

Robin M. Winter^a
Cheryll Tickle^b

^a Mothercare Department of
Paediatric Genetics, Institute of
Child Health, and

^b Department of Anatomy and
Developmental Biology,
University College and
Middlesex School of Medicine,
University College, London, UK

Syndactylies and Polydactylies: Embryological Overview and Suggested Classification

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Abstract

In 1978, Temtamy and McKusick classified isolated, non-syndromic polydactyly and syndactyly, using a logical anatomical approach, into five distinct types for each group. Since then, there have been considerable advances in the molecular embryology of the developing limb bud. These include the proposal that retinoic acid and/or related retinoids are the morphogens responsible for the morphogenetic gradient giving rise to anterior-posterior pattern formation of the limb bud, the suggestion that the HOX4 complex and other homeotic genes may also be involved in patterning, and a greater understanding of other mechanisms such as programmed cell death in the shaping of the final hand and foot. This paper briefly reviews the molecular embryology of limb development and outlines the 'end-organ responsiveness' of the limbs to a variety of single-gene mutations. An alternative classification of syndactylies and polydactylies is suggested. It is still too early to match specific defects to individual genes with precision, and it is obvious that many important developmental genes remain to be identified; nevertheless, it is envisaged that clues from molecular embryological studies will become increasingly more useful.
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Key Words

Polydactyly
Syndactyly
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Introduction

In 1978, Temtamy and McKusick classified isolated, non-syndromic polydactyly and syndactyly, using a logical anatomical approach, into five distinct types for each group. Since then, there have been considerable ad-

vances in the molecular embryology of the developing limb bud and a more developmental approach to the classification of syndactylies and polydactylies can now be attempted. Recent advances include the proposal that retinoic and/or related retinoids are the morphogens responsible for the morpho-

Table 1. Comparative times of development for the mouse and human

Developmental stage	Number of somites	Mouse ¹ days	Human ² days
Neural plate/first somites	1–3	7.5–8	20–21
Anterior neuropore closes	15–20	9–10	24–25
Upper limb bud appears	15–25	9–9.5	26–27
Posterior neuropore closes	21–29	9.5–10	26–27
Lower limb bud appears	30–35	10–10.5	28–30
Upper digits distinguishable		12.5–13.5	41–43
Lower digits distinguishable		13.5–14.5	44–46
Upper digits fully separated		15–15.5	52–53
Lower digits fully separated		16–17	54–55

The times are approximate and depend on the strain of mouse and different experimental studies in the human.

¹ From Johnson [44].

² From Moore [51].

genetic gradient giving rise to anterior-posterior pattern formation of the limb bud, the suggestion that HOX4 complex and other homeotic genes may also be involved in patterning, and a greater understanding of other mechanisms such as programmed cell death in the shaping of the final hand and foot [1].

Brief Embryological Overview

The upper limb bud in the human appears at 26–27 days post-fertilisation and the lower limb bud slightly later at 28–30 days (table 1). Initially, these structures are paddle-shaped and consist of a mesenchymal core with overlying ectoderm. Mesenchymal cells at the margin of the bud induce the overlying ectoderm to thicken into a columnar pseudostratified epithelium to form the apical ectodermal ridge (AER). The AER is essential for the proper elongation and differentiation of the underlying mesenchyme into separate digits with the formation of cartilage, bone and muscle. The AER itself is thought to be maintained by contact with the underlying meso-

derm. It has been postulated that this mesoderm produces an apical ectodermal maintenance factor (AEMF), without which the AER degenerates [2].

Digits in the upper limb become distinguishable at 41–43 days and are fully separated at 52–53 days. In the lower limbs these events occur at 44–46 and 54–55 days, respectively.

These complex embryological processes require the formation of the correct number of digits and the establishment of the correct pattern for each digit, such that a ‘thumb’ or ‘hallux’ is situated on the preaxial side of the limb and a morphological fifth digit is on the postaxial side. The differentiation of specific cell types and structures suggests that cells in the limb bud have positional information and this must relate to the proximodistal, antero-posterior (preaxial-postaxial) and the dorso-ventral axes.

Mesenchymal cells underneath the AER proliferate rapidly and form the ‘progress zone’. As the limb bud grows, cells leave the progress zone and become incorporated into the developing mesenchymal structures. It

has been proposed that positional information is gained by cells according to the length of time spent in the progress zone [3]. Thus cells leaving early will give rise to more proximal structures and cells leaving later will have spent more time in the progress zone and will therefore be 'set' to develop into more distal structures.

When cells from the proximal part of a chick leg bud which would normally form thigh are placed in the progress zone beneath the AER, they are reprogrammed and now give rise to the toes [4]. Recently it has been shown that two related homeobox genes, Hox-7.1 and Hox-8.1, are expressed in the mesenchyme at the tip of mouse limb buds (fig. 1c). Furthermore, grafting experiments show that their expression in the bud is position dependent and appears to be regulated by a signal from the apical ridge [5, 6]. Therefore, Hox-7.1 and Hox-8.1 are good candidates for genes involved in the specification of cell position that occurs in the progress zone. The growth factor, bone morphogenetic protein 2a (BMP-2a), [7], which is related to transforming growth factor β , is expressed in the AER [8]. There is a gradient of BMP-4 [9] that suggests a negative regulation by 5' members of the the Hox-4 complex (see below). The receptor for fibroblast growth-factor is also expressed strongly in the distal part of the limb bud [10].

Transcripts of a number of other genes are found in the progress zone and these could interact with the products of Hox-7.1 and Hox-8.1. These include members of the Wnt-related gene family which could act as short-range signals [11], and the transcription factor AP-2, which is expressed in neural-crest-derived tissues, limb bud mesenchyme and in mesometanephric regions [12].

Positional information in the anteroposterior axis is thought to be gained from a chemical gradient set up by a morphogen diffusing from a group of cells on the postaxial

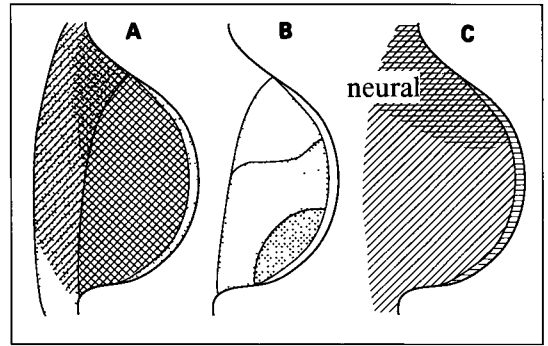


Fig. 1. Distribution of transcripts in early vertebrate limb buds. Expression is shown in both the mesenchyme cells and in the thickened apical ectodermal ridge that rims the bud. The section shown passes through the centre of the dorsoventral axis of the bud from the anterior down to the posterior. **A** ▨ = RARA; ▩ = RARB; ▧ = RARG. From Dollé et al. [16], and Schofield et al. [unpubl. data]. **B** Upper section = Hox-4.4; middle section = Hox-4.4 and -4.6; lower section = Hox-4.4, -4.6 and -4.8. From Dollé et al. [16] and Izpisua-Belmonte et al. [22]. **C** ▨ = Hox-7; ▩ = Hox-8. From Hill et al. [52] and Davidson et al. [5 and unpubl. data].

border of the limb bud – the zone of polarising activity (ZPA). It has been suggested that the morphogen involved is retinoic acid or a derivative [see review by Eichele, ref. 13], although more recently workers have questioned whether retinoids are the primary molecules involved [14].

If retinoids are important morphogens then cells must be able to respond to varying concentrations. At least three nuclear retinoic acid receptors (RARs), which are part of the steroid/thyroid family of ligand-dependent transcription factors, have been identified [reviewed by Brockes, ref. 15]. These three receptors, labelled alpha (A), beta (B) and gamma (G), are present in the limb bud. There is no evidence of a graded distribution across the limb during early development (fig. 1a), although RARB is mainly concentrated in interdigital mesenchyme and RARG

in cartilage in later stages of limb development [16]. In contrast, the cellular retinoic acid-binding protein (CRABP) shows evidence of being inversely distributed to the retinoic acid gradient in the limb bud (i.e. it is more concentrated preaxially). This has led to the suggestion that it could act in amplifying the retinoic acid signal [17].

In addition to the RARs there is another class of nuclear receptors, the RXRs (retinoid X receptors) which also exist in three main isoforms. All-trans retinoic acid is potent in transcriptional activation of both RARs and RXRs. Recently it has been shown that the ligand for RXR is 9-cis retinoic acid, which can be shown to be produced by cells in culture and in adult kidney and liver [18, 19].

Genes in the HOX4 complex have been found to be strongly expressed in the developing limb buds. There are known to be at least seven genes in the cluster [20]. Genes from the same complex are also strongly expressed in the fetal gonads. Dolle et al. [21] have shown that the more 3' genes in the complex are expressed earlier and have a more proximal expression boundary, whereas the more 5' genes begin to be expressed later and have a more distal distribution. Dolle et al. [21] have likened the pattern of expression to a set of nested Russian dolls. The focus of the zones of gene expression appears to be at the postaxial border of the limb bud, i.e. at or near the ZPA (fig. 1b).

Retinoic acid derivatives are known to be capable of regulating the transcription of homeotic genes and it is possible that the pattern of expression of the genes in the HOX4 complex is created by diffusion of a retinoid from the ZPA [22].

Hox-4 genes behave as though they encode positional values. When anterior cells are reprogrammed to form posterior structures by either application of retinoic acid or grafts of the polarising region, a mirror image pattern of Hox-4 genes is obtained which presages the

mirror image pattern of digits that will subsequently develop. The activation of the genes in the complex also proceeds in a 3' to 5' direction, reflecting the sequence of gene activation during bud development [22].

Paralogous genes in the Hox-1 complex are also expressed in the chick limb bud with semi-overlapping domains along the proximodistal axis. The most 5' genes in the complex are expressed at the tip of the limb bud, while those more 3' are expressed proximally. It has been suggested that the combination of Hox-4 and Hox-1 gene expression could specify cell position [23].

The limbs are further shaped by programmed cell death [24]. This can be demonstrated by staining with neutral red or Nile Blue sulphate which are taken up by macrophages scavenging dead cells. Mesenchymal cell death can be demonstrated between the future digits in the interdigital necrotic zones, on the anterior and posterior border of the limb bud (the anterior and posterior necrotic zone) and between the developing radius and ulna (the opaque patch). Cell death has been found to be under direct genetic control in *C. elegans* [25]. Some of the genes mentioned above are preferentially expressed in areas of programmed cell death. For example RARB, CRABP and HOX7 are expressed in the interdigital zones. The AER seems to be necessary for the induction of cell death, as interdigital tissue will not undergo cell death but will produce cartilaginous elements when the AER is removed [26].

Hox-3.3 and its homologues (e.g. XIHbox 1 in *Xenopus*) is expressed in the proximal anterior part of vertebrate forelimbs. Its pattern of expression appears to be reciprocal to Hox-4.4 [27]. Manipulations of chick wing buds show that expression can be switched on by retinoic acid. An increase in the domain of Hox-3.3 is associated with shoulder girdle abnormalities in the chicken [28].

Table 2. Syndactylies according to the Temtamy and McKusick classification [37]

Type	Fingers involved	Toes involved	Comment
I	3-4	2-3	
II	3-4	4-5	frequent mesoaxial polydactyly
III	4-5	-	possibly overlaps with oculodentodigital syndrome
IV	1-5	rarely affected	Haas type; polydactyly the rule
V	4-5 (metacarpals)	2-3; 4-5	variable cutaneous syndactyly of fingers

Table 3. Polydactylies according to the Temtamy and McKusick classification [37]

Type	Digits involved	Comment
Postaxial	postaxial extra digits	types A and B
Preaxial type I	duplication of thumb/great toes	associated with Fromont anomaly
Preaxial type II	triphalangeal thumb, duplication of great toes	thumbs opposable
Preaxial type III	absent thumb, one or two extra preaxial digits	preaxial digit may or may not be opposable; preaxial metacarpal has distal epiphysis
Preaxial type IV	broad thumbs, preaxial polysyndactyly, postaxial postminimus	overlaps with Greig's syndrome

Other genes involved in limb morphogenesis have been identified by a reverse genetic approach. The gene for limb deformity (ld) in the mouse has been isolated by insertional mutagenesis in a transgenic mouse. The ld mouse has longitudinally fused forelimb bones and complete syndactyly of disorganised bones in the paws and feet. The development of the AER is defective. Ld transcripts are present in both mesenchymal and ectodermal tissues of developing limbs. The ld gene codes for a highly conserved protein which is localised in the nucleus and has no homology to any known protein [29-31]. Cenani-Lenz syndrome in the human [32] is a possible homologue [33], although unlike the mouse mutants, patients with Cenani-Lenz syndrome have not been reported with renal agenesis.

The zinc finger protein GLI-3 appears to be interrupted in translocation cases of Greig's syndrome [34]. This gene is amplified in certain glioblastomas and is part of the GLI-Kruppel gene family which is known to play a key role in *Drosophila* and *Xenopus* development [35]. Other members of the same family could be candidates for genes important in limb development. Another limb abnormality created by insertional mutagenesis in the mouse is legless [36].

Classification

The opportunity arises to classify polydactylies and syndactylies according to whether pattern formation is normal or abnormal and

whether secondary modelling of the limb bud appears to be defective. Both of these processes seem to be capable of deviation from normal to give distinctive patterns of malformation. A review of the 'end-organ responsiveness' of the limb bud could be used to provide clues to the number and expression of candidate genes that might lead to malformations.

The Temtamy and McKusick [37] classifications of isolated syndactylies and polydactylies are given in tables 2 and 3. This classification was anatomical and did not include some multisystem disorders where the hand malformations have a 'unique' pattern not seen in isolation.

To the syndactyly classification could be added the 'Apert' hand (total, mitten-like syndactyly) [38], which is almost never seen in isolation, F-syndrome [39], where syndactyly is primarily preaxial involving the thumb and the first two fingers, and Cenani-Lenz syndrome [32] where the syndactyly is totally disorganised with abnormal development of pattern formation of the hand.

Syndactyly type II (synpolydactyly) and type IV (Haas type) seem to represent forms of polydactyly. The former manifests with syndactyly of the third and fourth fingers and the fourth and fifth toes but there is frequently partial or complete duplication of a digit within the syndactylous web [37]. The latter manifests with complete cutaneous syndactyly of fingers and thumb, or sometimes with a separate hypoplastic or triphalangeal thumb. Radiographs of the syndactylous web usually reveal at least six metacarpals [40].

Mirror hand or foot is a particularly interesting type of polydactyly and should be included in the classification. An autosomal-dominant condition has been described with bilateral absence of the tibiae, duplication of the fibula and a mirror foot with perhaps six or seven toes and no hallux. Pfeiffer and Roeskau [41] described a boy with bilateral

anomalies; his mother had unilateral involvement. A father and daughter were reported with mirror hands and feet associated with syndactyly and unusual facies [42]. In the father, the hands and feet were described as manifesting polydactyly and syndactyly at birth, and surgery was undertaken to remove supernumerary digits and release the syndactyly. The face was characterised by bilateral notches of the alae nasi. His daughter had similar facies with fixed flexion of the elbows and ten digits on each hand and foot with complete syndactyly. The ulnae were duplicated in the arms and the fibulae in the legs.

A tentative reclassification is given in table 4. This is based primarily on whether patterning is normal or abnormal, and on the location of syndactyly or polydactyly in a normally or abnormally patterned hand.

Discussion

This paper briefly reviews the molecular embryology of limb development and outlines the 'end-organ responsiveness' of the limbs to a variety of single-gene mutations.

It is likely that mutations involving the known HOX genes, growth factors or receptors will give rise to abnormalities of limb development. For example, by analogy to the proposed 'HOX-code' for specification of vertebral morphology in a cranial to caudal direction, which can be disrupted by gain or loss of function of individual HOX genes in a cluster [43], one can imagine that gain or loss of function of individual genes in the HOX4 complex might cause a shift in anteroposterior patterning to give, for example, a fingerised thumb. Mutations of the RAR genes would be expected to cause widespread abnormalities and early lethality, although it is possible that if only certain isoforms of the receptors were involved, localised malformations could arise.

Table 4. Suggested reclassification of syndactylies and polydactylies

<i>Normal patterning</i>	
Abnormal separation of digits (usually with normal number)	
Preaxial	F-syndrome
Mesoaxial	syndactyly type I
Postaxial	syndactyly types III and V
Total	Apert syndrome
Increased number of digits (sometimes with abnormal separation)	
Preaxial	preaxial polydactyly type I
Mesoaxial	synpolydactyly (syndactyly type II)
Postaxial	postaxial polydactyly types A and B
Pre- and postaxial	preaxial polydactyly type IV (Greig's syndrome)
Decreased number of digits	
Preaxial	radial ray defects
Mesoaxial	split hand/foot
Postaxial	ulnar ray defects

<i>Abnormal patterning</i>	
Preaxial	
Polydactyly of an index finger	(preaxial polydactyly type III)
Triphalangeal thumbs	(preaxial polydactyly type II)
Symmetrical with increased number of digits	
Mirror hands/feet	
Pre- and postaxial with increased number and abnormal separation of digits	
Haas-type polysyndactyly	(syndactyly type IV)
Chaotic with abnormal separation of digits	
Cenani-Lenz syndrome	

In many syndromes, pre- or postaxial polydactyly might be a relatively non-specific response to any abnormal gene that gives rise to widening of the limb bud.

Defects of programmed cell death would be expected to give rise to syndactyly, although the various anatomical distributions of the syndactylies would have to be explained by differential patterns of expression of 'cell death genes' in the limb buds. It should also be noted that diminution in size of the anterior or posterior necrotic zones, and the interdigital necrotic zones, has been postulated as a mechanism for the polydactyly mutants of the talpid series in the chick [44].

Lack of programmed cell death induced experimentally can also result in polydactyly [26].

Unusual forms of syndactyly or polydactyly have not so far been covered in this review. They could be defined as having an inconsistent pattern of abnormality, or as affecting limbs unilaterally or asymmetrically. It is recognised that vascular disruption might give rise to unilateral syndactyly or limb defects. The syndactyly of Poland syndrome is a possible example [45, 46]. Amniotic bands are another postulated cause of asymmetric, non-specific syndactyly or sometimes polydactyly [47]. Mouse mutants where subepi-

dermal blebs form early in embryonic development have been shown to give rise to syndactyly (without a specific pattern) and cryptophthalmia – attention has been drawn to the similarities to Fraser's syndrome in the human [48]. The mutant 'disorganisation' in the mouse can cause high degrees of undifferentiated, asymmetric or unilateral polydactyly [49]. Again, possible homologous cases in the human have been reported [50].

In summary, a wide range of anatomically specific syndactylies and polydactylies have been reported. Rapid advances in the molecular embryology of limb development have identified an array of 'candidate genes' whose malfunction might be expected to be implicated in some of these malformations – these are reviewed in the present paper and summarised in table 5. It is still too early to match specific defects to individual genes with preci-

Table 5. Candidate genes/processes for limb defects

Retinoid metabolism defects	zinc finger genes (e.g. GLI-Kruppel family)
Retinoid receptor defects	growth factors and receptors
AEMF defects	defective cell death
AEMF receptor defects	mechanical disruption
Hox defects	vascular abnormalities
Hox target defects	'disorganisation-like' gene

sion, and it is obvious that many important developmental genes remain to be identified. Linkage and mutation analysis of genetically inherited limb defects will continue to be somewhat 'hit and miss', but it is envisaged that clues from molecular embryological studies will become increasingly more useful.

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