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Report

Localization of a Gene for Syndactyly Type 1 to Chromosome 2q34-q36

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Syndactyly type 1 (SD1) is an autosomal dominant limb malformation characterized in its classical form by complete or partial webbing between the third and fourth fingers and/or the second and third toes. After exclusion of a candidate region previously identified for syndactyly type 2 (synpolydactyly), we performed a genomewide linkage analysis in a large German pedigree. We found evidence for linkage of SD1 to polymorphic markers on chromosome 2q34-q36, with a maximum LOD score of 12.40 for marker D2S301. Key recombination events in affected individuals defined a 9.4-cM region between markers D2S2319 and D2S344. The identification of the responsible gene will give further insights into the molecular basis of limb development.

Isolated syndactyly is one of the most common congenital malformations of the hands and feet. On the basis of an anatomic approach, isolated syndactyly has been subdivided into five types (Tentamy and McKusick 1978). Syndactyly type 1 (SD1 [MIM 185900]), also named "zygodactyly," is inherited as an autosomal dominant trait and accounts for the majority of isolated syndactylies, with an incidence of ~2–3/10,000 in newborns (Castilla et al. 1980; Tentamy 1990). In this common type of syndactyly, there is usually complete or partial webbing between the third and fourth fingers and the second and third toes, with occasional involvement of other digits. In some cases, the webbing between fingers is associated with bony fusion of the distal phalanges. Sometimes only the hands are affected and sometimes only the feet. The phenotype is extremely variable within and between families. Occurrence of skipped generations indicates that penetrance is <100% (Montagu 1953; Feller et al. 1981). Here, we describe the localization of the first SD1 locus and provide evidence that SD1 maps to the 2q34-q36 region in a large German family.

The family was originally described by Lueken (1938) and now extends over eight generations comprising 338 individuals, including 77 affected family members (fig. 1). After informed consent was obtained, we collected blood samples from 63 individuals, 27 of whom were affected. DNA was prepared according to standard methods. In 23 individuals, syndactyly was present in both hands and feet, whereas 4 individuals presented with malformations of only the feet. The spectrum of digital malformation reached from skin fusion between the second and third toes to complete webbing between the second to fifth fingers and first to fifth toes (fig. 2). Three persons presented with synostoses between distal phalanges of their fingers (fig. 2). Men and women were affected equally; no instance of nonpenetrance was observed. The present family is unique among the published families with SD1 because, in some affected members, the syndactyly involves the first toes. The involvement of the second fingers is also an extremely rare finding in SD1, since it has otherwise been reported in only a single individual from a family with SD1 (Tentamy and McKusick 1978). On the basis of the involvement of the second fingers and the first toes, Lenz and Majewski (1981) classified this family as having syndactyly type 1a (or "syndactyly type Lueken").

Thirty-four members of the family were analyzed for the initial exclusion of a candidate locus. Because mutations in the *HOXD13* gene on 2q31 are known to cause syndactyly type 2 (Muragarki et al. 1996), this gene deserved particular attention as a potential can-

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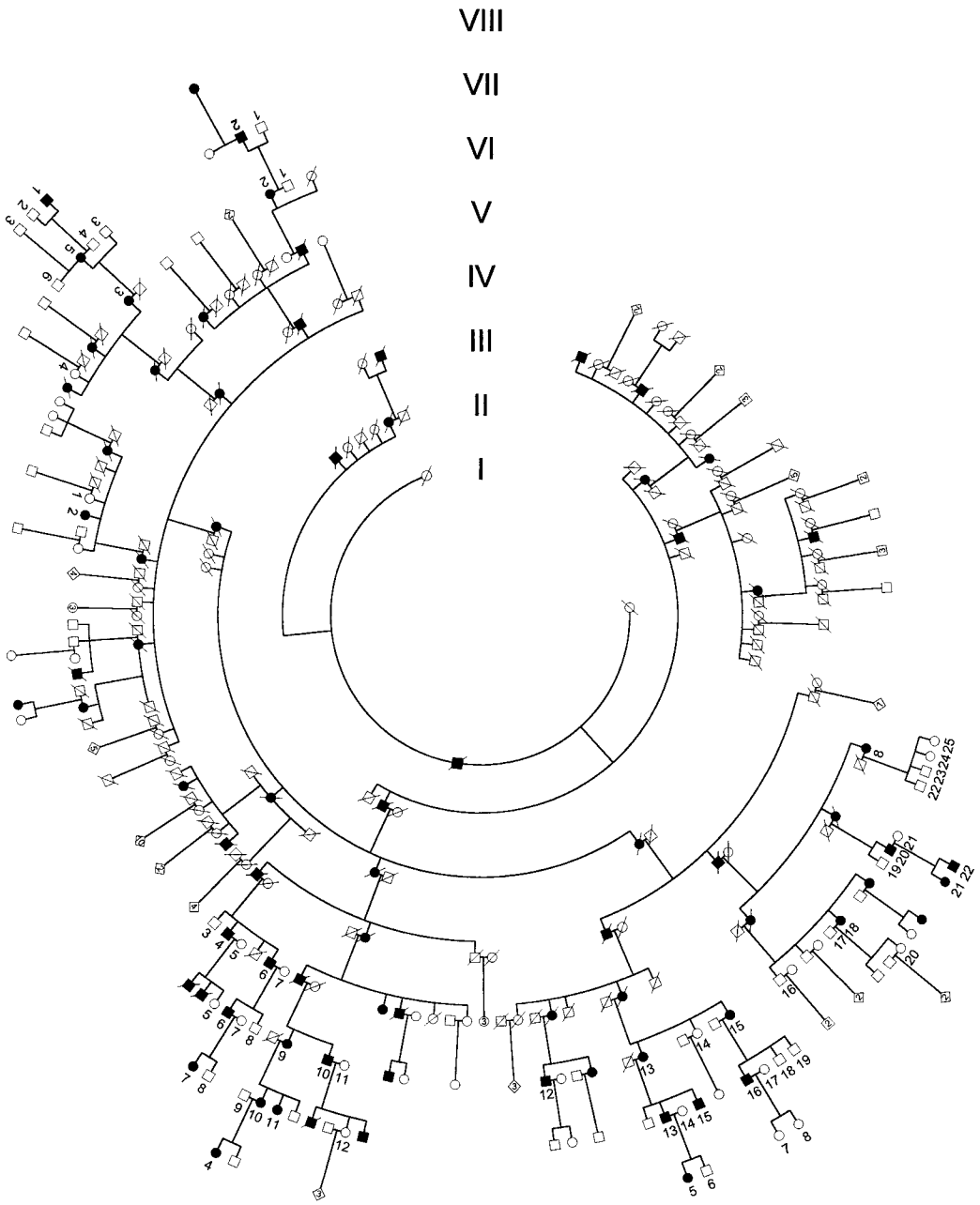

Figure 1 Pedigree of the SD1 family studied. Circles denote females; squares, males; and blackened symbols, affected individuals. Numbers below symbols denote individuals included in the linkage analysis. Numbers inside symbols indicate the number of siblings.

didate. However, a negative LOD score was obtained for this locus (data not shown).

Subsequently, a genomewide linkage scan using highly polymorphic microsatellite markers was performed in 42 individuals. Two-point LOD scores were calculated between each marker locus and syndactyly, using the LINKAGE version 5.21 software (Lathrop et al. 1984). Given the inheritance pattern observed in our family, LOD scores were calculated under the assumption of autosomal dominant inheritance with a penetrance of 100% (phenocopy rate 0), a frequency of .001 for the disease allele, and equal allele frequencies for each marker. Alternatively, we calculated LOD scores by applying a more conservative model with a penetrance of 95%. After performing linkage analysis for 48 markers, we found evidence for linkage to the marker D2S377. Fine mapping of the region by means of nine additional markers and genotyping of 21 additional family mem-

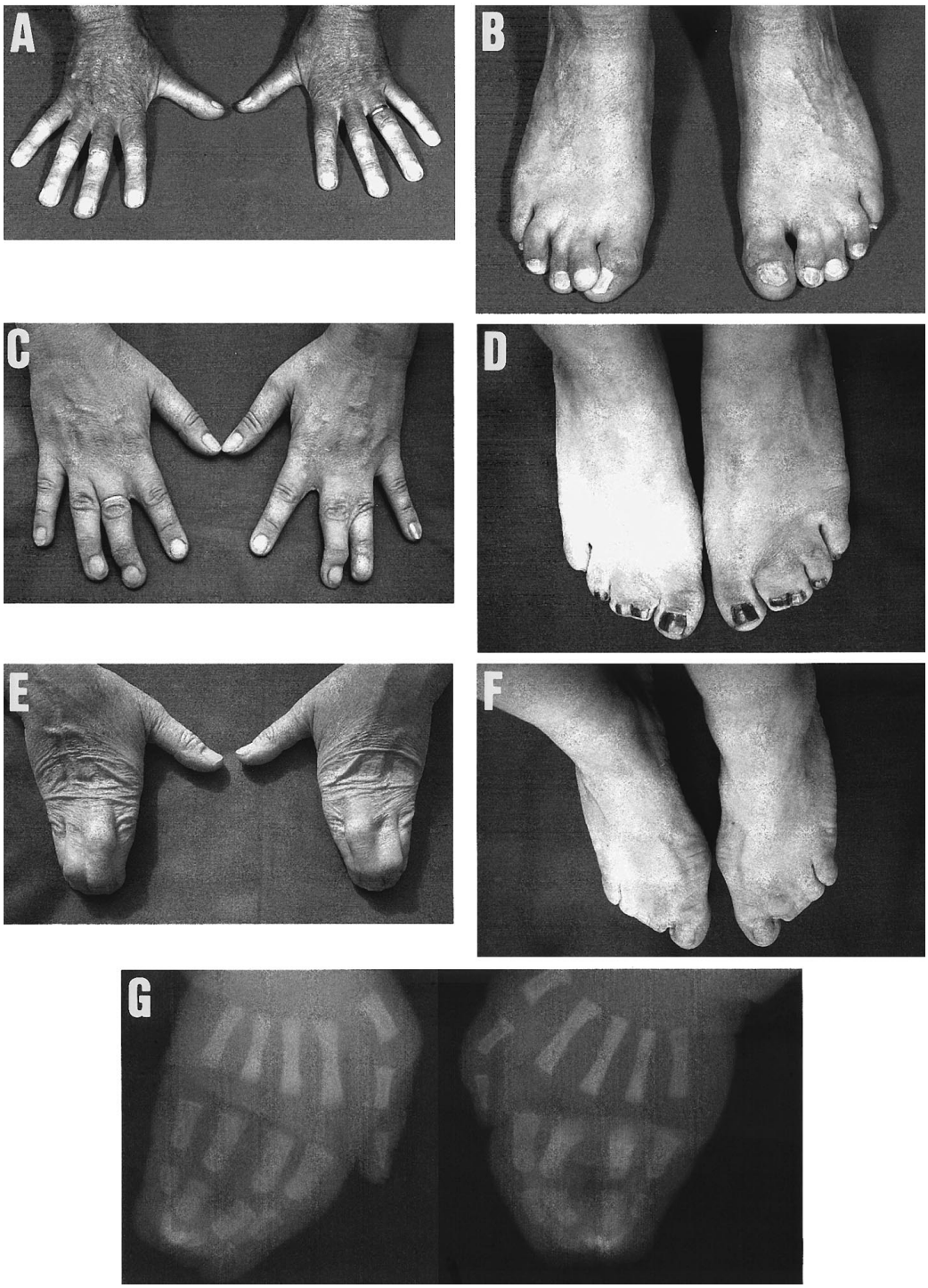

Figure 2 Phenotypic appearance of variably affected individuals. *A* and *B,* Hands and feet of individual VI:12, demonstrating a mild expression of SD1; the hands are unaffected, the feet show partial syndactyly between the second and third toes. *C* and *D,* Hands and feet of individual VII:11. Partial webbing is present between the second, third, and fourth fingers, more pronounced in the left hand than in the right hand. Both feet show complete syndactyly between the second and third toes and partial syndactyly between the third and fourth toes. *E* and *F,* Hands and feet of individual VI:2, demonstrating the most severe expression of SD1 in the family studied; complete webbing between the second to fifth fingers in both hands and syndactyly between the first to fourth toes in both feet. *G,* Radiographs of the hands of individual VII:2 taken shortly after birth. The distal phalanges of the third, fourth, and fifth fingers show a synostotic fusion in both hands.

bers was performed. A maximum two-point LOD score (Z_{max}) of 12.40 was obtained at recombination fraction (θ) 0 for marker D2S301 (table 1). Allowing for reduced penetrance had only minor effects on maximum LOD scores (data not shown). Haplotypes were then reconstructed to determine the critical recombination events. A common haplotype spanning a 9.4-cM region between markers D2S2319 and D2S344 segregated in all affected members. The critical recombinants defining the cosegregating interval both occurred in affecteds. The centromeric boundary of this interval was defined by a recombination between markers D2S2319 and D2S2382 in individual VII-2 (fig. 3*A*). The telomeric boundary of this interval corresponded to a recombination between markers D2S163 and D2S344, which was observed in individual VII-11 (fig. 3*B*).

The results of the present study have identified the first locus for SD1, on chromosome 2q34-q36. Our finding that a gene for SD1 is localized at least 24 cM telomeric to the *HOXD* gene cluster (Muragarki et al. 1996) suggests the existence of an additional gene involved in limb development located on the long arm of chromosome 2.

Of interest, brachydactyly type A-1 (BDA1) has recently been mapped to an 8.1-cM region on 2q35-q36 (Yang et al. 2000). This region overlaps with the interval in which we mapped the gene for SD1. The BDA1 phenotype of the two families studied by Yang et al. (2000) includes proportionate shortening of all fingers. The middle phalanges were either reduced, fused, or missing. Clinodactyly of the second, third, or fourth fingers was present in most of the affected members. In contrast, the affected individuals from our family with SD1 showed neither shortening of fingers nor clinodactyly. Although BDA1 and SD1 present with clinically different malformations of the limbs, it is possible that both disorders represent different mutations within a single gene. Alternatively, one can hypothesize that a cluster of genes with related function maps to chromosome 2q34-q36. In the case that BDA1 and SD1 represent allelic disorders, the candidate region could be narrowed to a 4.9-cM interval flanked by markers D2S301/D2S2248 and D2S344.

The formation of limbs involves numerous genes, such as those encoding proteins involved in the *Sonic hedgehog* (*SHH*) signalling pathway, fibroblast growth factors, bone morphogenetic proteins, cartilage-derived morphogenetic protein, and *Wnts* (Tickle 1995; Manouvrier-Hanu et al. 1999). GeneMap'99 lists a large number of expressed-sequence tags (ESTs) and cloned genes in the SD1 region. Among these, the *Indian hedgehog* (*IHH*) gene can be considered a potential candidate gene. *IHH* is a homolog of *SHH,* which is an important morphogen in vertebrates and plays a key role in the establishment of anteroposterior polarity of the limbs (Riddle et al. 1993; Chang et al. 1994). *IHH* has biological properties similar to those of *SHH,* and it has been shown that *IHH* functions in a pathway that regulates the rate of chondrocyte differentiation (Vortkamp et al. 1996).

In addition, a clone containing part of the human *WNT6* gene has been mapped to chromosome 2q35 (Rankin et al. 1999). Furthermore, GeneMap'99 contains an EST with high similarity to *Wnt6,* the EST being localized between markers D2S301/D2S164 and D2S163. The human *WNT6* gene might be a promising candidate gene, since *Wnt6* is known to be expressed in the limb bud of developing mouse embryos (Parr et al. 1993).

Yang et al. (2000) discussed the transcription factor *PAX3* as a candidate for BDA1. According to GeneMap'99, the *PAX3* gene is located outside our region, providing exclusion of this gene for SD1.

In summary, the data presented in this study identify

Table 1

Two-Point LOD Scores, between the Disease Locus and 10 Chromosome 2 Microsatellite Markers

Marker	Genetic-Map Location (cM)[a]	LOD Score at θ =					Maximum	
		.00	.01	.05	.10	.20	θ	Z_{max}
D2S143	217.0	−∞	4.34	5.09	4.85	3.73	.053	5.09
D2S2319	217.5	−∞	3.55	4.94	4.92	3.91	.050	4.94
D2S2382	220.7	7.70	7.65	7.30	6.67	5.06	0	7.70
D2S301	222.0	12.40	12.17	11.20	9.95	7.33	0	12.40
D2S1371	222.4	8.09	7.96	7.40	6.58	4.73	0	8.09
D2S2249	223.1	10.16	9.97	9.17	8.13	5.93	0	10.16
D2S173	223.2	10.87	10.64	9.70	8.50	6.02	0	10.87
D2S163	225.6	11.01	10.83	10.05	8.96	6.56	0	11.01
D2S344	226.9	−∞	2.76	3.06	2.83	2.04	.050	3.06
D2S377	228.2	−∞	6.92	7.01	6.46	4.90	.028	7.10

[a] Measured from 2qter and taken from the Généthon sex-averaged linkage map (Dib et al. 1996) and the Marshfield sex-averaged linkage map (Broman et al. 1998).

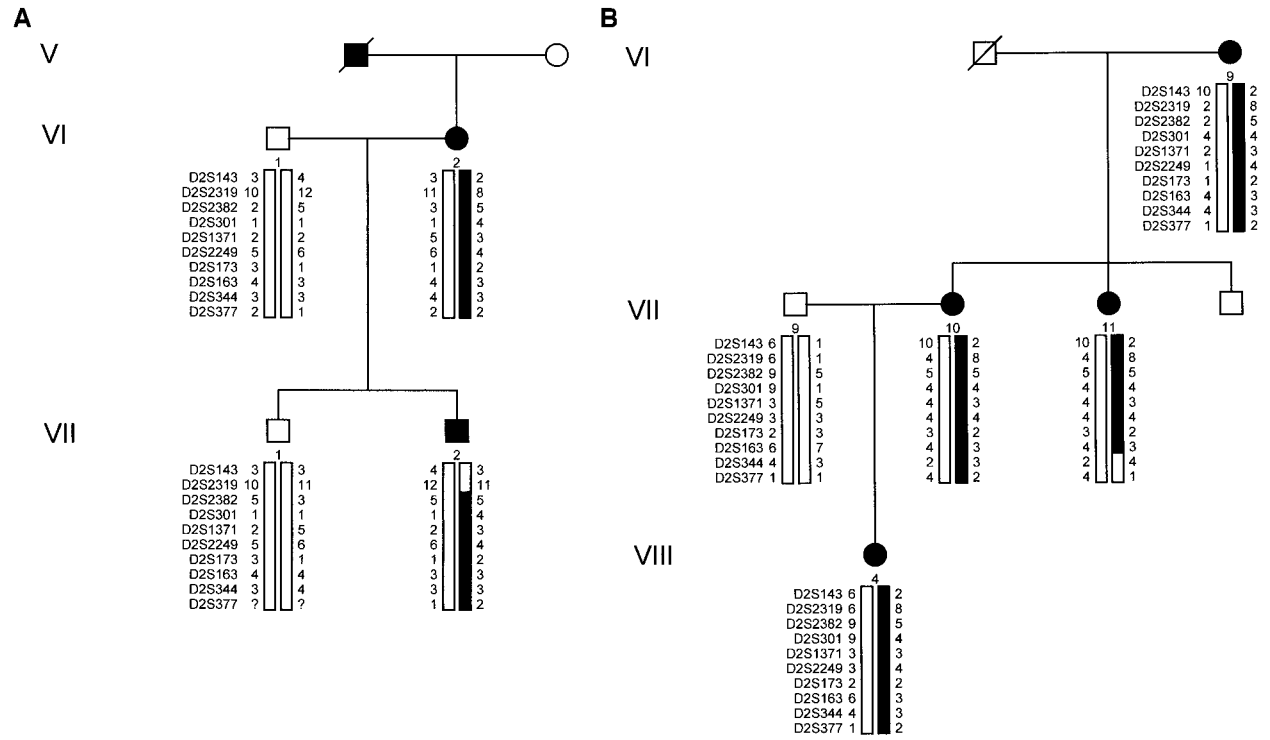

Figure 3 Haplotype analysis of two parts of our family with SD1, showing the two recombinants that delineate the 9.4-cM critical region between markers D2S2319 (proximal) in individual VII-2 (*A*) and D2S344 (distal) in individual VII-11 (*B*). Blackened bars denote segregating chromosomal segments and show regions of crossover.

a new locus for syndactyly. The identification of a gene for SD1 is anticipated to broaden our understanding of the molecular basics of limb differentiation.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/
GeneMap'99, http://www.ncbi.nlm.nih.gov/genemap99/
Généthon, http://www.genethon.fr/genethon_en.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SD1 [MIM 185900])

References

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63:861–869

Castilla EE, Paz JE, Orioli-Parreiras IM (1980) Syndactyly: frequency of specific types. Am J Med Genet 5:357–364

Chang DT, Lopez A, Von Kessler DP, Chiang C, Simandl BK, Zhao R, Seldin MF, et al (1994) Products, genetic linkage and limb patterning activity of a murine *hedgehog* gene. Development 120:3339–3353

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Feller AM, Pick CF, Enders H (1981) Syndactylism of the hand: pathogenetic and therapeutic aspects. Z Kinderchir 33: 166–174

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lenz W, Majewski F (1981) Fehlbildungen der Gliedmaßen. In: Schinz HR (ed) Lehrbuch der Röntgendiagnostik, Thieme Verlag, Stuttgart, pp 935–1032

Lueken KG (1938) Über eine Familie mit Syndaktylie. Z Menschl Vererb Konstit-Lehre 22:152–159

Manouvrier-Hanu S, Holder-Espinasse M, Lyonnet S (1999) Genetics of limb anomalies. Trends Genet 15:409–417

Montagu MFA (1953) A pedigree of syndactylism of the middle and ring fingers. Hum Genet 5:70–72

Muragarki Y, Mundlos S, Upton J, Olsen BR (1996) Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. Science 272:548–550

Parr BA, Shea MJ, Vassileva G, McMahon AP (1993) Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. Development 119:247–261

Rankin J, Strachan T, Lako M, Lindsay S (1999) Partial cloning and assignment of WNT6 to human chromosome band 2q35 by in situ hybridization. Cytogenet Cell Genet 84: 50–52

Riddle RD, Johnson RL, Laufer E, Tabin C (1993) *Sonic hedgehog* mediates the polarizing activity of the ZPA. Cell 75: 1401–1416

Tentamy SA, McKusick VA (1978) The genetics of hand malformations. Alan R. Liss, New York, pp 301–322

Tentamy SA (1990) Syndactyly. In: Buyse ML (ed) Birth defects encyclopedia. Blackwell Scientific Publications, Cambridge, MA, pp 1617–1618

Tickle C (1995) Vertebrate limb development. Curr Opin Genet Dev 5:478–484

Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ (1996) Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 273:613–622

Yang X, She C, Guo J, Yu ACH, Lu Y, Shi X, Feng G, et al (2000) A locus for brachydactyly type A-1 maps to chromosome 2q35-q36. Am J Hum Genet 66:892–903