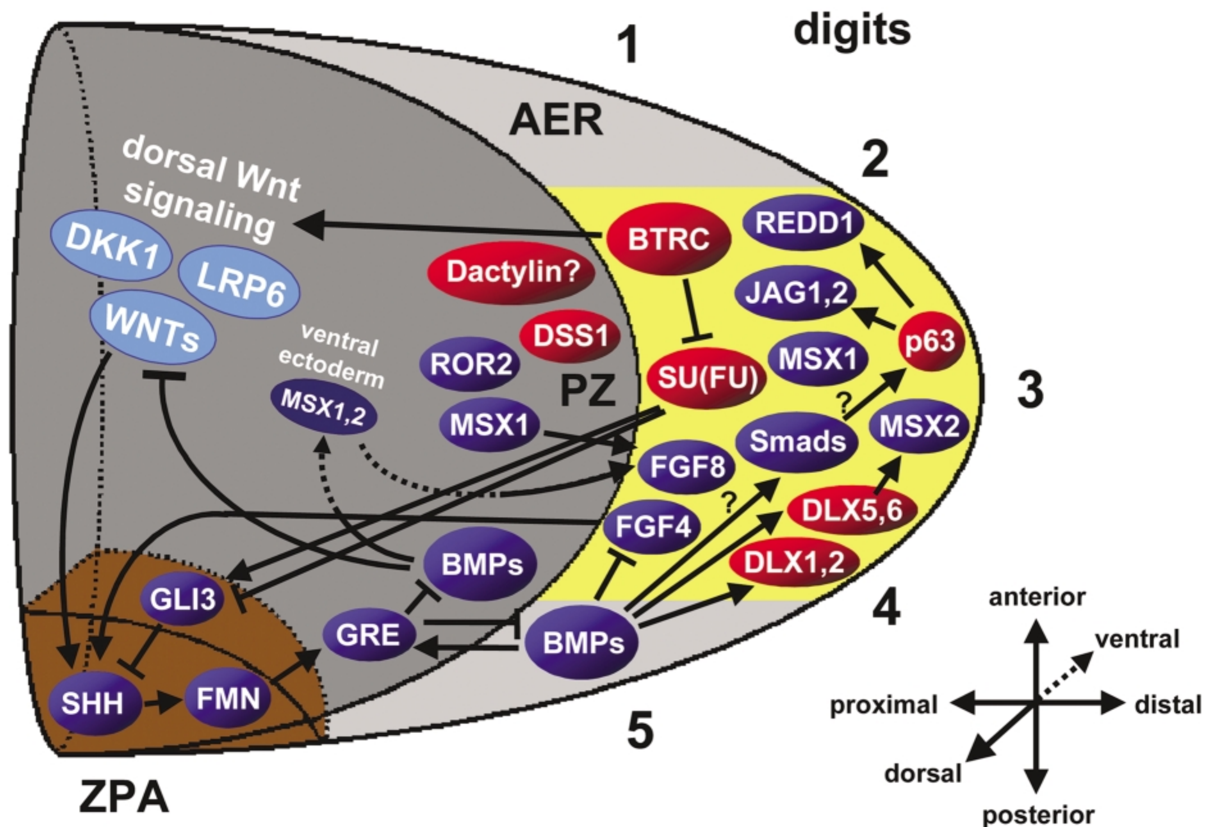

Figure 2. Signalling pathways in the developing limb bud. Failure to maintain the AER or defective AER signalling underlie SHFM. Correct signalling in the anterior and posterior apical ectodermal ridge (AER; light grey), but not in the median AER (yellow), may explain the relatively normal development of the anterior and posterior digits, respectively, while the median digits either develop very poorly or do not form at all. The positions of the AER (light grey and yellow), underlying progress zone (PZ; dark grey), and zone of polarizing activity (ZPA; brown) are indicated. Numbers 1–5 refer to the future positions of digits 1–5, respectively. Directions of the three-dimensional axes are indicated. Protein products from positional candidate genes for isolated SHFM are highlighted in red. Other molecules are shown in blue. Dorsally and ventrally expressed proteins are depicted in lighter and darker blue, respectively. Inhibitory and stimulatory effects are indicated with bars and arrows, respectively.

the AER, predominantly resulting in abnormalities of the distal limb structures.

A mouse mutant with absence of central rays was recently reported (44). In this mutant, ectrodactyly is caused by a defect in the cholesterol-modified form of Shh. This reduced the long-range signalling abilities of Shh, leaving only short range signalling intact in the posterior portion of the developing limb. Such mice lack digits 2 and 3. Mice that lack Shh completely have only a single digit, possibly a first digit. This observation is consistent with a model in which the first digit develops independent of Shh, under the influence of Bmp2. Development of the posterior digits depends on Shh short-range signalling and the central rays require combined Shh and Bmp signalling (27,44).

The *Lrp6* knockout mouse

The Wnt signalling pathway is implicated in a number of developmental processes, including dorso-ventral patterning of the limb. Activation of the pathway occurs through the binding of the Wnt ligand to its Frizzled receptor and either the Lrp5 or Lrp6 co-receptor. This triggers activation of Dishevelled, β-catenin and the LEF/TCF transcription factor (45).

Mice deficient in the *Lrp6* (*low-density lipoprotein receptor-related protein 6*) co-receptor gene exhibit a number of developmental abnormalities. These include neural tube defects, microphthalmia and axial skeletal, urogenital and limb anomalies (46). The limb defects are variable: digit 5 is consistently absent, but some mice lack additional posterior digits. In addition, the radius may be missing and the ulna malformed. Interestingly, a strongly reduced *Shh* expression and a subsequent failure to maintain the AER precede aberrant development in *Lrp6*$^{-/-}$ limbs. A similar failure of AER maintenance is seen upon ectopic expression of *Dkk1* (*Dickkopf 1*) in limb buds, leading to absent and fused digits. Dkk1 is an inhibitor of Wnt signalling, which acts through repression of Lrp6 (47). Since at least one of the Wnts (Wnt7a) can induce *Shh* expression in the posterior mesenchyme, the absence of Lrp6 may result in perturbed Wnt signalling downstream of the Frizzled receptor and hence aberrant induction of *Shh* expression. Shh is engaged in a feedback loop that activates Fgf4 in the AER (Fig. 2) (25). Thus, as in mice deficient in cholesterol-modified Shh, disturbance of the

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Shh-Fgf4 pathway is likely to underlie the ectrodactyly phenotype in *Lrp6*$^{-/-}$ mice.

HUMAN LOCI FOR ISOLATED SHFM

SHFM1 (chromosome 7q21; OMIM 183600)

A number of cases of either isolated SHFM or syndromic forms of ectrodactyly are associated with chromosomal aberrations involving the 7q21–q22 region (48–57). Families with SHFM and sensorineural deafness also show linkage to this locus (58). Extensive analysis of the locations of the deletions has narrowed down the SHFM1 critical region to 1.5 Mb and six breakpoints have been found within a 700 kb region (59,60). Three candidate genes are located in the common deletion interval: *DLX5*, *DLX6* and *DSS1* (61).

The murine orthologue of *DSS1* (*Deleted in Split-hand/Split-foot malformation 1*) is expressed in the branchial arches, genital tubercle and the developing limb bud. In the limb bud, expression is first detected throughout the mesenchyme, but not in the ectoderm. At later stages, expression is confined to the distal mesenchyme and ultimately to the interdigital mesenchyme only. Targeted deletion of both *Dlx5* and *Dlx6*, but not either one alone, results in typical ectrodactyly in addition to inner ear and severe craniofacial defects (30). The combined homozygous *Dlx5/6*$^{-/-}$ mice thus recapitulate the dominantly inherited limb and ear canal defects in SHFM1 (54). *Dss1* expression is normal in these mutant mice, suggesting that this gene does not contribute to the phenotype.

SHFM1 patients do not have mutations in the coding regions of *DLX5*, *DLX6* or *DSS1* (61). One possible explanation for this is that mutations of long distance transcriptional control elements cause SHFM1.

SHFM2 (chromosome Xq26; OMIM 313350)

Only a single SHFM family has been reported with X-chromosomal inheritance of isolated ectrodactyly (3). The patients from a large inbred Pakistani family exhibited mono- or bidactylous hands and typical ectrodactyly of the feet. Of a total of 36 individuals with the full expression, 33 were males and only three were female. A presumed X-chromosomal inheritance of the SHFM phenotype led to a more extensive clinical examination of the obligate heterozygous females. In about half of these heterozygous females, mild malformations of the hands and/or feet were observed (3). Cytogenetic studies ruled out the possibility of translocations or X-chromosomal rearrangements in this family. Linkage analysis then mapped the SHFM2 locus to chromosome region Xq26 (62). Possible candidate genes in the region include *FGF13* and *TONDU*. The latter is homologous to the *Drosophila* wing-development gene *vestigial* (63).

SHFM3 (chromosome 10q24; OMIM 600095)

A third locus for isolated SHFM was mapped to a 2 cM region on chromosome 10q24–q25 (59,64–66). The *Dactylaplasia* (*Dac*) mouse is considered a model for human SHFM3 as the human 10q24 region is homologous to the *Dac* locus on mouse chromosome 19. Yet no mutations in human *Dactylin* have been reported to date and other positional candidates should not be excluded. Expansion or contraction of a CGG trinucleotide repeat in *HOX11* might explain the anticipation observed in some SHFM3 families. However, mutations in this gene could not be identified (1). A number of further positional candidates are located in 10q24, including *FGF8*, *SUFU* and *BTRC*, but no mutations have been found in the former two genes (1,67). BTRC is the human orthologue of *Drosophila* Slimb (for supernumerary limbs), an F-box/WD40 repeat protein. Interestingly, Slimb is a regulator of both the Shh and the Wnt signalling pathways (68).

SHFM4 (chromosome 3q27; OMIM 605289)

Up to now, the 3q27 locus is the only SHFM locus for which the causative gene has been identified. Mutations underlying SHFM4 have been found in the *TP63* gene, which encodes a homologue of the tumour-suppressor p53. Despite this homology, p63 plays a key role in embryonic development, rather than in tumour suppression. The *TP63* gene encodes at least six different isoforms (69). Some of these act as transcriptional activators like p53, whereas some other isoforms have repressive activity towards p53- and p63-driven gene expression. The dominant-negative isoform ΔN-p63α is specifically found in the AER and in the basal cell layer of a number of epithelia, including the epidermis of the skin (69). Mutations in the *TP63* gene were first identified in patients with ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome (70). Later, *TP63* mutations were reported in patients with isolated ectrodactyly (71,72) as well as in a number of other syndromes (see below). In total, seven *TP63* mutations have been identified in non-syndromic SHFM families and isolated patients (71,72). Four of these are uniquely found in SHFM: two missense mutations in the DNA binding domain (K193E and K194E), a splice site mutation that predicts an amino acid insertion in the DNA binding domain, and two nonsense mutations that predict carboxy-terminal truncations of three and eight amino acids, respectively. The two other SHFM mutations, R280C and R280H, have also been encountered many times in EEC syndrome (Fig. 3) (72).

SHFM5 (chromosome 2q31; OMIM 606708)

Patients with deletions of chromosome region 2q24–q31 exhibit a number of abnormalities, including microcephaly, mental retardation, micrognatia, low-set ears, and limb abnormalities (73,74). Only some patients with such deletions present with typical ectrodactyly (75–77). Recent studies have revealed that the 2q24.3–q31 region can be subdivided into three distinct loci for limb abnormalities. Patients with deletions of the 2q24.3 locus exhibit a typical wide gap between the first and second toes in combination with flexion deformity of the fingers. Deletions of 2q31 removing the *HOXD10-HOXD13* and *EVX2* genes, as well as mutations in *HOXD13* cause synpolydactyly (SPD) (78–80). Finally, Goodman *et al.* (80) have suggested that a locus for ectrodactyly is present between these two loci. This putative ectrodactyly locus is situated centromeric to the *HOXD* gene cluster, between the *EVX2* gene

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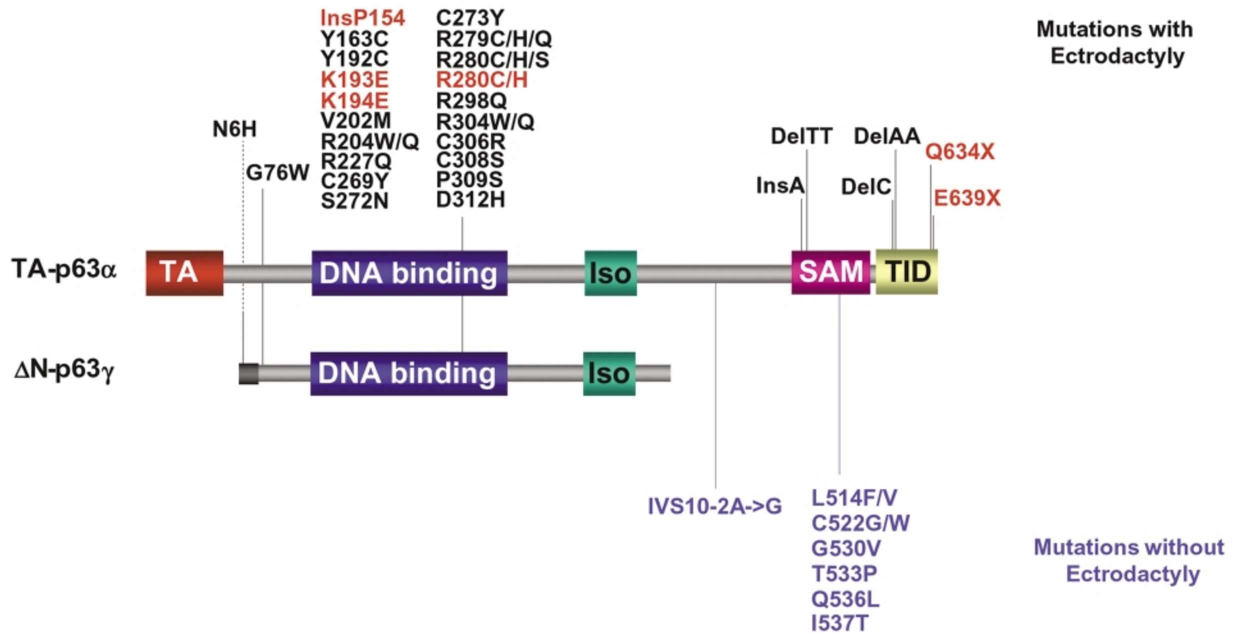

Figure 3. Mutations in p63. Two of at least six isoforms of the p63 protein are depicted: TA-p63α and ΔN-p63γ. Mutations causing syndromic SHFM are indicated in black (top), mutations causing isolated SHFM in red (top), and syndromic mutations without SHFM in blue (bottom). The latter all cause Hay–Wells syndrome. Mutations in black cause either EEC syndrome, ADULT syndrome, or LMS. The R280C and R280H mutations can either cause EEC syndrome or isolated ectrodactyly (see text for details). TA, transactivation domain; Iso, isomerization domain; SAM, sterile alpha motif; TID, transactivation inhibitory domain.

and microsatellite marker D2S294 at 2q31. This region also contains the 'digit enhancer' that controls the expression levels of the distal *HOXD* genes (*HOXD10–HOXD13*) (81). Candidate genes located in the critical SHFM5 interval include *DLX1* and *DLX2*, two homeobox genes expressed in the AER and the PZ. However, despite their role in limb development, heterozygous or homozygous knock-out mice of *Dlx1*, *Dlx2*, or both do not show any limb abnormalities at all (82). A comprehensive search for 2q31 microdeletions in additional patients with isolated SHFM should confirm the SHFM5 locus and facilitate its fine-mapping.

HUMAN ECTRODACTYLY SYNDROMES

Ectrodactyly is frequently seen in combination with other congenital anomalies. Such ectrodactyly syndromes may be caused by genetic factors or by exposure of the embryo to environmental factors. More than 50 syndromes and associations are distinguished in the London Dysmorphology Database (4). Several of these represent single case reports and neither their molecular basis nor their status as independent syndromes is known. A selection of recurrent pattern syndromes is listed in Table 1. Syndromes in which ectrodactyly is associated with other abnormalities can occur when two or more genes are affected by a chromosomal rearrangement. This explains the association of SHFM with other congenital anomalies in patients with deletions in 2q31 or 7q21. In contrast, syndromic ectrodactyly may also be the result of single gene defects. The most common and best known human SHFM syndrome is EEC syndrome, which is caused by missense mutations in the *TP63* gene (70,72). EEC syndrome is characterized by ectrodactyly, ectodermal dysplasia and clefting of the lip/palate. A number of similar disorders that are also caused by *TP63* mutations include limb-mammary syndrome (LMS) (83), acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome (84,85), and Hay–Wells syndrome (86). Interestingly, a clear genotype–phenotype correlation is observed for p63 mutations. EEC syndrome mutations cluster in the DNA binding domain of p63, while Hay–Wells syndrome, which does not comprise limb defects, is caused by mutations in the SAM protein–protein interaction domain (86). The patterns of multiple abnormalities in patients with *TP63* gene mutations allow the identification of specific domains within this gene that are most relevant for the limb phenotype (Fig. 3). Two mutations are of particular interest: R280C and R280H. Strikingly, the phenotypic consequences of these mutations appear to be consistent within families, giving rise to either the full-blown EEC syndrome or to non-syndromic SHFM. In the latter case, reduced penetrance of the R280 mutation has been observed. These observations suggest the involvement of genetic modifiers in these families (87–89). Thus, careful evaluation of specific mutations, such as R280C and R280H, may pave the way to the identification of genetic modifiers. A paradigm for the role of modifier genes in ectrodactyly is offered by the *Dac* mouse, a model for human SHFM3. The *Dactylaplasia* phenotype only develops in mice homozygous for the *mdac* modifier gene, located at mouse chromosome 13. This region is homologous to the human chromosome 9q22.31 region. Interestingly, ectrodactyly occurs in at least four unrelated patients with autosomal recessive Robinow syndrome (90) (Balci *et al.*, in preparation). These patients have homozygous loss-of-function mutations in the *ROR2* gene, which maps to this putative 9q22 modifier locus for SHFM3 (91,92).

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Perhaps, *mdac* is an allele of the *Ror2* gene, which has a crucial role in the chondrocyte lineage in late limb development (93). Other genes of interest on 9q22.31 include *GAS1*, a regulator of FGF8/FGF10 (94), and *PTCH*, a modulator of SHH signalling (44).

CONCLUSIONS

As observed in the mouse models for ectrodactyly, disturbances in AER signalling appear to be the major cause of SHFM. More specifically, ectrodactyly develops due to a failure to maintain median AER activity, either through increased cell death, or through reduced cell proliferation. This AER defect does not occur in the very earliest stages of limb development, since that would result in more severe limb malformations that are not limited to the autopod. The observation that defects in genes involved in antero-posterior signalling (*Shh*), dorso-ventral signalling (*Lrp6*, *Dkk1*), or proximo-distal signalling (*Tp63*, *Dlx5/6*) can all cause ectrodactyly demonstrates that signalling pathways acting in each of the three spatial dimensions are closely linked. For instance, the dorsalizing factor Wnt7a positively regulates posterior *Shh* expression, which in turn induces *Fgf4* expression in the AER through the Shh–Fgf4 feedback loop (25). Although in humans *TP63* is currently the only gene identified, a similar diverse array of SHFM genes is likely to exist. The existence of autosomal dominant, autosomal recessive, and X-linked inheritance and the extreme genetic heterogeneity of non-syndromic and syndromic ectrodactyly underlines this assumption. Thus, many more SHFM loci and genes remain to be discovered. The identification of the genetic defects underlying SHFM will aid in deciphering the molecular processes that govern normal AER function, as well as the pathogenesis of ectrodactyly and other limb malformations.

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