Clin Genet 1998: 53: 337–348
Printed in Ireland. All rights reserved


CLINICAL GENETICS
ISSN 0009-9163

## Developmental Biology: Frontiers for Clinical Genetics

# Limb development: molecular dysmorphology is at hand!

Innis JW, Mortlock DP. Limb development: molecular dysmorphology is at hand!
Clin Genet 1998: 53: 337–348. © Munksgaard, 1998

We present a review of limb development integrating current molecular information and selected genetic disorders to illustrate the advances made in this field over the last few years. With this knowledge, clinical geneticists can now begin to consider molecular mechanisms and pathways when investigating patients with limb malformation syndromes.

**Jeffrey W Innis and Douglas P Mortlock**

University of Michigan, Department of Human Genetics, Ann Arbor, MI, USA

Key words: limb development – molecular dysmorphology – pattern organs

Corresponding author: Jeffrey W Innis, University of Michigan, Department of Human Genetics, Med. Sci. II, M4708, Ann Arbor, MI 48109-0618, USA. Fax: +1 734 763 3784; e-mail: innis@umich.edu

Received 5 March 1998, revised and accepted for publication 6 March 1998

Developmental biology is now making astounding advances. The increasing correlation of specific embryological events with gene expression/function studies is providing clues to the fundamental cellular pathways that build animals and pattern organs. Connections to human disorders are made possible because of the remarkable functional conservation of many key patterning molecules and mechanisms between model systems and human development (1–5). It is now possible to explain in some detail how malformations occur, utilizing molecular models. From a clinical standpoint, it could be said that we have firmly begun the field of molecular dysmorphology. As stated by Wilson (6), 'a molecular biology of malformations would be greatly aided if the clinical science of dysmorphology could draw upon basic embryology for a clear set of rules'. These rules are now becoming clearer with the construction of molecular models (5, 7, 8).

Significant advances in the identification of genetic determinants for limb morphogenesis have been made in model systems, which has accelerated progress in elucidating the genetic causes for several heritable limb malformations in humans. Table 1 lists numerous genes involved in human and/or mouse limb malformation syndromes, including 25 human syndromes, nine spontaneous mouse mutants, and 20 mouse knockouts. The knowledge of the patterning mechanisms and genes involved in limb development is illustrative of the impact of contemporary biology and genetics on our understanding of clinical genetic dysmorphology.

### General principles of limb development

Much of what we know about the general mechanisms underlying limb development is the result of research using chickens and amphibians over the last 50 years; however, this work has shown that important tissue domains, and signaling molecules emanating from them, are functionally conserved across species (2–5, 9). The apical ectodermal ridge (AER), zone of polarizing activity (ZPA), the mesenchyme within the progress zone (PZ), and epithelial-mesenchymal inductive interactions between these cellular domains are necessary for normal morphogenesis (Figs. 1 and 2). The limb can be divided into three axes: proximal–distal (P–D), anterior–posterior (A–P), and dorsal–ventral (D–V). Development along these axes is mediated by the asymmetric expression of specific molecular triggers in the AER, ZPA, ectoderm, and mesenchyme. Growth of the limb bud mesenchyme is followed by formation of precartilaginous condensations which form in a defined order, beginning first in proximal regions through the processes of *de novo* aggregation, bifurcation or segmentation (Fig. 2; (10)). The regulated expression of downstream target genes of these key molecules modulates growth, death, adhesion and

Table 1. Genes involved in limb development, as revealed by characterized mutations in humans or mice

| Gene | Function | Human disorder(s) | Limb phenotype | Mouse mutant(s) | References |
|---|---|---|---|---|---|
| ZPA signaling and/or A-P patterning | | | | | |
| *Shh* | Secreted signal | | Hemimelia | Knockout | (72) |
| *PTC* | Shh receptor | BCNS | Short digital elements, polydactyly | Knockout | (70, 73, 74) |
| 7-cholesterol reductase | | Smith–Lemli–Opitz | polydactyly, various defects | | (75–77) |
| *Bmp7* | Secreted signal | | Preaxial polydactly | Knockout | (78, 79) |
| *GLI2* | TF | | Upper and middle limbs shortened | Knockout | (80) |
| *GLI3* | TF | Greig cephalopolysyndactyly<br>Pallister–Hall<br>Postaxial polydactyly type A | Polydactyly,<br>syndactyly, short limbs | *Xt* (*extra toes*), *add* (*anterior digit deformity*), *bph* (*brachyphalangy*),<br>*pdn* (*polydactyly Nagoya*) | (81–89) |
| AER/Mesenchymal signaling | | | | | |
| *FGFR1* | FGF receptor | Pfeiffer | Brachydactyly, syndactyly | | (90) |
| *FGFR2* | FGF receptor | Apert<br>Pfeiffer<br>Jackson–Weiss | Syndactyly, brachydactyly | | (91–94) |
| *Formin* | cytoskeleton | | Syndactyly, oligodactyly | *ld* (*limb deformity*) | (95, 96) |
| Dorsal/ventral patterning | | | | | |
| *Wnt-7a* | TF | | Distal limbs ventralized | Knockout | (69) |
| *En-1* | TF | | Distal limbs dorsalized, syndactyly, postaxial polydactyly, digital reductions | Knockout | (97, 71) |
| Patterning and growth of cartilagenous condensations | | | | | |
| *SOX9* | TF | Campomelic dysplasia (CMPD1) | Short, bent femurs and tibiae; phalangeal defects | | (98, 99) |
| *TBX3* | TF | Ulnar–Mammary | Ulnar ray defects | | (28) |
| *TBX5* | TF | Holt–Oram | Radial ray defects | | (29, 30) |
| *TWIST* | TF | Saethre–Chotzen | Brachydactyly, syndactyly | | (100, 101) |
| *CRABPII* | TF | | Postaxial polydactyly | Knockout | (102) |
| *Hoxa10* | TF | | Upper limb defects | Knockout | (103, 104) |
| *Hoxa11* | TF | | Middle limb defects | Knockout | (105, 106, 48) |
| *Hoxa13* | TF | Hand–foot–genital (HFG) | Digital reductions | *Hd* (*Hypodactyly*), knockout | (107, 59, 108, 49) |
| *Hoxd10* | TF | | Minor hindlimb defects | Knockout | (109) |
| *Hoxd11* | TF | | Middle limb defects | Knockout | (48, 110, 111) |
| *Hoxd12* | TF | | Digital reductions | Knockout | (50) |
| *Hoxd13* | TF | Synpolydactyly (SPD) | Syndactyly, polydactyly, brachydactyly | Knockout | (50, 56, 57, 54, 112, 58) |
| HoxD locus | † | | Ulnar dysplasia | *Ul* (*Ulnaless*) | (61, 62) |
| *eEF1* | TF | | Short bones, carpal and tarsal fusions | Knockout | (113) |
| *Alx-4* | TF | | Polydactyly | Knockout | (114) |
| *p107* | Rb-like TF | | Slightly short, thickened bones | Knockout | (115) |
| *PAX3* | TF | Waardenburg type III | digital defects | | (116) |
| *SALL1* | TF | Townes-Brocks | preaxial polydactyly | | (117) |
| Bone/cartilage maturation and growth | | | | | |
| *smCDMP1* | Secreted signal | Hunter–Thompson<br>Grebe<br>AD brachydactyly type C | Short bones, phalangeal segmentation defects | *bp* (*brachypodism*) | (118–121) |

Table 1. (Continued)

| Gene | Function | Human disorder(s) | Limb phenotype | Mouse mutant(s) | References |
|---|---|---|---|---|---|
| *CBFA1* | TF | Cleidocranial dysplasia (CCD) | Brachydactyly§ | Knockout; *Ccd* | (122–126) |
| *FGFR3* | FGF receptor | Achondroplasia | Short bones | Knockout (bone overgrowth) | (127–129) |
| | | Thanatophoric dysplasia I, II | | | |
| *FGD1* | RAS signalling | Aarskog (FGD) | Brachydactyly | | (130) |

The genes have been grouped according to general developmental processes for organizational clarity, though some genes have roles in more than one category. Limb phenotype refers to the general category of defects, in humans and/or mice, caused by the spectrum of known mutations in the gene. For the sake of a cogent summary, specific correlations between phenotypes and individual mutations are not presented. In some syndromes, defects are often variable or incompletely penetrant. TF, transcription factor. 'Knockout', targeted mutagenesis.

* Humans and mice that are heterozygous for Shh deficiency generally have holoprosencephaly without limb defects, while mice that are homozygous for Shh deficiency have severe embryonic defects including limb truncations.

** Defective cholesterol metabolism may impair normal post-translational processing of Shh.

† Ulnaless may be a regulatory mutation within the *HoxD* gene complex.

§ Brachydactyly was reported in one CCD family that had an unusual alanine repeat expansion in CBFA1.

differentiation, as well as the subsequent growth of the cartilage and bone. Evolutionary or pathological differences in skeletal shape and size can be attributed to alterations in these signals.

## When, where, how many and what type?

The central problem in the developing embryo is to promote appropriately-timed development of a certain number of limbs in specified positions each with a particular morphology (forelimb versus hindlimb). It has been demonstrated that the initial outgrowth of the limb buds results from reduced mesenchymal proliferation on either side of the future limb bud territory, which continues to proliferate (9, 11), suggesting that only mesoderm at the appropriate levels receives key signals that stimulate limb bud growth. The position of the presumptive limb field is prefigured prior to limb bud outgrowth (Fig. 1) and almost certainly involves signals from the Hox genes (9, 12). In different vertebrates, somite position is not correlated with limb position, but is correlated closely with Hox gene expression (13). An ectopic mouse forelimb can be induced by changing the anterior extent of expression of *Hoxb8* (14), and, anterior displacement of the position of the endogenous forelimb bud can be induced by inactivating mouse *Hoxb5* (15).

### FGF10: the best candidate limb inducer

The intermediate mesoderm (IM) is important in the initiation and positioning of the early limb bud (Fig. 1; (16–19)). Critical signals for D–V and A–P patterning and growth induction are present long before a morphological limb bud is apparent (4, 20–25). A strong candidate for the inducer is FGF10, based on correlative expression and misexpression experiments. FGF10 is expressed early in the intermediate and lateral plate mesoderm limited to the presumptive limb territories, and is capable of inducing expression of FGF8 in the undifferentiated surface ectoderm overlying the lateral plate mesoderm prior to AER formation, and sonic hedgehog (*Shh*) expression in the mesoderm (20). The entire lateral flank has the capacity to form limbs and several fibroblast growth factors (FGFs) can initiate a signalling cascade that results in fully-formed limbs when applied to non-limb forming mesoderm in the flank (1, 20, 23, 26).

Once organized, the limb bud is able to self-organize itself, since transplantation of limb buds to ectopic locations results in fully-formed normal limbs (9). Cells derived from the initial proliferation from the lateral plate mesoderm will give rise to cartilage, blood vessels and connective tissue in a specific pattern. Nerves, neural crest cells and the somitic cells which will develop into muscles enter the limb secondarily (Fig. 2; (27)).

### Holt–Oram and Ulnar–Mammary syndromes: disorders of T-box genes

Other factors are involved in the determination of limb type. The isolation of the T-box family of genes and the elucidation of defects in these genes in Holt–Oram (TBX5) and Ulnar–Mammary (TBX3) syndromes has been particularly instructive in the elucidation of the basis for radial and ulnar ray defects (28–32). T-box genes, so-called because of a highly homologous DNA binding region shared with the brachyury gene or T (for tail) gene in mice, are transcription factors that are

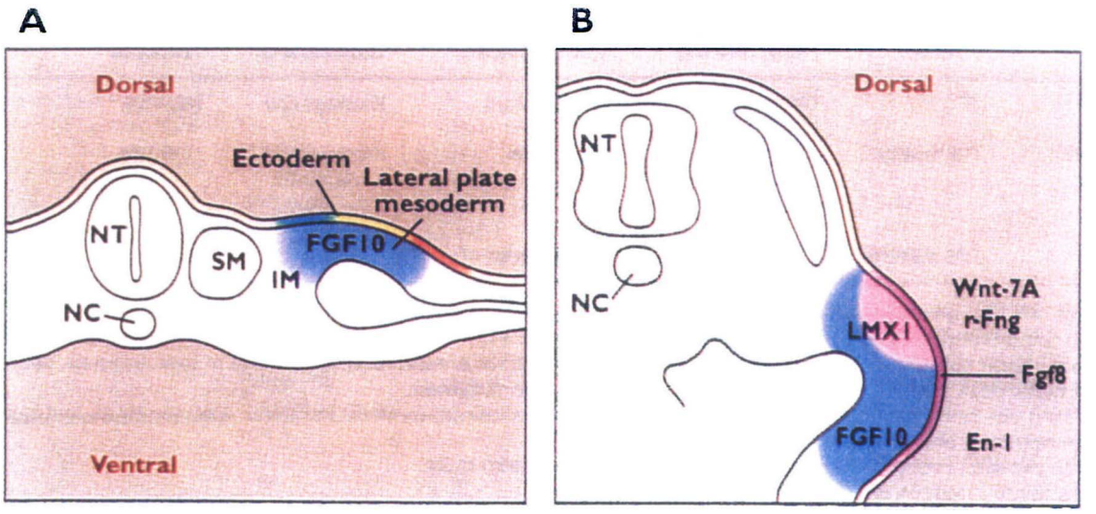


*Fig. 1.* Early stages in limb bud formation. A) Schematic diagram depicting a transverse section through an early stage embryo at the somite level where a limb bud will form. The shading depicts FGF10 expression within the mesoderm that will contribute to limb bud mesenchyme, including the lateral plate mesoderm (LPM) and part of the intermediate mesoderm (IM). FGF10 may be a key signal initiating limb bud growth. Cells that will contribute to the dorsal limb ectoderm (green) are closer to the neural tube, while those that will contribute to the ventral ectoderm (orange) are more lateral. Between these regions is a rather broad domain of ectoderm (yellow) that will give rise to the AER (see text). NT, neural tube; NC, notochord; SM, somitic mesoderm; IM, intermediate mesoderm. B) The same transverse level of section at a slightly later stage in development. FGF8 is now expressed in the bud ectoderm prior to AER formation, perhaps induced by the FGF10 signal from the LPM. Wnt-7a and r-Fng are expressed in the dorsal bud ectoderm, while En-1 is expressed in the ventral ectoderm. Wnt-7a expression triggers Lmx-1 in the underlying dorsal mesenchyme.

expressed in many regions of the developing embryo (31). Regional restriction of expression of these genes in the developing limb buds probably plays a role in the defects observed for these disorders (32). Differential expression of *Tbx* genes (T-box genes) *Tbx5* and *Tbx4* may be involved in the distinction between forelimb and hindlimb, probably regulated in part by region-specific Hox gene expression (32, 33).

*Fig. 2.* How to make a limb. Panels 1–4 depict the growth of the bud through time, while 5–8 show the phases of HOX gene expression during the same time points as panels 1–3. Note the orientation of the proximal-distal and anterior-posterior axes. Schematic expression domains of various genes are shown. Examples of malformations caused by stage-specific defects are also given. 1) Early limb budding. Early FGF10 expression in the LPM probably stimulates this process. The onset of Hox gene expression also occurs at this stage. Failure of limb bud formation at this stage could result in amelia. 2) The AER has formed along the anterior-posterior axis of the bud ectoderm; secreted FGFs stimulate mitosis in the underlying mesenchyme (PZ). Cells that are proximal to the PZ are condensing to form the humerus. A region of posterior mesenchyme (ZPA) secretes Shh, which is sensed by the surrounding mesenchyme and the AER and provides an anterior-posterior patterning signal. The limb bud is now an independent, self-organizing domain. A failure of AER or ZPA function at this stage could also prevent further bud growth, resulting in distal limb truncation. Neural and muscle precursors are migrating into the limb field. 3) The distal bud has enlarged and flattened, and the ZPA has moved distally along the bud. The radius and ulna are forming as cells aggregate in a branching pattern at the distal end of the cartilagenous humerus, which is maturing and lengthening. FGF4 is expressed in the posterior two-thirds of the AER. While the TBX5 and TBX3 genes have wide and complex expression patterns, defects in TBX5 or TBX3 function cause primarily anterior or posterior defects in the Holt–Oram and Ulnar–Mammary syndromes, respectively. 4) Late limb bud development. The AER and ZPA are no longer active. All the major cartilage elements of the limb skeleton have condensed and their basic shapes and relative orientations are apparent. Cell death between the digits allows them to separate. Achondroplasia and Grebe syndrome are examples of defective limb bone maturation. 5) Hox gene expression: In the limb, there are three major phases of Hox gene expression where multiple Hox genes are expressed. These roughly correlate with the growth and differentiation of the proximal, middle and distal limb skeleton. A particular Hox gene can be expressed in more than one phase. Phase 1 of Hox expression occurs early in a crescent pattern just underlying the ectoderm. 6) Phase 1 still persists, while phase 2 has begun in the posterior mesenchyme. 7) Phase 1 has shut off. Phase 2 has extended anteriorly but does not follow the distal growth of the bud, where phase 3 develops. Phases 2 and 3 are well correlated with the gowth and differentiation of the radius/ulna and digits, respectively. Hand–foot–genital (HFG) and synpolydactyly (SPD) are examples of syndromes, caused by defective *Hox* genes, that involve hand/foot defects. Dorsal–ventral patterning. Panel 8 depicts a view looking at the distal tip of the limb bud toward the body wall. Compare with Fig. 1. LPM, lateral plate mesoderm; AER, apical ectodermal ridge; ZPA, zone of polarizing activity; PZ, progress zone; M, myoblasts; N, neural precursors; H, humerus; R, radius; U, ulna.

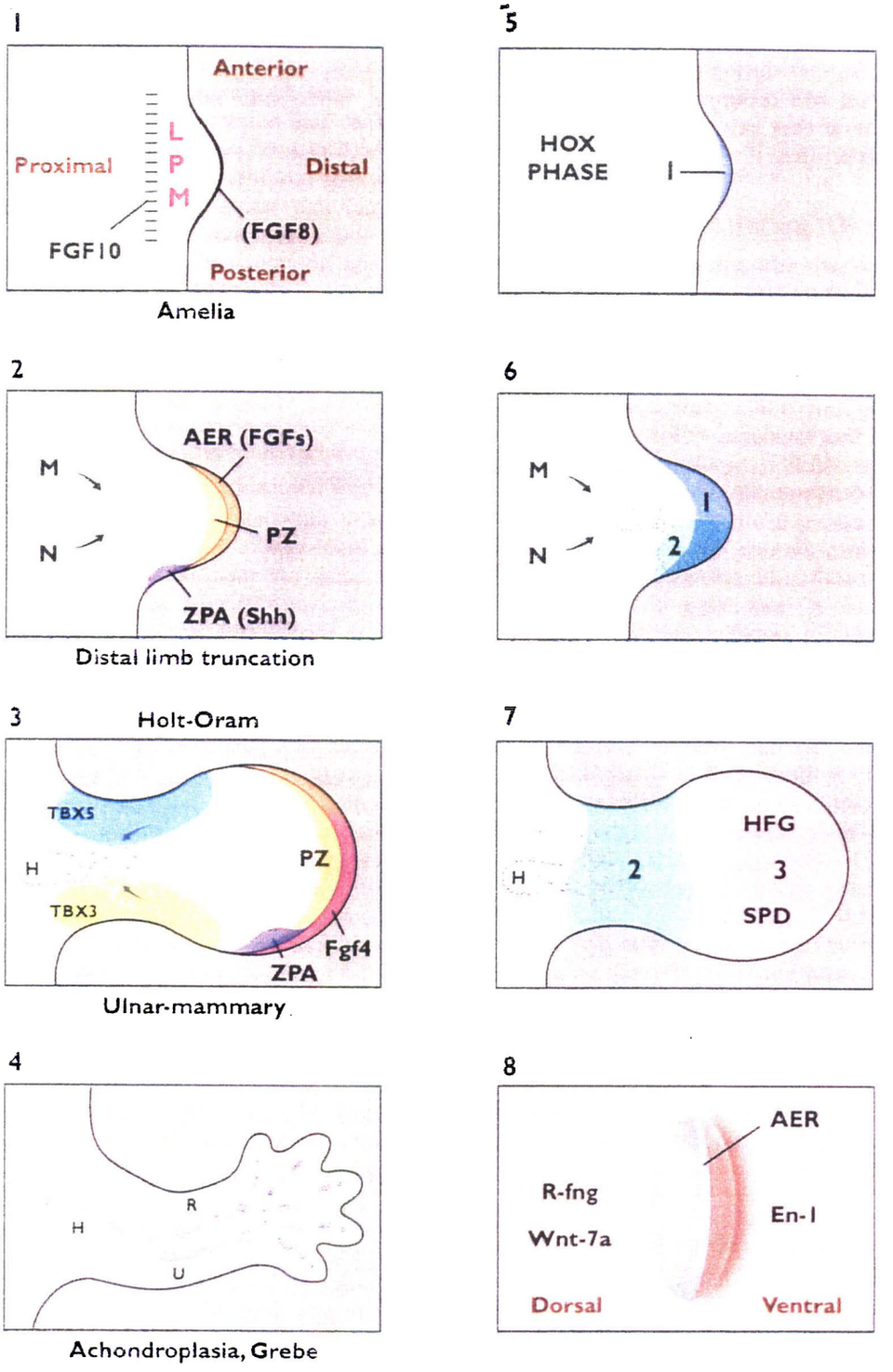


*Fig. 2.*

### Outgrowth and proximal-distal patterning

The limb mesoderm determines limb type and orientation, and is necessary to induce the ectoderm to elongate and to form the AER, a population of cells which extend along the distal tip of the limb bud in the A–P dimension at the boundary of the dorsal and ventral ectoderm (Fig. 2). The AER is responsible for continued distal growth of the limb and produces FGFs which stimulate the underlying mesoderm, known as the

PZ, to proliferate in an undifferentiated state (4). In the progress zone model, cells that leave the progress zone sooner during growth and extension of the limb bud will occupy more proximal positions, while those that exit the PZ later will form more distal structures.

### Induction of the AER and limb truncation phenotypes

In addition to currently unknown signals from the mesoderm, another key molecular mediator of ridge formation is radical fringe (*r-Fng*) (34, 35). An ectodermal border, defined by *r-Fng*-expressing cells adjacent to non-expressing cells at the tip of the developing limb bud, creates a critical 'molecular boundary' that is necessary for the formation of the AER. If the AER is experimentally removed at any stage of development, further distal limb development ceases, causing truncations.

In experimental models, removal of a portion of the AER can result in asymmetries, such as the loss of digits; however, increasing the extent of the AER can result in polydactyly (4). In the chick mutants *limbless* and *wingless*, an AER is not properly formed, and FGF8 and other FGFs needed for early mesenchymal proliferation are not expressed (36). In the mouse mutant *legless*, hindlimbs fail to develop due to absence of the AER (37). FGF4 is normally expressed within the posterior two-thirds of the AER. Ectopic anterior expression of FGF4 in the AER is associated with polydactyly in the chick mutant *talpid* and mouse mutants *Strong's luxoid*, *extra toes*, and *Rim4* (4). Defective 'formin' expression in *limb deformity* (*ld*) mice leads to insufficient FGF4 expression in the AER, and consequent reduction in *Shh* expression, resulting in a multitude of bony reductions and fusions (38, 39). Therefore, early stages in the induction of the limb bud and the formation of the AER are critical to establishment of the limb bud and instructive as to potential defects underlying human amelia (40).

### Lumping or splitting in split hand–split foot malformation 1

Human ectrodactyly or split hand–split foot malformation (SHFM) is genetically heterogeneous with autosomal loci on human chromosome 7q (*SHFM1*) and 10q (*SHFM3*). On 7q, a critical interval with three genes, *DLX5*, *DLX6*, and *DSS1*, has been defined on the basis of the positions of distinct translocations and six interstitial deletion patients (41). Due to the absence of direct interruption of any of these genes by the translocations, it has been hypothesized that alterations in the regulation of expression of these three genes, either alone or in combination, leads to SFHM1.

The mouse Dactylaplasia (*Dac*) mutation results in a semi-dominant phenotype that resembles SFHM. *Dac* maps to mouse chromosome 19, and the limb phenotype also depends on another locus, *mdac* (for modifier of *Dac*), that acts in a recessive manner and maps to chromosome 13 (42). The *Dac* locus is within a region of chromosome 19 syntenic to human chromosome 10q25 where the *Fgf8* locus resides; however, no mutations have been found in this gene (43). *Dac* mutants exhibit excessive cell death within the AER, a potential mechanism for SHFM syndromes (43).

### *HOX* mutations in hand–foot–genital syndrome and synpolydactyly

Growth and patterning along the P–D and A–P axes also require the products of the Hox genes. Hox genes are members of a highly conserved set of transcription factor genes that are found in all animal species (44, 45). They are expressed very early in embryonic development along the body axis, in specific spatial domains and in a temporal succession that correlates with the relative order of the genes in a particular complex, a feature termed colinearity. Colinearity of Hox expression is an ancient regulatory mechanism that distinguishes animals from other multicellular living organisms.

Several *Hoxa* and *Hoxd* genes are expressed within the developing limb buds in a complex, dynamic pattern, with three phases of expression roughly correlating with the appearance of the stylopod (humerus/femur), zeugopod (radius/ulna or tibia/fibula), and the autopod (digital arch) (Fig. 2; (5, 46, 47)). The construction of putatively null alleles of these *Hox* genes by homologous recombination in mice, followed by creation of compound mutants of several *Hox* genes, has been very instructive for understanding their role in limb development (48–53). These results suggest that the Hox genes influence the growth rates of mesenchymal condensations within their respective regions of expression, and can have effects at more than one stage of development in the timing of cartilage growth and differentiation. In addition, *Hox* genes mutated in combination were observed to have synergistic effects, implying that they have overlapping as well as unique functions in the specification of body morphology or growth (50, 54, 51, 55, 52, 5, 53).

Mice are not the only mammals to have mutated *Hox* genes. Recently, two human syndromes have been identified to be the result of mutations within genes of the *HOXD* and *HOXA* complexes. One of

these, synpolydactyly (SPD), primarily affects the digit pattern due to a gain-of-function mutation in *HOXD13* (56–58). In this syndrome, syndactyly involving the third and fourth fingers (and occasionally the fourth and fifth toes) with partial or complete duplication of individual fingers and toes, is observed. The hand–foot–genital syndrome (HFG) affects both digital and genitourinary organ development, due to a premature stop codon mutation in the homeodomain of *HOXA13* (59). The identification of these mutations reveals the importance of these genes for human morphogenesis, which was predicted based on studies in mice and flies. Their digital arch malformations are consistent with the roles of these genes in controlling growth rates and allocation of mesenchyme in selected regions, since alterations in these processes would be expected to result in additional digits or deleted or hypoplastic elements. Genitourinary anomalies in HFG are consistent with the expression of this gene in developing caudal trunk structures and parallel those observed in *Hoxa13* knockout mice (49). The occurrence of similar combined malformations in other human syndromes is compelling for potential alteration of Hox gene expression (44, 60).

### Hox genes expressed in the wrong place: a case of superiority

Misexpression of Hox genes in places where they are not normally expressed can also result in deficiencies. For example, in the mouse mutant *Ulnaless*, severe truncation of the radius/ulna and tibia/fibula is associated with aberrant proximal expression of *Hoxd13* in the mesenchymal territories that give rise to these bones (61, 62). These effects have parallels in flies and illustrate the principle of posterior prevalence: Hox genes normally active in more posterior regions are dominant if misexpressed in more anterior domains. Other limb misexpression studies with retroviruses in chick and mice support these observations and have revealed an 'early' role for Hox genes in mesenchymal proliferation and a 'late' role in growth of the proliferating cartilage of long bones (55, 63–65). This information teaches us that intercalary deficiencies or deletion malformations may result from regulatory mutations or induced misexpression of Hox genes, and not necessarily from deficient or abnormal HOX proteins.

## A–P patterning: what's all this about a hedgehog?

The ZPA within the developing limb bud is a cellular structure, located at the postero–distal margin of the limb bud, that received its name based on its characteristics in reciprocal transplantation studies (Fig. 1; (4, 5, 9)). The ZPA directs A–P pattern formation and is formed early before substantial budding occurs. When cells from this region are excised and placed within the anterior segment of a limb bud, mirror-image duplication of the digits occurs, and the extent of the duplication depends on the number of transplanted cells, suggesting that the ZPA is the source of a secreted polarizing signal. Mirror-image digit duplications have been observed in humans, and probably result from ectopic ZPA formation. Retinoic acid signalling is essential for ZPA development since competitive inhibitors prevent *Hoxb8* expression and ZPA development (25). Digits fail to develop when the ZPA is removed. *Sonic hedgehog* (*Shh*), a vertebrate homolog of the *Drosophila* segment polarity gene *hedgehog*, can provide the ZPA polarizing signal and its effects are mediated by the action of *Patched* (*Ptc*), *cubitus interruptus* (*Gli* in vertebrates), bone morphogenetic proteins (BMPs), HOX proteins, and other *Shh* pathway effectors (5, 66). Expression of *Shh* from the ZPA is maintained by AER-specific signals; FGF-containing beads can provide these signals when the AER is removed (4). AER survival requires continued expression of *Shh*. Therefore, a feedback loop between *sonic*-expressing ZPA cells and the posterior AER is necessary for A–P patterning and distal growth of the limb. *Shh* expression is also dependent on signals from the dorsal ectoderm, which can be replaced functionally by ectopic application of Wnt-7A (4).

### Gli3: an impressive allelic series

The *Gli* protein family composed of *Gli1*, *Gli2*, and *Gli3* are vertebrate homologs of the *Drosophila Ci* protein which is an important mediator of *hedgehog* signaling (5). An interesting allelic series of mutations in human *Gli3* has been discovered. Translocations or deletions interrupting *Gli3* are associated with Greig cephalopolysyndactyly (Table 1). Since these mutations likely create haploinsufficiency for this gene product, it appears that dosage is important for regulating interdigital cell death and proliferation of mesenchyme. Other human *Gli3* mutations may result in Pallister–Hall syndrome or postaxial polydactyly type A; these phenotypes may correlate with the position of the mutation in the gene relative to certain functional domains (67).

### Dorso-ventral patterning: the up-side and the down-side

The formation of the AER at the dorso-ventral boundary of the limb bud tip is a process separable from that which determines the dorsal-ventral axis (5). Dorso-ventral patterning requires expression of Wnt-7A in the dorsal ectoderm, which induces expression of Lmx-1 in the dorsal mesoderm (68). However, En-1 is necessary to restrict expression of Wnt-7A and r-Fng to the dorsal ectoderm, thereby creating a D-V boundary and helping to position the AER. Failure to set up these appropriate molecular boundaries can lead to abnormally-patterned limbs. Indeed, *Wnt-7A* mouse null mutants have 'ventralized' limbs (69), and *En-1* mouse mutants have 'dorsalized' limbs (Figs. 1 and 2 (71)). In addition, Wnt-7A induces expression of Lmx-1 in the dorsal mesoderm, imparting 'dorsal' specification; ectopic expression of Lmx-1 in the ventral mesoderm is sufficient to induce dorsal characteristics (68).

### Conclusions and future directions

The molecular rules for limb formation are rapidly being assembled. Critical advances have utilized animal model systems and mutants, expression methodologies *in situ*, and the ability to misexpress molecules of interest with viruses, transgenic mice, or from surgically-implanted factor-containing beads. By positional cloning and candidate gene approaches, human mutations have been identified as the basis for limb malformations and syndromes. We have learned the identification of key molecules that define functional cellular domains, and that there is a remarkable conservation of the rules between disparate species. This latter aspect is perhaps the most satisfying conclusion of the work of developmental biology over the last 10 years – model systems are indeed useful and more directly applicable to the interpretation of development and its anomalies in humans than previously believed. Over the next several years, we will learn more about the identity and function of factors that are important for positioning the limbs along the body axis through regulation of FGF expression, the mesodermal signals needed for AER induction, and the discovery of new genes involved in limb morphogenesis or malformation. It is safe to say that we have entered onto a new vista in clinical genetics where we are now capable of inferring the involvement of specific developmental pathways or genes in human malformations.

### Acknowledgements

We apologize for omitting discussion of general skeletal dysplasias and many other interesting limb malformation syndromes due to space limitations. This work was supported in part by grants from the NIH (HD34059) and the University of Michigan Multipurpose Arthritis and Musculoskeletal Diseases Center (Center grant 5 P60 AR20557) to J.W.I. D.P.M. is supported by a Postdoctoral Training Grant from the University of Michigan Center for Organogenesis (NIH 5 T32 HD07505-02).

### References

1. Cohn MJ, Tickle C. Limbs: a model for pattern formation within the vertebrate body plan. Trends Genet 1996: 12: 253-257.
2. Shubin N, Tabin C, Carroll S. Fossils, genes and the evolution of animal limbs. Nature 1997: 388: 639-648.
3. Panganiban G, Irvine SM, Lowe C, Roehl H, Corley LS, Sherbon B, Grenier JK, Fallon JF, Kimble J, Walker M, Wray GA, Swalla BJ, Martindale MQ, Carroll SB. The origin and evolution of animal appendages. Proc Natl Acad Sci USA 1997: 94: 5162-5166.
4. Niswander L. Limb mutants: what can they tell us about normal limb development? Curr Opin Gen Dev 1997: 7: 530-536.
5. Johnson RL, Tabin CJ. Molecular models for vertebrate limb development. Cell 1997: 90: 979-990.
6. Wilson GN. Molecular approach to understanding human malformations. In: Roger E Stevenson, Judith G Hall, Richard M Goodman (eds.) Human Malformations and Related Anomalies. Oxford: Oxford Monographs on Medical Genetics 1993 (27): 233.
7. Tickle C. Genetics and limb development. Dev Genet 1996: 19: 1-8.
8. Erlebacher A, Filvaroff EH, Gitelman SE, Derynck R. Toward a molecular understanding of skeletal development. Cell 1995: 80: 371-378.
9. Tickle C, Eichele G. Vertebrate limb development. Ann Rev Cell Biol 1994: 10: 121-152.
10. Shubin N, Alberch P. A morphogenetic approach to the origin and basic organization of the tetrapod limb. In: Hecht MK, Wallace B, Prance GT, eds. EvolutionaryBiology, vol. 20. New York: Plenum, 1986: 319-387.
11. Searls RL, Janners MY. The initiation of limb bud outgrowth in the embryonic chick. Dev Biol 1971: 24: 198-213.
12. Stratford TH, Kostakopoulou K, Maden M. *Hoxb-8* has a role in establishing early anterior-posterior polarity in chick forelimb but not hindlimb. Development 1997: 124: 4225-4234.
13. Burke AC, Nelson CE, Morgan BA, Tabin C. Hox genes and the evolution of vertebrate axial morphology. Development 1995: 121: 333-346.
14. Charite J, deGraaff W, Shen S, Deschamps J. Ectopic expression of *Hoxb8* causes duplication of the ZPA in the forelimb and homeotic transformation of axial structures. Cell 1994: 78: 589-601.
15. Rancourt DE, Tsuzuki T, Capecchi MR. Genetic interaction between *hoxb-5* and *hoxb-6* is revealed by nonallelic noncomplementation. Genes Dev 1995: 9: 108-122.
16. Stephens TD, McNulty TR. Evidence for a metameric pattern in the development of the chick humerus. J Embryol Exp Morphol 1981: 61: 191-205.
17. Smith DM, Torres RD, Stephens TD. Mesonephros has a role in limb development and is related to thalidomide embryopathy. Teratology 1996: 54: 126-134.

18. Fernandez-Teran M, Piedra ME, Simandl BK, Fallon JF, Ros MA. Limb initiation and development is normal in the absence of the mesonephros. Dev Biol 1997: 189: 246–255.
19. Geduspan JS, Solursh M. A growth-promoting influence from the mesonephros during limb outgrowth. Dev Biol 1992: 151: 242–250.
20. Ohuchi H, Nakagawa T, Yamamoto A, Araga A, Ohata T, Ishimaru Y, Yoshioka H, Kuwana T, Nohno T, Yamasaki M, Itoh N, Noji S. The mesenchymal factor FGF10, initiates and maintains the outgrowth of the chick limb bud through interaction with FGF8, an apical ectodermal factor. Development 1997: 124: 2235–2244.
21. Altabef M, Clarke JDW, Tickle C. Dorso–ventral ectodermal compartments and origin of apical ectodermal ridge in developing chick limb. Development 1997: 124: 4547–4556.
22. Michaud JL, Lapointe F, Le Douarin NM. The dorsoventral polarity of the presumptive limb is determined by signals produced by the somites and by the lateral somatopleure. Development 1997: 124: 1453–1463.
23. Crossley PH, Minowada G, MacArthur CA, Martin GR. Roles for FGF8 in the induction, initiation, and maintenance of chick limb development. Cell 1996: 84: 127–136.
24. Helms JA, Kim CH, Eichele G, Thaller C. Retinoic acid signaling is required during early chick limb development. Development 1996: 122: 1385–1394.
25. Lu H-C, Revelli J-P, Goering L, Thaller C, Eichele G. Retinoid signalling is required for the establishment of a ZPA and for the expression of *Hoxb-8*, a mediator of ZPA formation. Development 1997: 124: 1643–1651.
26. Cohn M, Izpisua-Belmonte JC, Abud H, Heath J, Tickle C. Fibroblast growth factors induce additional limb development from the flank of chick embryos. Cell 1995: 80: 739–746.
27. Carlson BM. Patten Foundations of Embryology. New York: McGraw-Hill, 1988: 393–421.
28. Bamshad M, Lin RC, Law DJ, Watkins WS, Krakowiak PA, Moore ME, Franceschini P, Lala R, Holmes LB, Gebuhr TC, Bruneau BG, Schinzel A, Seidman JG, Seidman CE, Jorde LB. Mutations in human *TBX3* alter limb, apocrine and genital development in ulnar-mammary syndrome. Nat Genet 1997: 16: 311–315.
29. Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Soults J, Grayzel D, Kroumpouzou E, Traill TA, Lablanc-Straceski J, Renault B, Kucherlapati R, Seidman JG, Seidman CE. Mutations in human *TBX5* cause limb and cardiac malformations in Holt–Oram syndrome. Nat Genet 1997: 15: 30–35.
30. Li QY, Newbury-Ecob RA, Terret JA, Wilson DI, Curtis ARJ, Yi CH, Gebuhr T, Bullen PJ, Robson SC, Strachan T, Bonnet D, Lyonnet S, Young ID, Raeburn JA, Buckle AJ, Law DJ, Brook JD. Holt–Oram syndrome is caused by mutations in *TBX5*, a member of the *Brachyury* (T) gene family. Nat Genet 1997: 15: 21–29.
31. Smith J. *Brachyury* and the T-box genes. Curr Opin Gen Dev 1997: 7: 474–480.
32. Gibson-Brown JJ, Agulnik SI, Chapman DL, Alexiou M, Garvey N, Silver LM, Papaioannou VE. Evidence of a role for T-box genes in the evolution of limb morphogenesis and the speculation of forelimb/hindlimb identity. Mech Dev 1996: 56: 93–101.
33. Ohuchi H, Takeuchi J, Yoshioka H, Ishimaru Y, Ogura K, Takahashi N, Ogura T, Noji S. Correlation of wing-leg identity in ectopic FGF-induced chimeric limbs with the differential expression of chick *Tbx5* and *Tbx4*. Development 1998: 125: 51–60.
34. Rodriguez-Esteban C, Schwabe JWR, De La Pena J, Foys B, Eshelman B, Izpisua-Belmonte J-C. *Radical fringe* positions the apical ectodermal ridge at the dorsoventral boundary of the vertebrate limb. Nature 1997: 386: 360–366.
35. Laufer E, Dahn R, Orozco OE, Yeo C-Y, Pisenti J, Henrique D, Abbott UK, Fallon JF, Tabin C. Expression of *Radical fringe* in limb-bud ectoderm regulates apical ectodermal ridge formation. Nature 1997: 386: 366–373.
36. Grieshammer U, Minowada G, Pisenti J, Abbott U, Martin GR. The chick *limbless* mutation causes abnormalities in limb bud dorsal-ventral patterning: implications for the mechanism of apical ridge formation. Development 1996: 122: 3851–3861.
37. Singh G, Supp D, Schreiner C, McNeish J, Merker H-J, Copeland N, Jenkins N, Potter S, Scott W. *legless* insertional mutation: morphological, molecular, and genetic characterization. Genes Dev 1991: 5: 2245–2255.
38. Chan DC, Wynshaw-Boris A, Leder P. Formin isoforms are differentially expressed in the mouse embryo and are required for normal expression of *fgf-4* and *shh* in the limb bud. Development 1995: 121: 3151–3162.
39. Zeller R, Jackson-Grusby L, Leder P. The limb deformity gene is required for apical ectodermal ridge differentiation and anteroposterior limb pattern formation. Genes Dev 1989: 3: 1481–1492.
40. Froster-Iskenius UG, Baird PA. Amelia: incidence and associated defects in a large population. Teratology 1990: 41: 23–31.
41. Crackower MA, Scherer SW, Rommens JM, Hui CC, Poorkaj P, Soder S, Cobben JM, Hudgins L, Evans JP, Tsui LC. Characterization of the split hand/split foot malformation locus SHFM1 at 7q21.3–q22.1 and analysis of a candidate gene for its expression during limb development. Hum Mol Genet 1996: 5: 571–579.
42. Johnson KR, Lane PW, Ward-Bailey P, Davisson MT. Mapping the mouse Dactylaplasia mutations, *Dac*, and a gene that controls its expression, *mdac*. Genomics 1995: 29: 457–464.
43. Seto ML, Nunes ME, MacArthur CA, Cunningham ML. Pathogenesis of ectrodactyly in the *Dactylaplasia* mouse: aberrant cell death of the apical ectodermal ridge. Teratology 1997: 56: 262–270.
44. Innis JW. Role of HOX genes in human development. Curr Opin Ped 1997: 9: 617–622.
45. Duboule D, Morata G. Colinearity and functional hierarchy among genes of the homeotic complexes. Trends Genet 1994: 10: 358–364.
46. Yokouchi Y, Sasaki H, Kuroiwa A. Homeobox gene expression correlated with the bifurcation process of limb cartilage development. Nature 1991: 353: 443–446.
47. Nelson CE, Morgan BA, Burke AC, Laufer E, DiMambro E, Murtaugh LC, Gonzales E, Tessarollo L, Parada LF, Tabin C. Analysis of Hox gene expression in the chick limb bud. Development 1996: 122: 1449–1466.
48. Davis AP, White DP, Hsieh-Li HM, Potter SS, Capecchi MR. Absence of radius and ulna in mice lacking *hoxa-11* and *hoxd-11*. Nature 1995: 375: 791–795.
49. Fromental-Romain C, Warot X, Messadecq N, LeMeur M, Dollé P, Chambon P. *Hoxa-13* and *Hoxd-13* play a crucial role in the patterning of the limb autopod. Development 1996: 122: 2997–3011.
50. Davis AP, Capecchi MR. A mutational analysis of the 5′ *HoxD* genes: dissection of genetic interactions during limb development in the mouse. Development 1996: 122: 1175–1185.

51. Duboule D. Vertebrate *Hox* genes and proliferation: an alternative pathway to homeosis? Curr Biol 1995: 5: 525–528.
52. Capecchi MR. Function of homeobox genes in skeletal development. NY Acad Sci 1996: 785: 34–37.
53. Rijli FM, Chambon P. Genetic interactions of Hox genes in limb development: learning from compound mutants. Curr Opin Genet Dev 1997: 7: 481–487.
54. Zákány J, Duboule D. Synpolydactyly in mice with a targeted deficiency in the *HoxD* complex. Nature 1996: 384: 69–71.
55. Goff DJ, Tabin CJ. Analysis of *Hoxd13* and *Hoxd11* misexpression in chick limb buds reveals that Hox genes affect both bone condensation and growth. Development 1997: 124: 627–636.
56. Akarsu AN, Stoilov I, Yilmaz E, Sayil BS, Sarfarazi M. Genomic structure of *HOXD13* gene: a nine polyalanine duplication causes synpolydactyly in two unrelated families. Hum Mol Genet 1996: 5: 945–952.
57. Muragaki Y, Mundlos S, Upton J, Olsen BR. Altered growth and branching patterns in synpolydactyly caused by mutations in *HOXD13*. Science 1996: 272: 548–551.
58. Goodman FR, Mundlos S, Muragaki Y, Donnai D, Giovannucci-Uzielli ML, Lapi E, Majewski F, McGaughran J, McKeown C, Reardon W, Upton J, Winter RM, Olsen BR, Scambler PJ. Synpolydactyly phenotypes correlate with size of expansions in HOXD13 polyalanine tract. Proc Natl Acad Sci USA 1997: 94: 7458–7463.
59. Mortlock DP, Innis JW. Mutation of *HOXA13* in hand–foot–genital syndrome. Nat Genet 1997: 15: 179–180.
60. Pinsky L. A community of human malformation syndromes involving the Müllerian ducts, distal extremities, urinary tract, and ears. Teratology 1973: 9: 65–80.
61. Herault Y, Fraudeau N, Zakany J, Duboule D. *Ulnaless* (*Ul*), a regulatory mutation inducing both loss-of-function and gain-of-function of posterior Hoxd genes. Development 1997: 124: 3493–3500.
62. Peichel CL, Prabhakaran B, Vogt TF. The mouse *Ulnaless* mutation deregulates posterior HoxD gene expression and alters appendicular patterning. Development 1997: 124: 3481–3492.
63. Yokouchi Y, Nakazato S, Yamamoto M, Goto Y, Kameda T, Iba H, Kuroiwa A. Misexpression of *Hoxa-13* induces cartilage homeotic transformation and changes cell adhesiveness in chick limb buds. Genes Dev 1995: 9: 2509–2522.
64. van der Hoeven F, Zakany J, Duboule D. Gene transpositions in the *HOXD* complex reveal a hierarchy of regulatory controls. Cell 1996: 85: 1025–1035.
65. Knezevic V, De Santo R, Schughart K, Huffstadt U, Chiang C, Mahon KA, Mackem S. *Hoxd-12* differentially affects preaxial and postaxial chondrogenic branches in the limb and regulates *Sonic hedgehog* in a positive feedback loop. Development 1997: 124: 4523–4536.
66. Riddle R, Johnson R, Laufer E, Tabin C. *Sonic hedgehog* mediates the polarizing activity of the ZPA. Cell 1993: 75: 1401–1416.
67. Biesecker L. Strike three for *Gli3*. Nat Genet 1997: 15: 259–260.
68. Vogel A, Rodriguez C, Warnken W, Izpisua-Belmonte J-C. Dorsal cell fate specified by chick *Lmx1* during vertebrate limb development. Nature 1995: 378: 716–720.
69. Parr BA, McMahon AP. Dorsalizing signal *Wnt-7a* required for normal polarity of D-V and A-P axes of mouse limb. Nature 1995: 374: 350–353.
70. Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DRJr, Scott MP. Human homolog of *patched*, a candidate gene for the basal cell nevus syndrome. Science 1996: 272: 1668–1671.
71. Loomis CA, Harris E, Michaud J, Wurst W, Hanks M, Joyner AL. The mouse *Engrailed-1* gene and ventral limb patterning. Nature 1996: 382: 360–363.
72. Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. Nature 1996: 383: 407–413.
73. Hahn H, Wicking C, Zaphiropoulos PG, Gailani MR, Shanley S, Chidambram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toffgrad R, Chenevix-Trench G, Wainwright B, Bale AE. Mutations of the human homolog of Drosophila *patched* in the nevoid basal cell carcinoma syndrome. Cell 1996: 85: 841–851.
74. Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse *patched* mutants. Science 1997: 277: 1109–1113.
75. Tint GS, Irons M, Elias ER, Batta AK, Frieden R, Chen TS, Salen G. Defective cholesterol biosynthesis associated with the Smith–Lemli–Opitz syndrome. N Engl J Med 1994: 330: 107–113.
76. Cunniff C, Kratz LE, Moser A, Natowicz MR, Kelley RI. Clinical and biochemical spectrum of patients with RSH/Smith–Lemli–Opitz syndrome and abnormal cholesterol metabolism. Am J Med Genet 1997: 68: 263–269.
77. OMIM # 14882.
78. Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev 1995: 9: 2795–2807.
79. Luo G, Hofmann C, Bronckers ALJJ, Sohocki M, Bradley A, Karsenty G. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes Dev 1995: 9: 2808–2820.
80. Mo R, Freer AM, Zinyk DL, Crackower MA, Michaud J, Heng HH, Chik KW, Shi XM, Tsui LC, Cheng SH, Joyner AL, Hui C. Specific and redundant functions of *Gli2* and *Gli3* zinc finger genes in skeletal patterning and development. Development 1997: 124: 113–123.
81. Johnson DR. Brachyphalangy, an allele of *extra-toes* in the mouse. Genet Res 1969: 13: 275–280.
82. van der Hoeven F, Schimmang T, Vortkamp A, Rüther U. Molecular linkage of the morphogenetic mutation *add* and the zinc finger gene *Gli3*. Mammalian Genome 1993: 4: 276–277.
83. Schimmang T, Oda SI, Rüther U. The mouse mutant Polydactyly Nagoya (*Pdn*) defines a novel allele of the zinc finger gene *Gli3*. Mammalian Genome 1994: 5: 384–386.
84. Wild A, Kalff-Suske M, Vortkamp A, Bornholdt D, Konig R, Grzeschik KH. Point mutations in human *GLI3* cause Greig syndrome. Hum Mol Genet 1997: 6: 1979–1984.
85. Vortkamp A, Gessler M, Grzeschik KH. *GLI3* zinc-finger gene interrupted by translocations in Greig syndrome families. Nature 1991: 352: 539–540.
86. Radhakrishna U, Wild A, Grzeschik KH, Antonarakis SE. Mutation in *GLI3* in postaxial polydactyly type A. Nat Genet 1997: 17: 269–271.
87. Kang S, Jr JMG, Olney AH, Biesecker LG. *GLI3* frameshift mutations cause autosomal dominant Pallister-Hall syndrome. Nat Genet 1997: 15: 266–268.
88. Hui C, Joyner AL. A mouse model of Grieg cephalopolysyndactyly syndrome: the *extra-toes* (J) mutation contains an intragenic deletion of the *Gli3* gene. Nat Genet 1993: 3: 241–246.

89. Vortkamp A, Franz T, Gessler M, Grzeschik KH. Deletion of *GLI3* supports the homology of the human Greig cephalopolysyndactyly syndrome (GCPS) and the mouse mutant *extra toes* (*Xt*). Mammalian Genome 1992: 3: 461–463.
90. Muenke M, Schell U, Hehr A, Robin NH, Losken HW, Schinzel A, Pulleyn LJ, Rutland P, Reardon W, Malcolm S, Winter RM. A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. Nat Genet 1994: 8: 269–274.
91. Lajeunie E, Ma HW, Bonaventure J, Munnich A, Merrer ML. *FGFR2* mutations in Pfeiffer syndrome. Nat Genet 1995: 9: 108.
92. Jabs EW, Li X, Scott AF, Meyers G, Chen W, Eccles M, Mao JI, Charnas LR, Jackson CE, Jaye M. Jackson–Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2. Nat Genet 1994: 8: 275–279.
93. Wilkie AOM, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, Hayward RD, David DJ, Pulleyn LJ, Rutland P, Malcolm S, Winter RM, Reardon W. Apert syndrome results from localized mutations of *FGFR2* and is allelic with Crouzon syndrome. Nat Genet 1995: 9: 165–172.
94. Rutland P, Pulleyn LJ, Reardon W, Baraitser M, Hayward R, Jones B, Malcolm S, Winter RM, Oldridge M, Slaney SF, Poole MD, Wilkie AOM. Identical mutations in the *FGFR2* gene cause both Pfeiffer and Crouzon syndrome phenotypes. Nat Genet 1995: 9: 173–176.
95. Woychik RP, Maas RL, Zeller R, Vogt TF, Leder P. Formins': proteins deduced from the alternative transcripts of the *limb deformity* gene. Nature 1990: 346: 850–853.
96. Maas RL, Zeller R, Woychik RP, Vogt TF, Leder P. Disruption of formin-encoding transcripts in two mutant *limb deformity* alleles. Nature 1990: 346: 853–855.
97. Wurst W, Auerbach AB, Joyner AL. Multiple developmental defects in *Engrailed-1* mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. Development 1994: 120: 2065–2075.
98. Foster JW, Dominguez-Steglich MA, Guioli S, Kowk G, Weller PA, Stevanovic M, Weissenbach J, Mansour S, Young ID, Goodfellow PN, Brook JD, Schafer AJ. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994: 372: 525–530.
99. Wagner T, Wirth J, Meyer J, Zabel B, Held M, Zimmer J, Pasantes J, Bricarelli FD, Keutel J, Hustert E, Wolf U, Tommerup N, Schempp W, Scherer G. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the *SRY*-related gene *SOX9*. Cell 1994: 79: 1111–1120.
100. El Ghouzzi V, Merrer ML, Perrin-Schmidt F, Lajeunie E, Benit P, Renier D. Mutations of the *TWIST* gene in the Saethre–Chotzen syndrome. Nat Genet 1997: 15: 42–46.
101. Howard TD, Paznekas WA, Green ED, Chiang LC, Ma N, Luna RIOD, Delgado CG, Gonzalez-Ramos M, Kline AD, Jabs EW. Mutations in *TWIST*, a basic helix-loop-helix transcription factor, in Saethre–Chotzen syndrome. Nat Genet 1997: 15: 36–41.
102. Fawcett D, Pasceri P, Fraser R, Colbert M, Rossant J, Giguère V. Postaxial polydactyly in forelimbs of *CRABP-II* mutant mice. Development 1995: 121: 671–679.
103. Rijli FM, Matyas R, Pellegrini M, Dierich A, Gruss P, Dollé P, Chambon P. Cryptorchidism and homeotic transformations of spinal nerves and vertebrae in *Hoxa-10* mutant mice. Proc Natl Acad Sci USA 1995: 92: 8185–8189.
104. Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in *Hoxa10*-deficient mice. Nature 1995: 374: 460–463.
105. Small KM, Potter SS. Homeotic transformations and limb defects in *Hoxa11* mutant mice. Genes Dev 1993: 7: 2318–2328.
106. Hsieh-Li HM, Witte DP, Weinstein M, Branford W, Li H, Small K, Potter SS. *Hoxa11* structure, extensive antisense transcription, and function in male and female fertility. Development 1995: 121: 1373–1385.
107. Robertson KE, Chapman MH, Adams A, Tickle C, Darling SM. Cellular analysis of limb development in the mouse mutant Hypodactyly. Dev Genet 1996: 19: 9–25.
108. Mortlock DP, Post LC, Innis JW. The molecular basis of hypodactyly (*Hd*): a deletion in *Hoxa13* leads to arrest of digital arch formation. Nat Genet 1996: 13: 284–289.
109. Carpenter E, Goddard JM, Davis AP, Nguyen TP, Capecchi MR. Targeted disruption of *Hoxd-10* affects mouse hindlimb development. Development 1997: 124: 4505–4514.
110. Favier B, Meur ML, Chambon P, Dollé P. Axial skeleton homeosis and forelimb malformations in *Hoxd-11* mutant mice. Proc Natl Acad Sci USA 1995: 92: 310–314.
111. Davis AP, Capecchi MR. Axial homeosis and appendicular skeleton defects in mice with a targeted disruption of *hoxd-11*. Development 1994: 120: 2187–2198.
112. Dollé P, Dierich A, LeMeur M, Schimmang T, Schuhbaur B, Chambon P, Duboule D. Disruption of the *Hoxd-13* gene induces localized heterochrony leading to mice with neotenic limbs. Cell 1993: 75: 431–441.
113. Takagi T, Moribe H, Kondoh H, Higashi Y. *EF1*, a zinc finger and homeodomain transcription factor, is required for skeleton patterning in multiple lineages. Development 1998: 125: 21–31.
114. Qu S, Niswender KD, Ji Q, Meer RVD, Keeney D, Magnuson MA, Wisdom R. Polydactyly and ectopic ZPA formation in *Alx-4* mutant mice. Development 1997: 124: 3999–4008.
115. Cobrinik D, Lee MH, Hannon G, Mulligan G, Bronson RT, Dyson N, Harlow E, Beach D, Weinberg RA, Jacks T. Shared role of the pRB-related p130 and p107 proteins in limb development. Genes Dev 1996: 10: 1633–1644.
116. Hoth CF, Milunsky A, Lipsky N, Sheffer R, Clarren SK, Bladwin CT. Mutations in the paired domain of the human *PAX3* gene cause Klein–Waardenburg syndrome (WS-III) as well as Waardenburg syndrome type I (WS-I). Am J Med Genet 1993: 52: 455–462.
117. Kohlhase J, Wischermann A, Reichenbach H, Froster U, Engel W. Mutations in the *SALL1* putative transcription factor gene cause Townes–Brocks syndrome. Nat Genet 1998: 18: 81–83.
118. Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee S. Limb alterations in *brachypodism* mice due to mutations in a new member of the TGFβ-superfamily. Nature 1994: 368: 639–643.
119. Thomas JT, Kilpatrick MW, Lin K, Erlacher L, Lembessis P, Costa T, Tsipouras P, Luyten FP. Disruption of human limb morphogenesis by a dominant negative mutation in *CDMP1*. Nat Genet 1997: 17: 58–64.
120. Polinkovsky A, Robin NH, Thomas JT, Irons M, Lynn A, Goodman FR, Reardon W, Kant SG, Brunner HG, Burgt IVD, Chitayat D, McGaughran J, Donnai D, Luyten FP, Warman ML. Mutations in CDMP1 cause autosomal dominant brachydactyly type C. Nat Genet 1997: 17: 18–19.

121. Thomas JT, Lin K, Nandedkar M, Camargo M, Cervenka J, Luyten FP. A human chondrodysplasia due to a mutation in a TGF-β superfamily member. Nat Genet 1996: 13: 315–317.
122. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of *Cbfa1* results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 1997: 89: 755–764.
123. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GWH, Beddington RSP, Mundlos S, Olsen BR, Selby PB, Owen MJ. *Cbfa1*, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 1997: 89: 765–772.
124. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JHM, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR. Mutations involving the transcription factor *CBFA1* cause cleidocranial dysplasia. Cell 1997: 89: 773–780.
125. Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J, Geoffroy V, Ducy P, Karsenty G. Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor *OSF2/CBFA1* in cleiodcranial dypslasia. Nat Genet 1997: 16: 307–310.
126. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. *Osf2/Cbfa1*: A transcriptional activator of osteoblast differentiation. Cell 1997: 89: 747–754.
127. Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, Winokur ST, Wasmuth JJ. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. Cell 1994: 78: 335–342.
128. Tavormina PL, Shiang R, Thompson LM, Zhu YZ, Wilkin DJ, Lachman RS, Wilcox WR, Rimoin DL, Cohn DH, Wasmuth JJ. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. Nat Genet 1995: 9: 321–328.
129. Deng C, Wyndshaw-Boris A, Zhou F, Kuo A, Leder P. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell 1996: 84: 911–921.
130. Pasteris NG, Cadle A, Logie LJ, Porteous ME, Schwartz CE, Stevenson RE, Glover TW, Wilroy RS, Gorski JL. Isolation and characterization of the faciogenital dysplasia (Aarskog–Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor. Cell 1994: 79: 669–678.