A Locus for Brachydactyly Type A-1 Maps to Chromosome 2q35-q36

Xinping Yang,[1] Chaowen She,[4] Jingzhi Guo,[5] Albert CH Yu,[2,6] Yingjin Lu,[1] XiaoLiu Shi,[7] Guoying Feng,[1] and Lin He[1,2,3]

[1]Shanghai Institute of Physiology, Chinese Academy of Sciences, [2]Shanghai Research Center of Life Sciences, and [3]College of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai; [4]Biology Department, Huaihua Teachers' College, Hunan; [5]Guiyang Women and Children's Hospital, Guiyang, P. R. China; [6]Department of Biology, the Hong Kong University of Science and Technology, Hong Kong; and [7]The Second Affiliated Hospital, Hunan Medical University, Changsha, P. R. China

Brachydactyly type A-1 (BDA1) was, in 1903, the first recorded example of a human anomaly with Mendelian autosomal dominant inheritance. Two large families, the affected members of which were radiographed, were recruited in the study we describe here. Two-point linkage analysis for pedigree 1 (maximum LOD score [Z_{max}] 6.59 at recombination fraction [θ] 0.00) and for pedigree 2 ($Z_{max} = 5.53$ at $\theta = 0.00$) mapped the locus for BDA1 in the two families to chromosome 2q. Haplotype analysis of pedigree 1 confined the locus for family 1 within an interval of <8.1 cM flanked by markers D2S2248 and D2S360, which was mapped to chromosome 2q35-q36 on the cytogenetic map. Haplotype analysis of pedigree 2 confined the locus for family 2 within an interval of <28.8 cM flanked by markers GATA30E06 and D2S427, which was localized to chromosome 2q35-q37. The two families had no identical haplotype within the defined region, which suggests that the two families were not related.

Introduction

Inherited brachydactyly was classified by Bell (1951) into five types—A, B, C, D, and E—on the basis of malformation of the digits. Short middle phalanges are the main feature of type A brachydactyly, of which there are several subtypes. Bell recognized types A-1, A-2, and A-3. Brachydactyly type A-1 (BDA1; MIM 112500) is characterized by shortening of the middle phalanges, which may be fused to the distal ones. The trait of type A-1 brachydactyly in humans was the first to be interpreted in terms of Mendelian dominance (Farabee, 1903). In Bell brachydactyly type A-2, the shortening is confined to the middle phalanx of the index finger, and, in Bell type A-3, it is confined to the middle phalanx of digit 5. McKusick (1975) added types A-4 and A-5. In McKusick type A-4, the middle phalanges of digits 2 and 5 are short, and there is radial clinodactyly of finger 4. In McKusick type A-5, the middle phalanges are missing. Fitch (1979), after re-examining 44 radiographs of BDA1, made the identification of BDA1 more comprehensive. The hands are broad, with proportionate shortening of all the digits; all the hand bones may be shorter than those in normal hands, but the middle phalanges

are the most severely shortened. Fitch gave a manifestation spectrum of BDA1: at one end, all the middle phalanges were missing (McKusick type A-5), and, at the other end, only the phalanges of digits 2 and 5 were shortened (McKusick type A-4). Therefore, in Fitch's classification, McKusick types A-4 and A-5 belong to Bell type A-1.

BDA1 may also be present with other manifestations, such as ankylosis of the thumbs and mental retardation in women (Piussan et al. 1983); Slavotinek et al. (1998) found Klippel-Feil anomaly in a Japanese child with BDA1 who carried a balanced reciprocal translocation between 5q11.2 and 17q23.

Despite the presence of a detailed clinical description of BDA1, to our knowledge, no molecular genetic study of this malformation has been performed. However, some progress has been achieved in other subtypes of brachydactyly. Weinstein et al. (1990) detected mutations of the *GNAS1* gene on 20q in patients with syndromic forms (Albright hereditary osteodystrophy) of brachydactyly type E. Polinkovsky et al (1997) detected mutations in CDMP1 (cartilage-derived morphogenetic protein 1, a member of the transforming growth factor–β superfamily) on chromosome 12 in unrelated families with BDC.

In this article, we report phenotypes of 33 patients in two unrelated families with BDA1 and linkage mapping of the BDA1 locus to chromosome 2q35-q36. We examined the radiographs of patients in the two large families; we provide evidence that supports Fitch's (1979) description of nonsyndromic BDA1. We suggest

that the two families' mutations have a different origin, because they do not share a common haplotype within the same defined interval.

Subjects and Methods

Patients and Family Database

Two large families, one from Hunan Province, China (fig. 1), and another from Guizhou Province, China (fig. 2), were recruited in this study. Twenty-five of 33 affected individuals were further examined by radiography. Various manifestations of BDA1, as described by Fitch (1979), were revealed. Nine of the affected individuals (individual 32 of family 1 and individuals 2, 4, 5, 7, 8, 11, 18, and 23 of family 2) had the proximal phalanges of digit 5 shortened, and four of the affected individuals (individuals 6 and 16 of family 1 and individuals 5 and 7 of family 2) had shortened distal phalanges and shortened metacarpals, traits beyond the manifestations described by Fitch (1979).

Phenotype

The defects found in the affected individuals in the two families were confined to the hands and feet. The affected members of both families had normal stature and mental status compared with the unaffected family members or the local population.

Family 1 had 18 affected members available for study. The hands of the affected family members were short, with proportionate shortening of all digits, but the defects were mainly confined to the middle phalanges, which led to the lack of distal interphalangeal creases. Fourteen of the affected family members were examined by radiography. X-ray examination revealed an anomaly of hand bones (fig. 3; table 1). Individuals 6, 14, and 20 had all the middle phalanges missing. Other individuals had less-severely affected middle phalanges, with the middle phalanges of digits 2, 4, and 5 missing or fused (individuals 8 and 16), middle phalanges of digits 2 and 5 missing or fused (individuals 23, 26, and 29), or the middle phalange of digit 5 missing (individuals 3, 12, 30, 31, 32, and 36). All the remaining middle phalanges were severely shortened. All affected adults in this family have metacarpals and proximal phalanges with thin shafts and broad epiphyses; radiographs of three affected children (individual 31, aged 11 years; individual 32, aged 9 years; and individual 36, aged 5 years) did not show such this feature. Clinodactyly of the second, fourth, or third fingers was present in most of the affected members (individuals 3, 8, 16, 18, 20, 23, 26, 29, 30, 31, 32, 36, and 37). The proximal phalanges of the thumbs were markedly shortened and broadened in most of the affected family members (individuals 3, 12, 14, 16, 20, 23, 26, and 29–32). All the

manifestations described above are common to other reports reviewed by Fitch (1979). In addition, however, affected family members had some manifestations beyond Fitch's description. The individual with the most severe manifestation was individual 6, whose middle phalanges were missing; in addition, the distal phalanges of digits 2–5 were severely shortened, as was the third metacarpal of the right hand. The distal phalanges of digits 1, 3, and 5 and the third metacarpal of the left hand were also affected in individual 16. Individual 32 had the proximal phalanges of digit 5 shortened. To our knowledge, the severely shortened distal phalanges, shortened third metacarpals, and shortened proximal phalanges of digit 5 have not yet been reported elsewhere as being associated with BDA1.

Family 2 had 15 affected members available for study. The affected members had phenotypes similar to those of family 1 (fig. 4). Eleven of the affected family members were examined by radiography. In most of them (individuals 2, 4, 5, 7, 8, 11, 18, 22, 23, and 24), the middle phalanges were missing. Only individual 19 had unfused shortened middle phalanges in digits 2, 3, and 4. Like the affected members of family 1, the affected adults also had metacarpals and proximal phalanges with thin shafts and broad epiphyses. Clinodactyly was present in individuals 1, 5, 7, 8, 11, 18, 19, 22, and 23. The proximal phalanges of the thumb were markedly shortened and broadened in most of the affected family members (individuals 2, 4, 5, 7, 8, 11, 18, 22, 23, and 24). These manifestations are common features of BDA1 (Fitch 1979). Like family 1, family 2 also had affected individuals who had shortened distal phalanges and shortened metacarpals. Individual 5 was the most severely affected individual in this family. In individual 5, the middle phalanges were missing or fused, the distal phalanges were severely shortened, and severe clinodactyly was present in finger 4. The third and the fifth metacarpals were shortened, and thus finger 4 was the longest finger. Another severely affected individual, individual 7, had the middle phalanges missing, and some of the distal phalanges were severely shortened. Individuals 2, 4, 5, 7, 8, 11, 18, and 23 had shortened and malformed proximal phalanges of digit 5. Table 1 summarizes the phenotype variability of the two families.

Genotyping and Linkage Analysis

Samples of peripheral blood DNA were taken from all available family members, and DNA was isolated by means of standard procedure. A slightly modified Weber screening set (Sheffield et al. 1995) of 380 polymorphic microsatellite markers was recruited in the genome scan. In the denser mapping of the critical chromosomal region, marker maps from Généthon (Dib et al. 1996) and the Cooperative Human Linkage Center were used.
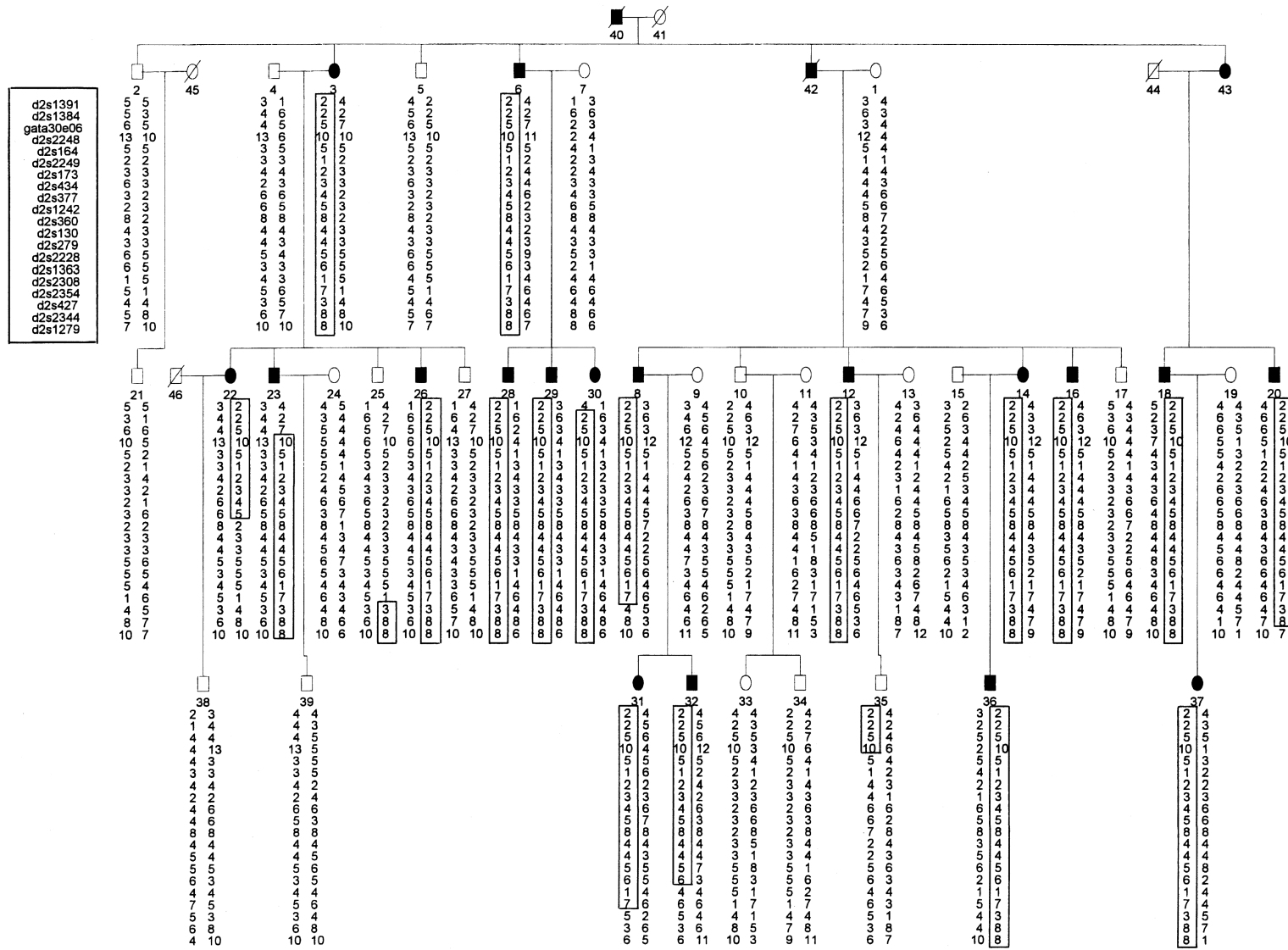
Figure 1 Pedigree of BDA1 family 1 showing haplotypes for the polymorphic markers in the region of 2q32-q37. Marker order was determined from the Généthon sex-averaged genetic map, the CHLC sex-averaged genetic map, and the Genome Database. Open symbols indicate unaffected individuals, blackened symbols indicate affected individuals, squares indicate men, and circles indicate women. Boxed markers are the 20 contiguous-marker disease-linked haplotype or part of the haplotype.
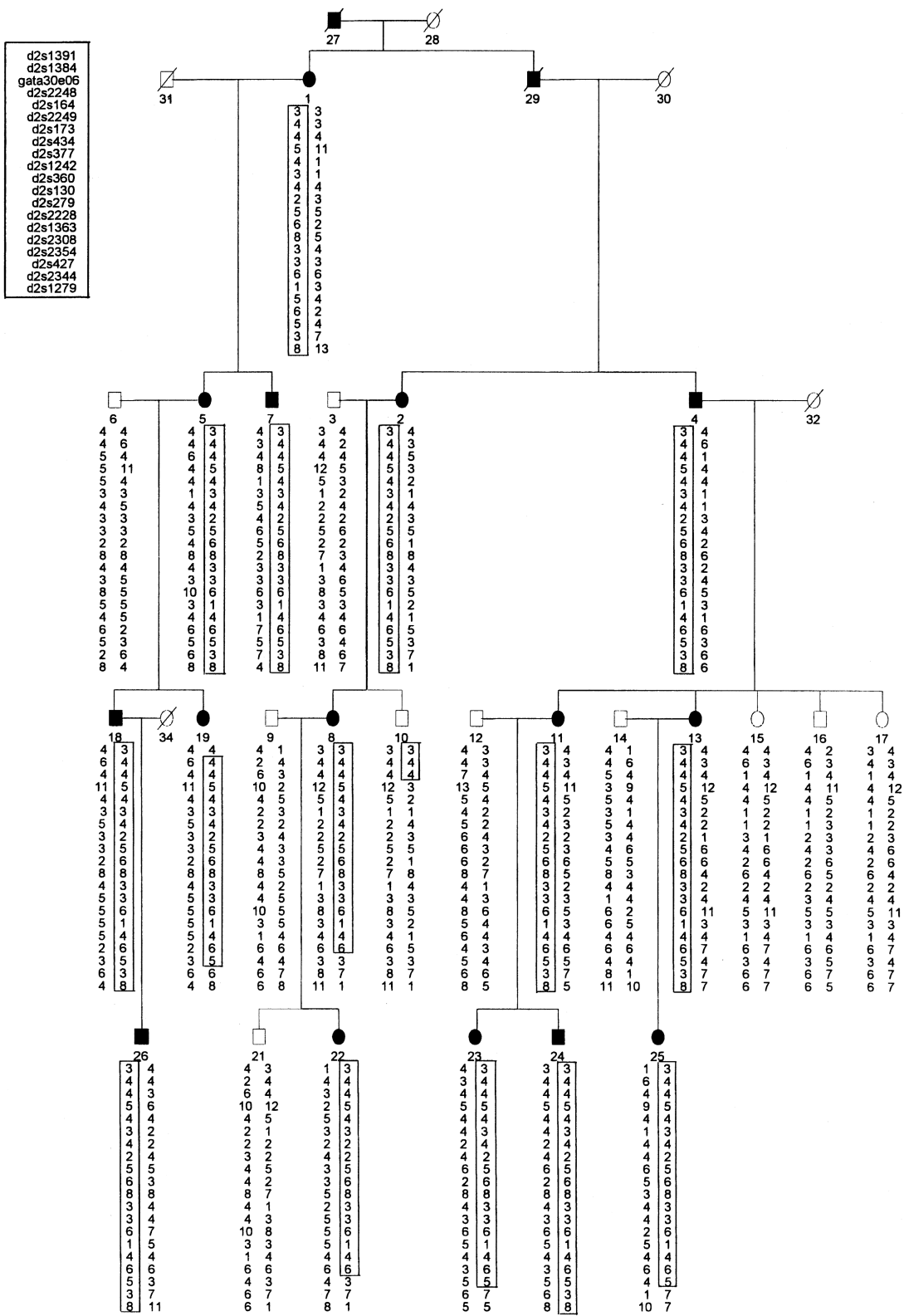
Figure 2 Pedigree of BDA1 family 2 showing haplotypes for the polymorphic markers in the region of 2q32-q37. Marker order was determined from the Généthon sex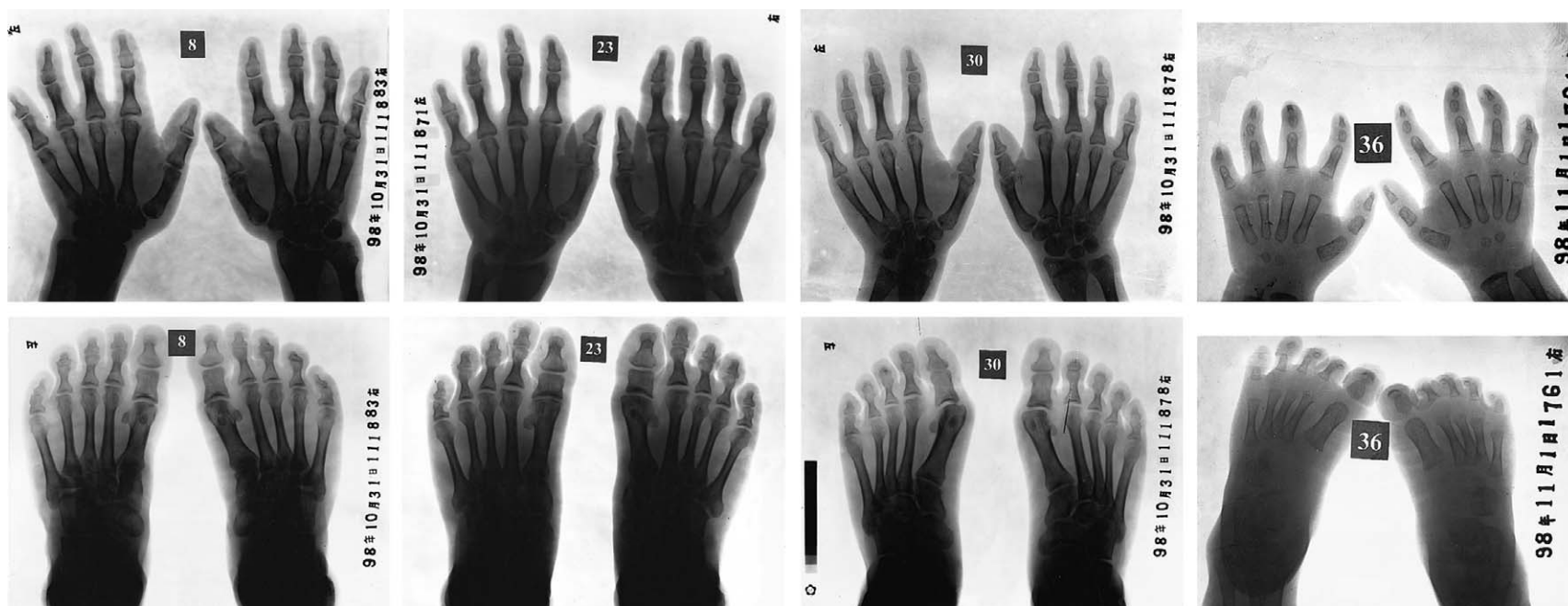-averaged genetic map, the CHLC sex-averaged genetic map, and the Genome Database. Symbols are as indicated in the legend to figure 1. Boxed markers are the 20 contiguous-marker disease-linked haplotype or part of the haplotype.
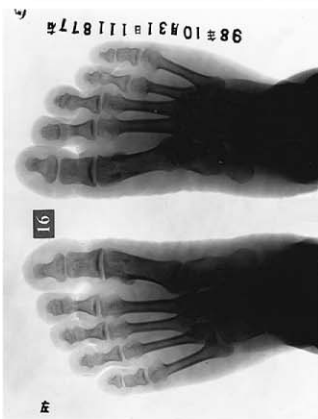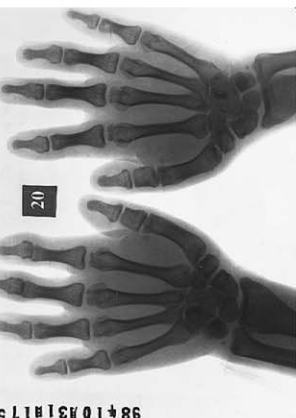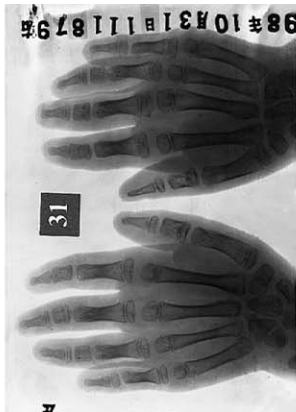
Figure 3   Finger and toe features of family 1. The pictures provide the numbers of the individuals. Anomalies were mainly confined to the middle phalanges. In some affected individuals, all the middle phalanges were missing or fused to the distal ones (e.g., individuals 6 and 20). In some affected family members, one to three fingers had middle phalanges missing or fused (e.g., individual 30 had the middle phalanges of digit 5 missing; individual 23 had the middle phalanges of digits 2 and 5 missing; and individuals 8 and 16 had the middle phalanges of digits 2, 4, and 5 missing). The proximal phalanges of the thumbs were markedly shortened and broadened in most of the affected members (e.g., individuals 16, 20, 23, 30, and 31). All affected adults in this family had the feature of thin shafts and broad epiphyses of metacarpals and proximal phalanges, but affected children did not have such a feature (e.g., individual 31, 11 years old; and individual 36, 5 years old). The clinodactyly of the second, fourth, or third fingers was present in most of the affected family members (e.g., individuals 8, 16, 23, 30, 31, and 36). All the manifestations described above are common to other reports reviewed by Fitch (1979). The distal phalanges and metacarpals were also shortened in two of the affected family members: individual 6 and individual 16. Individual 6 had very short distal phalanges in digits 2, 3, 4, and 5 and had a shortened metacarpal 3 of the right hand. Individual 16 had short distal phalanges in digits 1, 3, and 5 and a short third metacarpal of the left hand.

Table 1

Phenotype Variability of Individuals Affected with BDA1

Family and Affected Individual	Age (years)	Presence of Anomaly of Hand (Bones)										
		1				2	3		4	5	6	7
		A	B	C	D		E	F				
Family 1:												
3	56	+				+	+	+	+	−	−	−
6	50				+	+	−	−	−	+	+	−
8	37			+		+	+	+	−	−	−	−
12	29	+				+	−	−	+	−	−	−
14	28				+	+	−	−	+	−	−	−
16	24			+		+	+	−	+	+	+	−
18[a]	29						+	+				
20	26				+	+	+	+	+	−	−	−
22[a]	29						+	−				
23	27		+			+	+	+	+	−	−	−
26	24		+			+	+	+	+	−	−	−
28[a]	22						+	+				
29	19		+			+	+	−	+	−	−	−
30	17	+				+	+	+	+	−	−	−
31	11	+				0	+	+	+	−	−	−
32	9	+				0	+	+	+	−	−	+
36	5	+				0	+	+	+	−	−	−
37[a]	5						+	+				
Family 2:												
1[a]	80						+	−				
2	59				+	+	−	−	+	−	−	+
4	57				+	+	−	−	+	−	−	+
5	57				+	+	+	−	+	−	−	+
7	50				+	+	+	−	+	+	+	+
8	34				+	+	+	−	+	−	+	+
11	32				+	+	+	−	+	−	−	+
13[a]	29						+	−				
18	27				+	+	+	−	+	−	−	+
19	20	+				+	+	+	+	−	−	−
22	7				+	0	+	−	+	−	−	+
23	10				+	0	+	−	+	−	−	+
24	8				+	0	+	−	+	−	−	+
25[a]	3						−	−				
26[a]	7						−	−				

NOTE.—A plus sign (+) indicates presence, a minus sign (−) indicates absence, and a question mark (?) indicates that presence or absence of the anomaly could not be determined because the individual was too young. Anomalies are numbered, as follows: 1 = all middle phalanges severely shortened; some were fused or missing; 2 = thin shafts and broad epiphyses of metacarpals and proximal phalanges; 3 = clinodactyly; 4 = proximal phalanges of the thumbs shortened and broadened; 5 = third metacarpal, fifth metacarpal, or both shortened; 6 = shortened distal phalanges; 7= proximal phalange of digit 5 shortened and broadened; A = middle phalange of digit 5 missing; B = middle phalange of digits 2 and 5 missing; C = middle phalange of digits 2, 4, and 5 missing; D = all middle phalanges missing; E = radial clinodactyly of fingers 4 and 5; and F = ulnar clinodactyly of fingers 2 and 3.
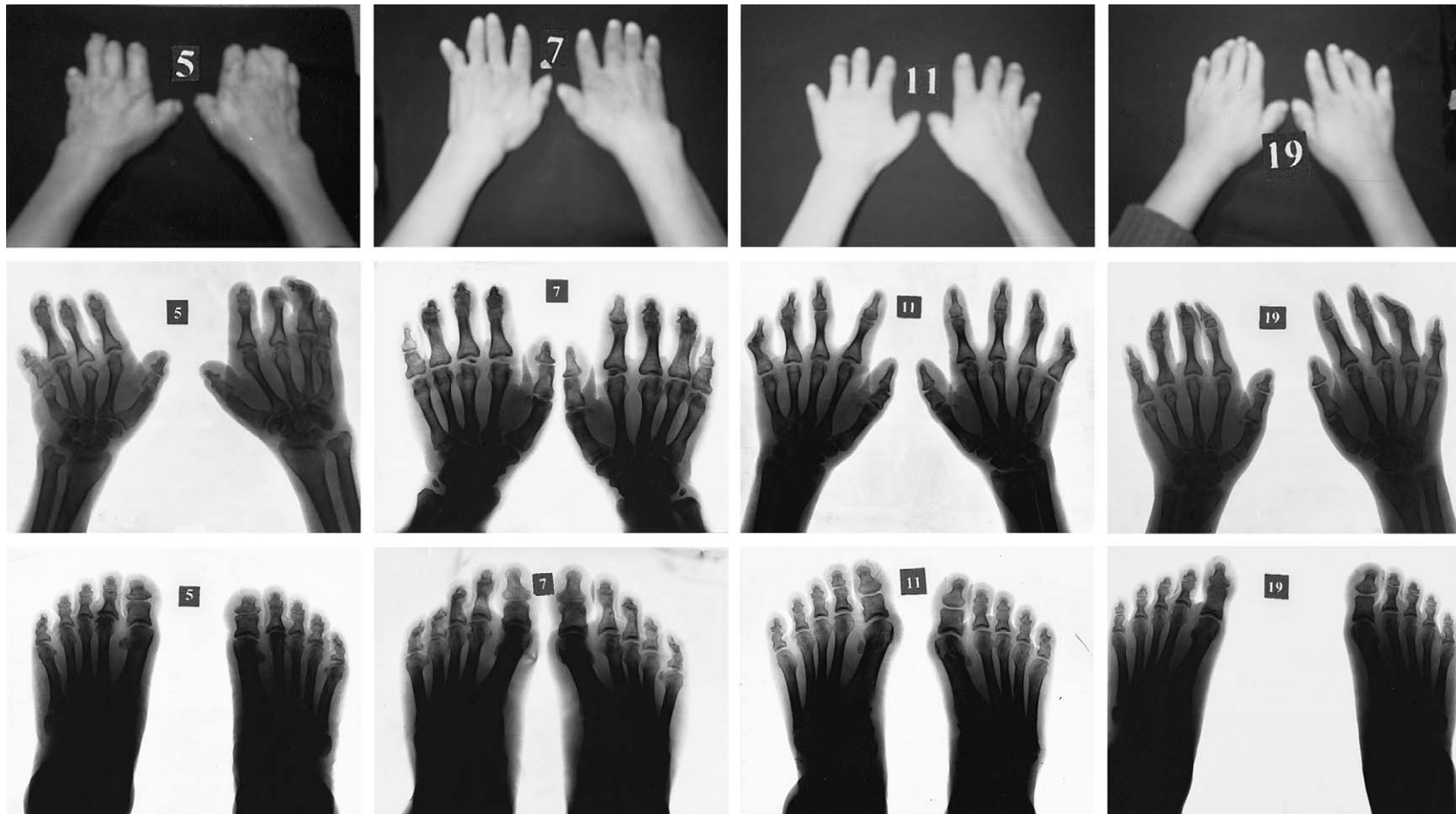
[a] Individual not examined by radiography.

Most primer sequences were from the Généthon collection (Dib et al. 1996). The primer sequences and allele sizes of markers D2S1242, D2S279, and D2S1279 were from the Genome Database (D2S1242, GDB 309429; D2S279, GDB 198733; and D2S1279, GDB 315905). We also consulted the Genome Database for cytogenetic maps of markers.

All the primers were commercially synthesized. A sequence of 5′-GAGAGAAAGGGAAGGGAG-3′ was tagged to the 5′ end of the forward primer of each pair of primers. A universal primer, which had the same sequence as the added tag, was fluorescently labeled (Pharmacia).

PCRs were performed in a total volume of 10 μl containing 100 ng of genomic DNA, 1.5 mM MgCl$_2$, 0.2 μM tagged forward primer, 0.8 μM universal primer, 1.0

Figure 4   Finger and toe features of family 2. The pictures provide the numbers of the individuals. All the affected members had phenotypes similar to those of family 1. In most affected individuals examined by radiography, the middle phalanges were missing (e.g., individuals 5, 7, and 11). Individual 19 had shortened middle phalanges in digits 2, 3, and 4. The proximal phalanges of the thumbs and digit 5 were markedly shortened and broadened in most of the affected members (e.g., individuals 5, 7, and 11). The affected family members also have thin shafts and broad epiphyses of metacarpals and proximal phalanges. The clinodactyly of fingers 5 and 4 was present in some affected individuals (e.g., individual 18 had clinodactyly in finger 5, and individuals 5 and 19 had clinodactyly in finger 4). Most of the affected had the proximal phalanges of digit 5 shortened, broadened, or both (e.g., individuals 5, 7, 11, and 18). Distal phalanges and metacarpals were also shortened in two affected members of this family (e.g., in individual 5, the distal phalanges, metacarpal 3, and metacarpal 5 were shortened; in individual 7, the distal phalanges were severely shortened).

Table 2

Two-Point Linkage Data for Pedigree 1

	LOD SCORE AT θ =						
LOCUS	.00	.001	.01	.05	.10	.20	.30
D2S1391	$-\infty$	−1.95	−5.03	−1.22	.08	.82	.75
D2S1384	$-\infty$	−1.07	.87	1.96	2.17	1.91	1.31
GATA30E06	$-\infty$	−2.69	.24	1.99	2.45	2.38	1.80
D2S2248	$-\infty$	1.02	1.96	2.39	2.34	1.89	1.24
D2S164	.85	.85	.83	.78	.72	.58	.42
D2S2249	6.50	6.49	6.40	5.96	5.40	4.17	2.78
D2S173	6.34	6.32	6.23	5.78	5.21	3.98	2.65
D2S434	3.50	3.50	3.45	3.24	2.94	2.25	1.45
D2S377	6.59	6.58	6.48	6.04	5.46	4.21	2.80
D2S1242	6.41	6.40	6.30	5.87	5.29	4.04	2.64
D2S360	$-\infty$	2.58	3.51	3.87	3.74	3.11	2.21
D2S130	$-\infty$	2.63	3.55	3.91	3.78	3.12	2.21
D2S279	$-\infty$	3.24	4.17	4.52	4.35	3.56	2.45
D2S2228	2.45	2.45	2.40	2.21	1.96	1.42	.83
D2S1363	$-\infty$	2.01	2.93	3.30	3.18	2.60	1.81
D2S2308	$-\infty$	−1.90	1.00	2.65	2.97	2.63	1.80
D2S2354	$-\infty$	1.54	3.44	4.37	4.37	3.65	2.51
D2S427	$-\infty$	−8.30	−3.39	−.28	.72	1.14	.87
D2S2344	$-\infty$	−2.68	−.72	.47	.80	.83	.54
D2S1279	$-\infty$	−1.58	−4.66	−.84	.48	1.22	1.08

μM reverse primer, and 2 U of *Taq* DNA polymerase (Promega). PCR was performed on a Hybaid OmniGene thermal cycler. The following profile was used for DNA amplification: 95°C for 5 min; 35 cycles at 94°C for 30 s; 55°–57°C for 30 s; 72°C for 90 s; and 72°C for 30 min. PCR products were separated on an automated laser-fluorescence DNA sequencer (ALF Express Pharmacia). The genotype data were processed with the AL-LELELINKS computer program (Pharmacia).

Two-point LOD scores were calculated between each marker and the disease locus by use of the MLINK program of the LINKAGE software package (version 5.1; Lathrop et al. 1984). The brachydactyly was assumed to be a fully penetrant autosomal dominant model. The disease-gene frequency was set at .0001 for LOD score calculations. Equal recombination frequencies were assumed between male and female family members.

Results

Two-Point Linkage Analysis

The 39 individuals we studied from family 1 (fig. 1) were subjected to a genome screen with markers from the Weber screening set. Five adjacent markers on chromosome 2q provided a positive LOD score (D2S1384, GATA30E06, D2S434, D2S1363, and D2S427).

Denser markers between GATA30E06 and D2S434, D2S434 and D2S1363, D2S1363 and D2S427, and two additional markers, D2S2344 and D2S1279 on the distal side of D2S427, are analyzed in pedigree 1. A two-point

LOD score for 20 markers on chromosome 2q for pedigree 1 are summarized in table 2. The results indicate that the disease gene is localized to chromosome 2q, with the highest LOD score (Z_{max}) of 6.59 (recombination fraction [θ] 0) at marker D2S377.

The 25 individuals we studied from pedigree 2 (fig. 2) were subjected to genotyping and linkage analysis of the same 20 markers on chromosome 2q that were used in the analysis of pedigree 1. Two-point LOD scores for pedigree 2 are summarized in table 3. The linkage data suggested a locus similar to that suggested for pedigree 1, with Z_{max} = 5.53 (θ = 0) at marker D2S2248.
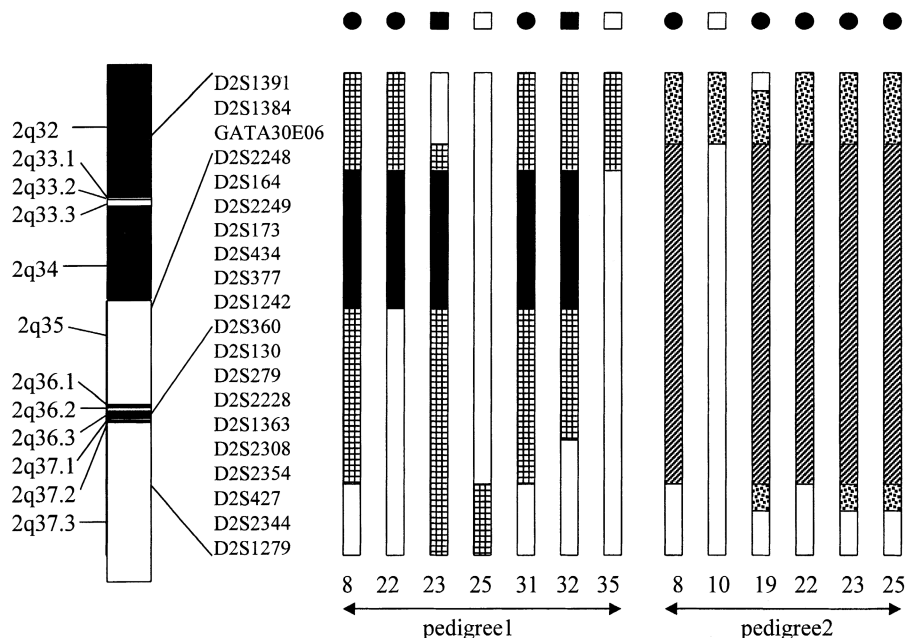
Haplotype Analyis

We constructed haplotypes of the two pedigrees with the computer program Cyrillic (version 2.0; Cherwell Scientific). The markers listed in tables 1 and 2 were used, and the haplotypes were checked by view inspection.

In pedigree 1 (fig. 1), the disease-linked haplotype seen in affected individuals 3, 6, 12, 14, 16, 18, 20, 26, 28, 29, 30, 36, and 37 represents the ancestral haplotype. Recombination events were found in five affected individuals (individuals 8, 22, 23, 31, and 32) and two unaffected individuals (individuals 25 and 35). Recombination events in individuals 22, 23, and 35 in pedigree 1 indicated that the locus for BDA1 was probably within an interval of <8.1 cM flanked by markers D2S2248 and D2S360, which were localized to chromosome 2q35-q36 (fig. 5).

Table 3

Two-Point Linkage Data for Pedigree 2

	LOD SCORE AT θ =						
LOCUS	.00	.001	.01	.05	.10	.20	.30
D2S1391	$-\infty$	−5.39	−2.43	−.52	.14	.54	.53
D2S1384	$-\infty$	−3.20	−1.22	.06	.50	.71	.60
GATA30E06	$-\infty$	−3.07	−1.10	.13	.52	.68	.56
D2S2248	5.53	5.52	5.44	5.07	4.59	3.56	2.43
D2S164	1.36	1.36	1.35	1.29	1.18	.91	.61
D2S2249	3.90	3.89	3.82	3.47	3.01	2.10	1.26
D2S173	$-\infty$	.49	1.44	1.92	1.93	1.63	1.16
D2S434	3.72	3.71	3.65	3.35	2.97	2.16	1.31
D2S377	2.92	2.91	2.87	2.66	2.40	1.84	1.24
D2S1242	4.22	4.21	4.14	3.83	3.43	2.57	1.65
D2S360	3.91	3.90	3.84	3.56	3.20	2.42	1.58
D2S130	3.97	3.97	3.89	3.54	3.10	2.18	1.31
D2S279	1.71	1.71	1.68	1.53	1.34	.95	.58
D2S2228	4.82	4.81	4.74	4.43	4.02	3.16	1.84
D2S1363	4.73	4.72	4.64	4.29	3.84	2.88	2.21
D2S2308	2.93	2.92	2.88	2.65	2.37	1.77	1.13
D2S2354	1.77	1.77	1.73	1.58	1.39	.99	.59
D2S427	$-\infty$	.31	1.26	1.74	1.76	1.45	.98
D2S2344	$-\infty$	−1.06	−5.12	−1.87	−.69	.15	.32
D2S1279	$-\infty$	−7.25	−3.31	−.76	.11	.62	.58

Figure 5 Haplotype analysis of pedigree 1 and pedigree 2, illustrating recombination events between the disease locus and chromosome 2q markers. Note that the genetic distances between markers are not shown. Pedigree 1 shows parts of the haplotype inherited from the founder (*trellised bars together with blackened bars*); note the region that had been confined as the disease locus region for family 1 that cosegregated with the BDA1 (*blackened bars*). Pedigree 2 shows parts of the haplotype inherited from the founder of family 2 (*dotted bars together with hatched bars*); note the region that had been confined as the disease locus region for family 2 (*hatched bars*). The two pedigrees do not share a common haplotype within the defined region. The cytogenetic positions of markers are from the Genome Database.

In pedigree 2 (fig. 2), the disease-linked haplotype in affected individuals (individuals 1, 2, 4, 5, 7, 11, 13, 18, 24, and 26) represented the ancestral haplotype. Five affected individuals (individuals 8, 19, 22, 23, and 25) and one unaffected individual (individual 10) were found to have recombination events. The haplotype analysis of pedigree 2 suggested a locus within an interval of 28.8 cM flanked by markers GATA30E06 and D2S427, which were mapped to chromosome 2q35-q37.

Discussion

Both families described in this paper have features of BDA1 that are similar to those reported elsewhere in other populations (reviewed by Fitch 1979). Common to all BDA1 families are the shortening of middle phalanges (which may be fused to the distal ones or may be absent); the thin shafts and broad epiphyses of metacarpals and proximal phalanges; and, in some cases, either the shortened proximal phalanges of the thumbs and radial clinodactyly of fingers 4 and 5, the ulnar clinodactyly of fingers 2 and 3, or both.

The two families had physical manifestations beyond those described of BDA1 by Fitch (1979). Individuals 6 and 16 of family 1 and individuals 5 and 7 of family

2 had severely shortened distal phalanges and shortened third metacarpals, fifth metacarpals, or both. Individual 32 in family 1 and most affected individuals in family 2 had the proximal phalanges of digit 5 shortened, broadened, or both (figs. 3 and 4).

We classify the manifestations of the affected individuals of the two families into seven items (table 1). The first six items exist in both families; the seventh exists only in family 2, suggesting that the affected members of the two families have very similar phenotypes. The first two items exist in all the affected individuals, and the other items are not always present. This leads to variability of symptoms among the affected family members (table 1; figs. 3 and 4).

We have mapped BDA1 to the locus within chromosome 2q35-q37 because strong linkage was observed between the BDA1 locus and markers on chromosome 2q35-q37 (tables 2 and 3). The two families have similar manifestations, and the haplotypes of the disease chromosomes in each family cover the same contiguous markers (fig. 5). We deduce that the two families may have the same disease-causing gene, but the disease-causing mutations in the two families might originate from different ancestors, since they do not share a common haplotype.

The recombination events in individuals 22, 23, and

35 of pedigree 1 reduced the interval to 8.1 cM between D2S2248 and D2S360, which roughly coincides with the band 2q35-q36 on a cytogenetic map. Evaluation of the human gene map within 2q35-q36 (NCBI MIM gene map) suggests the candidate gene *PAX3* (paired box homeotic gene 3).

The *PAX3* gene is within the locus defined for BDA1. Different defects in the *PAX3* gene cause various phenotypes (Waardenburg syndrome type 3 [WS3], MIM 148820; and craniofacial-deafness-hand syndrome, MIM 122880). Affected individuals in WS3 have skeletal upper-limb hypoplasia (Hoth et al. 1993; Tassabehji et al. 1995), and individuals with craniofacial-deafness-hand syndrome have hypoplasia in the nasal bones and maxilla (Asher et al. 1996). The mouse *Pax3* gene is expressed in many structures, including limb budding in early developing mouse embryos (Mouse Genome Informatics), so it is likely that some kind of mutations in *PAX3* can cause BDA1.

Homeobox (HOX) genes comprise a diverse multigene family encoding transcription factors that may control the pattern formation (timing and local growth rate) during early development. The structures and functions have been conserved through animal evolution. The expansions of polyalanine stretch in the amino-terminal region of HOXD13 cause synpolydactyly (Muragaki et al. 1996; Goodman et al. 1997). It is reported that HOXA13 and HOXD13 play a crucial role in early limb development (Fromental-Ramain et al. 1996; Heraultet al. 1999).

Acknowledgments

Electronic-Database Information

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

Cooperative Human Linkage Center, The, http://www.chlc .org/ (for marker maps)

Généthon, http://mapper.wustl.edu/genethon_frame/ (for 1996 marker maps)

Genome Database, The, http://gdbwww.gdb.org/ (for primer sequences; allele sizes of markers D2S1242, D2S279, and D2S1279; and cytogenetic map of markers)

Mouse Genome Informatics, http//:www.Informatics.Jax.Org/ (for expression data of *PAX3*)

National Center for Biotechnology Information OMIM gene map, http://www.ncbi.nlm.nih.gov/Omim/searchmap.html

Online Mendelian Inheritance in Man (OMIM), http://www .ncbi.nlm.nih.gov/Omim/ (for BDA1 [MIM 112500], WS3,

[MIM 148820], and craniofacial-deafness-hand syndrome [MIM 122880])

References

Asher JH Jr, Sommer A, Morrell R, Friedman TB (1996) Missense mutation in the paired domain of PAX3 causes craniofacial-deafness-hand syndrome. Hum Mutat 7:30–35

Bell J (1951) On brachydactyly and symphalangism. In: Penrose LS (ed) Treasury of human inheritance. Vol 5. Cambridge University Press, London, pp 1–31

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

Farabee WC (1903) Hereditary and sexual influence in meristic variation: a study of digital malformations in man. PhD thesis, Harvard University

Fitch N (1979) Classification and identification of inherited brachydactylies. J Med Genet 16:36–44

Fromental-Ramain C, Warot X, Messadecq N, LeMeur M, Dolle P, Chambon P (1996) Hoxa-13 and Hoxd-13 play a crucial role in the patterning of the limb autopod. Development 122:2997–3011

Goodman FR, Mundlos S, Muragaki Y, Donnai D, Giovannucci-Uzielli ML, Lapi E, Majewski F, et al (1997) Synpolydactyly phenotypes correlate with size of expansions in HOXD13 polyalanine tract. Proc Natl Acad Sci USA 94: 7458–7463

Herault Y, Beckers J, Gerard M, Duboule D (1999) Hox gene expression in limbs: colinearity by opposite regulatory controls. Dev Biol 208:157–165

Hoth CF, Milunsky A, Lipsky N, Sheffer R, Clarren SK, Baldwin CT (1993) 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 Hum Genet 52:455–462

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Easy calculation of LOD scores and genetic risks on small computers. Am J Hum Genet 36:460–465

McKusick V (ed) (1975) Mendelian inheritance in man. Johns Hopkins University Press, Baltimore

Muragaki Y, Mundlos S, Upton J, Olsen BR (1996) Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. Science 272:548–551

Piussan C, Lenaerts C, Mathieu M, Boudailliez B (1983) Regular dominance of thumb ankylosis with mental retardation transmitted over 3 generations. J Hum Genet 31:107–114

Polinkovsky A, Robin NH, Thomas JT, Irons M, Lynn A, Goodman FR, Reardon W, et al (1997) Mutations in CDMP1 cause autosomal dominant brachydactyly type C. Nat Genet 17:18–19

Sheffield VC, Weber JL, Buetow KH, Murray JC, Even DA, Wiles K, Gastier JM, et al (1995) A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution human genome-wide linkage maps. Hum Mol Genet 4:1837–1844

Slavotinek A, Donnai D (1998) A boy with severe manifestations of type A1 brachydactyly. Clin Dysmorphol 7:21–27

Tassabehji M, Newton VE, Liu XZ, Brady A, Donnai D, Kra-

jewska-Walasek M, Murday V, et al (1995) The mutational spectrum in Waardenburg syndrome. Hum Mol Genet 4: 2131–2137

Weinstein LS, Gejman PV, Friedman E, Kadowaki T, Collins RM, Gershon ES, Spiegel AM (1990) Mutations of the Gs α-subunit gene in Albright hereditary osteodystrophy detected by denaturing gradient gel electrophoresis. Proc Natl Acad Sci USA 87:8287–8290