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Brachydactyly Type B: Linkage to Chromosome 9q22 and Evidence for Genetic Heterogeneity

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Summary

Brachydactyly type B (BDB), an autosomal dominant disorder, is the most severe of the brachydactylies and is characterized by hypoplasia or absence of the terminal portions of the index to little fingers, usually with absence of the nails. The thumbs may be of normal length but are often flattened and occasionally are bifid. The feet are similarly but less severely affected. We have performed a genomewide linkage analysis of three families with BDB, two English and one Portugese. The two English families show linkage to the same region on chromosome 9 (combined multipoint maximum LOD score 8.69 with marker *D9S257*). The 16-cM disease interval is defined by recombinations with markers *D9S1680* and *D9S1786*. These two families share an identical disease haplotype over 18 markers, inclusive of *D9S278–D9S280*. This provides strong evidence that the English families have the same ancestral mutation, which reduces the disease interval to <12.7 cM between markers *D9S257* and *D9S1851* in chromosome band 9q22. In the Portuguese family, we excluded linkage to this region, a result indicating that BDB is genetically heterogeneous. Reflecting this, there were atypical clinical features in this family, with shortening of the thumbs and absence or hypoplasia of the nails of the thumb and hallux. These results enable a refined classification of BDB and identify a novel locus for digit morphogenesis in 9q22.

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Introduction

Brachydactyly (derived from the Greek meaning "short fingers") occurs in diverse congenital disorders, either as an isolated malformation or with other skeletal manifestations. A search for this term in OMIM produces 138 entries. The classification of the isolated brachydactylies was proposed originally by Bell (1951), who analyzed 124 dominantly inherited pedigrees and placed them into five types, A–E, on the basis of the pattern of digit malformation. A later reappraisal by Fitch (1979) led to substantial reclassification, but the type B form remained a distinct entity.

Brachydactyly type B (BDB; MIM 113000) is the most severe of the inherited brachydactylies and exhibits relatively little overlap with the other groups. The first report of BDB, in the medical literature, was of a family from Uxbridge, west London, in which 10 affected generations were described. A letter from Mr. L. to Dr. Kellie states: "On examining their hands, the thumbs only appeared perfect; instead of fingers, they had only the first phalanx of each finger, and the first and second of the ring-finger of the left hand. The fingers had no nails"(Kellie 1808, p. 252). Detailed clinical and radiological studies were reported by MacArthur and McCullough (1932), in a three-generation Canadian family known to originate from southern England. These authors termed the disorder "apical dystrophy," reasoning that the terminal phalanges are absent and that the middle phalanges may adopt the appearance of the terminal phalanges. Several other facts also point toward this conclusion and are reviewed by Fitch (1979). Overall, BDB is characterized by hypoplasia or complete absence of the terminal phalanges of the index to little fingers, with frequent shortening or absence of the middle phalanges and with absent or hypoplastic nails. The thumbs are less severely affected but can be broad and flattened and sometimes have bifid or duplicated terminal phalanges. The feet are similarly affected, but to a lesser degree. The severity varies among individuals, but the limbs tend to be symmetrically affected. Other features can include soft-tissue syndactyly and sym-

phalangism. A particular facial appearance, including a prominent nose with a bulbous or beaked tip, hypoplastic alae nasae, and a short philtrum was suggested by Houlston and Temple (1994) and Santos (1995), in families of English and Portuguese origin, respectively. BDB generally occurs as an isolated malformation but in Sorsby syndrome is associated with macular coloboma (Thompson and Baraitser 1988).

There have been no reports of molecular genetic studies of BDB, although two loci have been identified in syndromic brachydactyly and two in isolated brachydactyly. Brachydactyly with hypertension has been linked to chromosome 12p (Schuster et al. 1996), and deletions of 2q37 have been identified in brachydactyly with mental retardation (Wilson et al. 1995). Linkage of two loci to brachydactyly type C has been described, at 12q24 (Polymeropoulos et al. 1996) and 20q (Polinkovsky et al. 1997). Cartilage-derived morphogenetic protein 1 (*CDMP1*), a member of the transforming growth-factor β (TGFβ) superfamily, maps to 20q, and mutations of this gene have been identified in unrelated families with brachydactyly type C (Polinkovsky et al. 1997). This, however, remains the only causative gene identified for any of the isolated brachydactylies.

To elucidate the molecular basis of BDB, we performed a genomewide linkage analysis of the English and Portuguese families described by Houlston and Temple (1994) and Santos et al. (1981), respectively, together with a large unpublished English family. We present evidence that both of the English families show linkage to chromosome 9q22 and are in fact related, but the Portuguese family is excluded, indicating that BDB is genetically heterogeneous.

Subjects and Methods

Subjects

Approval for the study was obtained from the Central Oxford Research Ethics Committee, and informed consent was given by participating family members. Partial pedigrees of the three families analyzed in this study are shown in figure 1 (only individuals who gave samples and their intermediate relatives are shown). In family 1, which, until now, has been unpublished, samples were obtained from 13 affected individuals and from 7 unaffected offspring at 50% a priori risk. The affected individuals had the classic manifestations of BDB, including hypoplasia and anonychia of digits 2–5 and normal, broad, or bifid thumbs with normal or split thumb nails. These manifestations were associated with mild facial changes, notably a prominent nose with a bulbous tip. Four individuals had undergone hand surgery. One of these, patient 6, manifested a severe phenotype with a bifid distal phalanx of digit 1 and soft-tissue syndactyly

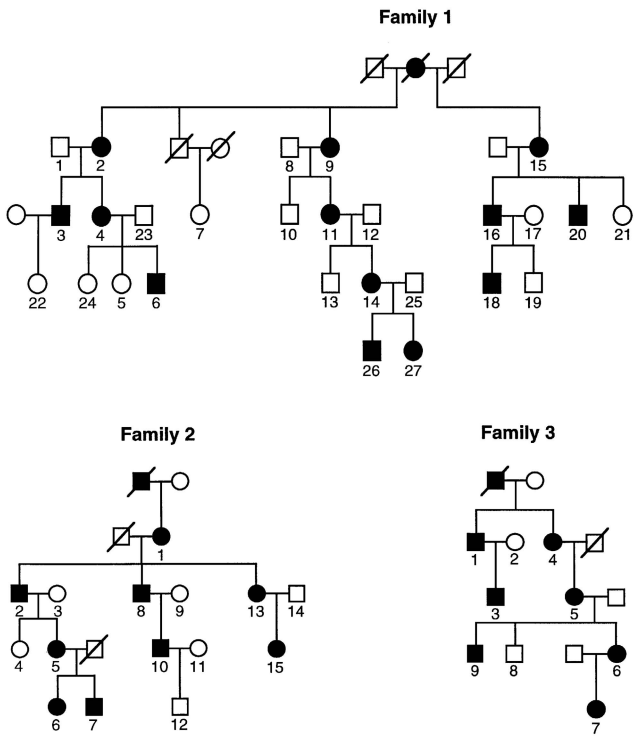

Figure 1 Partial pedigrees of families 1–3, showing individuals from whom samples were collected (identified by numbers). Affected individuals are denoted by blackened symbols.

of digits 3 and 4 (fig. 2). The deceased, affected mother of individuals 2, 9, and 15 originated from Manchester, England. Family 2, described by Houlston and Temple (1994), comprised nine affected individuals and two unaffected offspring and originated from Lincolnshire, England. This family also displayed the classic phenotype of BDB, including a prominent nose with a bulbous tip, wide-spaced eyes, and a short philtrum. High-resolution cytogenetic analysis of one affected person (patient 2) was normal (46,XY). Family 3 resides in Portugal and comprised seven affected individuals and one unaffected individual. This family was described by Santos et al. (1981) and Santos (1995) as affected with BDB with absence of the nails. The affected members had a phenotype suggestive of BDB, but, in some individuals, the fingers were relatively mildly affected with nail hypoplasia yet the thumbs always showed complete absence of the nails and the great toes showed absence or severe hypoplasia of the nails. There was also an association of a characteristic facies with a prominent beaked nose. High-resolution cytogenetic analysis of one affected person (patient 5) was normal (46,XX).

Genotyping

Peripheral blood samples were obtained from all individuals except those numbered 22–27 in family 1,

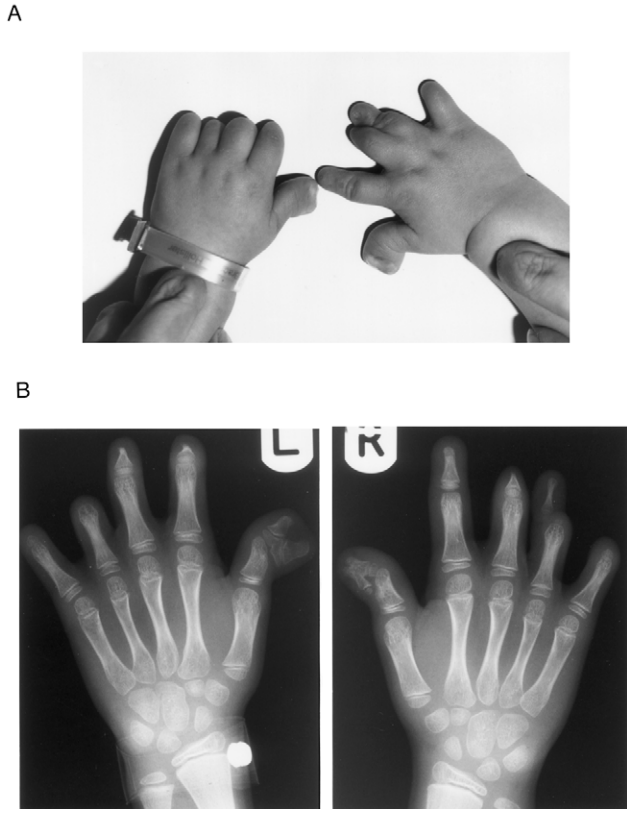

Figure 2 Preoperative appearance of hands of patient 6 from family 1. *A,* Clinical phenotype at age 1.5 years. *B,* Radiological features at age 8.5 years. The middle and terminal phalanges are either completely absent or are replaced by a single rudimentary bone. In the right hand, there is some soft-tissue syndactyly between digits 3 and 4, and the terminal bone of digit 4 is displaced radially. Both thumbs are very broad and have duplicated terminal phalanges.

from whom buccal cells were obtained from mouth swabs. Genomic DNA was prepared from blood samples by standard phenol/chloroform extraction and from buccal samples by the Puregene Buccal DNA Isolation Kit (Gentra Systems). We genotyped subjects, using a standard set of 270 highly polymorphic microsatellite markers at an average interval of 25 cM (Davies et al. 1994; Reed et al. 1994). The markers were amplified in microtiter plates in a volume of 15 μl. Each reaction contained 50 ng DNA, 0.4 units *Taq* Gold polymerase (Perkin-Elmer), 5–15 pmol each of forward and reverse primer (one of which carried a fluorescent label), and a final Mg^{2+} concentration with a range of 1–3 mM. Amplification products were electrophoresed through 6% acrylamide by an Applied Biosystems 377 Automated DNA Sequencer, as described by Reed et al. (1994). Alleles were sized by Applied Biosystems' GENESCAN (2.0.2) and GENOTYPER (1.1). After the initial linkage was found, we performed manual genotyping, using Généthon and Cooperative Human Linkage Center (CHLC) markers (Research Genetics) from the surrounding region (Sheffield et al. 1995; Dib et al. 1996).

Linkage Analysis

Two-point LOD scores between the disease locus and markers were calculated by MLINK of the FASTLINK 4.0 software package (Lathrop and Lalouel 1984; Cottingham et al. 1993; Schaffer et al. 1994). The disease was specified to be an autosomal dominant trait with a disease-allele frequency of .00001. Two liability classes were defined: in the first, which contained affected individuals and normal spouses, the disorder was specified to be fully penetrant, and in the second, which contained phenotypically normal offspring at 50% risk, the disorder was given a penetrance of .99. The allele frequencies for each marker were assumed to be equal, as were the recombination frequencies in males and females.

We performed multipoint analyses, using the LINKMAP program of the FASTLINK package. Marker orders and distances were taken from the Généthon linkage map (Dib et al. 1996). Markers *D9S15, D9S1680, D9S257, D9S1796, D9S1809, D9S1786,* and *D9S118* were used, with intermarker recombination fractions (θ) of .232, .024, .053, .074, .011, and .40, respectively. Because of computer memory constraints, the number of alleles had to be reduced: two-point analyses were performed with the renumbered alleles, to check that the LOD scores between the disease locus and the markers were unchanged. Sequential five-point analyses were performed as described by Terwilliger and Ott (1994). Identification of the disease haplotype, with use of Généthon and additional CHLC markers (*D9S906, D9S318*), was performed manually. Marker order and intermarker distances (in cM) were based on data of Dib et al. (1996) and the Whitehead/MIT STS map (Hudson et al. 1995). We note that conflicting marker orders for the *D9S196–D9S287* region have been published elsewhere (Povey et al. 1997; Xie et al. 1997; Blair et al. 1998).

Results

Two-Point Linkage Analysis

After we conducted the initial genomewide screen, we found that only one marker was strongly suggestive of linkage. *D9S176* gave LOD scores for families 1, 2, and 3 of 2.28, 1.80, and $-\infty$, respectively, at $\theta = 0$ (the combined LOD score for families 1 and 2 was 4.08). Other markers from the surrounding region were analyzed, and a maximum LOD score (Z_{max}) was obtained for marker *D9S257* (family 1, $Z\text{max} = 4.79$ at $\theta = 0$; family 2, $Z\text{max} = 2.40$ at $\theta = 0$). This resulted in a combined $Z\text{max} = 7.19$ for families 1 and 2, but family 3 still showed a LOD score of $-\infty$ at $\theta = 0$ and therefore seemed unlikely to show linkage to the same locus as

families 1 and 2. After this linkage was discovered, four more meioses from family 1 (subjects 22, 24, 26, and 27) were obtained, and the Z_{max} was increased to 5.99, with marker *D9S257,* giving a combined Z_{max}, for families 1 and 2, of 8.39 at $\theta = 0$. Table 1 shows the two-point LOD scores for critical markers across the linked region, for families 1–3 and families 1 and 2.

The disease interval for family 2 was defined by recombinations in affected individuals 2 and 8, with markers *D9S15* and *D9S118,* respectively, as indicated by the LOD scores of $-\infty$ at $\theta = 0$. This spans a region >40 cM and encompasses 33 markers with positive LOD scores at $\theta = 0$ (Zmax = 2.70 at *D9S283*). The results for family 1 reduced this interval significantly. Recombinations in affected individuals 16 and 2, with markers *D9S1680* and *D9S1786,* respectively, reduced the disease interval to 16 cM, including 21 markers with positive LOD scores at $\theta = 0$ (Zmax = 5.99 at *D9S257*). At *D9S1786,* patient 2 inherited, from her deceased mother, the opposite allele compared with patients 9 and 15. The linkage program allowed for the possibility of the mother being homozygous and transmitting the disease on both alleles; hence, the LOD score at $\theta = 0$ was -3.41 rather than $-\infty$. Homozygosity is very unlikely, because there was no history of consanguinity, and because the mother was reported to have had a typical BDB phenotype and gave birth to four unaffected children. A recombination event in patient 2 is a more probable explanation. Family 3 gave negative LOD scores at $\theta = 0$, for all markers analyzed between *D9S15* and *D9S118,* but could not confidently be excluded by use of two-point analysis in this region.

Multipoint Linkage Analysis

Results from the sequential five-point analyses are shown in figure 3 for families 1–3 and families 1 and 2. The Z_{max} for family 1 remained at 5.99, but the multipoint LOD score for family 2, across the interval *D9S257– D9S1786,* was increased, from 2.40 to 2.70. This gave a Z_{max}, for families 1 and 2, of 8.69 at *D9S257.* The phenotype appeared fully penetrant in these two families, since all unaffected offspring at 50% a priori risk inherited the low-risk allele. Between *D9S1680* and *D9S1786,* the interval within which the disorder was mapped in families 1 and 2, the multipoint LOD score for family 3 did not rise above -1.8, indicating that localization of the disorder within this region is highly unlikely, in family 3.

Common Disease Haplotype

It was noted that families 1 and 2 inherited the same size disease-associated allele, for a large number of markers. On further analysis, it was found that they shared a common disease haplotype spanning 18 markers bounded by *D9S257* and *D9S1851,* which were the first markers not to share the same size disease-associated allele (table 2). On the basis of published heterozygosities, the probability of this observation occurring by chance is $<10^{-9}$. This provides strong evidence that families 1 and 2 share a common ancestor and reduces the disease interval to <12.7 cM between these markers. Comparison of marker positions in the Genetic Location Database indicates that the disorder maps within chromosome band 9q22, because multiple markers lying both centromeric and telomeric to the candidate interval have been mapped within this band (Collins et al. 1996).

Table 1

Two-Point LOD Scores between BDB and Chromosome 9 Markers

Marker and Family	LOD Score at θ = .0	.01	.05	.1	.2	.3	.4
D9S15:							
1 and 2	$-\infty$	−3.72	−1.25	−.29	.36	.40	.22
1	$-\infty$	−2.91	−1.08	−.35	.16	.22	.12
2	$-\infty$	−.81	−.17	.06	.20	.18	.10
3	$-\infty$	−2.65	−1.31	−.77	−.31	−.11	−.02
D9S1680:							
1 and 2	$-\infty$	2.05	2.49	2.45	2.05	1.47	.78
1	$-\infty$	.87	1.38	1.44	1.24	.89	.47
2	1.20	1.18	1.11	1.01	.81	.58	.31
3	$-\infty$	−2.00	−.92	−.48	−.15	−.04	−.01
D9S257:							
1 and 2	8.39	8.26	7.72	7.02	5.54	3.80	1.95
1	5.99	5.90	5.52	5.03	3.96	2.76	1.43
2	2.40	2.36	2.20	1.99	1.54	1.04	.52
3	$-\infty$	−1.96	−.88	−.45	−.12	−.03	−.00
D9S1796:							
1 and 2	6.89	6.78	6.34	5.75	4.48	3.08	1.56
1	4.79	4.72	4.42	4.02	3.15	2.18	1.12
2	2.10	2.06	1.92	1.73	1.33	.90	.44
3	$-\infty$	−1.96	−.66	−.18	.15	.21	.14
D9S1809:							
1 and 2	6.33	6.24	5.81	5.25	4.07	2.75	1.35
1	5.13	5.05	4.69	4.23	3.25	2.17	1.03
2	1.20	1.19	1.12	1.02	.82	.58	.32
3	−4.45	−1.16	−.50	−.25	−.06	.00	.00
D9S1786:							
1 and 2	−1.61	3.47	3.77	3.56	2.81	1.90	.97
1	−3.41	1.71	2.17	2.17	1.83	1.32	.71
2	1.80	1.76	1.60	1.39	.98	.58	.26
3	−4.40	−.95	−.30	−.07	.09	.11	.07
D9S118:							
1 and 2	$-\infty$	−7.99	−4.18	−2.52	−1.05	−.39	−.09
1	$-\infty$	−3.95	−2.05	−1.20	−.47	−.16	−.04
2	$-\infty$	−4.04	−2.13	−1.32	−.58	−.23	−.05
3	−5.23	−1.54	−.78	−.46	−.18	−.06	−.01

Discussion

Phenotypic and Genetic Heterogeneity in BDB

We have mapped BDB, in two of three families, to a locus in chromosome 9q22, which we designate

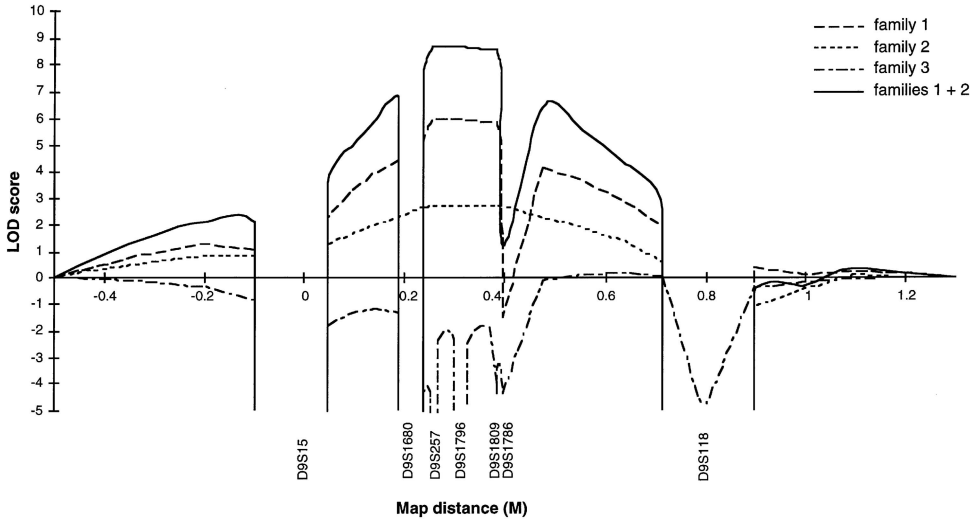

Figure 3 Multipoint LOD scores for families 1–3 and families 1 and 2, between BDB and markers *D9S15, D9S1680, D9S257, D9S1796, D9S1809, D9S1786,* and *D9S118. D9S15* is set at map position 0.

"*BDB1.*" The disease interval initially was defined by recombinations at markers *D9S1680* and *D9S1786,* for family 1. However, family 3 has, within this region, a peak multipoint LOD score of −1.8 and is therefore almost certainly excluded (fig. 3). The phenotype of family 3 resembles that of families 1 and 2, and it was suggested previously that families 2 and 3 share a similar facial appearance (Santos 1995). However, affected members of family 3 consistently exhibit complete absence of nails on the thumbs and absence or severe hypoplasia of the nails on the great toes. The terminal phalanx of the thumb is never bifid but frequently is reduced in size. The thumbs and great toes of affected individuals in families 1 and 2 all possess nails, the only defect being broadening of the terminal phalanges, with occasional phalangeal duplication and/or splitting of the nail. Family 3 therefore defines a new subset of BDB, one that is genetically and phenotypically distinct. However, the relatively small size of the available family precludes an independent disease localization.

A Locus for BDB in 9q22

The observation that families 1 and 2 share a common disease haplotype that spans 18 markers, *D9S278–D9S280* (table 2), provides strong evidence that the two families are related. If this is assumed, then the disease locus lies within the shared haplotype interval bounded by markers *D9S257* and *D9S1851* (<12.7 cM). No known genealogical relationship exists between these families, for at least two generations back from the pedigrees illustrated in figure 1. However, it is of note that founder individuals of these families originated from Manchester and Lincolnshire in England, because families with BDB that have originated from both regions have been described elsewhere. Thus, the six-generation family described by Wells and Platt (1947) came from Manchester, and both the seven-generation family described by MacKinder (1857) and the-five generation family described by Cragg and Drinkwater (1916) were from Lincolnshire (MacArthur and McCullough 1932). The two earliest descriptions of BDB (Kellie 1808; MacKinder 1857) independently recount a very similar legend concerning the origins of the disease (the legend involves the theft of a clergyman's apples by his pregnant wife), suggesting that these two families were also related. Combining the historical record with our finding of a conserved haplotype between two major branches of this family, we speculate that most or all of the English families with BDB share an ancestral mutation. Supporting this is our confirmation that families 1 and 2 share the same disease alleles, for nine consecutive microsatellite markers (*D9S1836–D9S197*), as the two BDB families investigated independently by Gong et al. (1999 [in this issue]) (M. Oldridge and A. O. M. Wilkie, unpublished data). It is unlikely that the mutation is unique, however, because, in three other families, from Germany, the United States, and Mexico, segregating a classic BDB phenotype, the malformation is reported to have arisen de novo (Degenhardt and Geipel 1954; Bass 1968; Cuevas-Sosa and Garcia-Segur 1971). Investigation of individuals from these and other independent families will be important in distinguishing between mutations and polymorphisms within candidate genes in 9q22.

In the evaluation of candidate genes for *BDB1,* the possible pathophysiological mechanisms of the limb ab-

Table 2

Disease Haplotype of Families 1 and 2

Recombination Fraction (cM)	Marker	Disease-Associated Allele Size (bp)	
		Family 1	Family 2
1.6	D9S1812	254	260
2.4	D9S1680	211	211
2.6	D9S257	267	271
0.0	D9S278	269	269
	D9S906[b]	1	1
1.1	D9S283	189	189
0.0	D9S1820	180	180
1.1	D9S1797	240	240
	D9S318[b]	2	2
0.5	D9S1836	205	205
0.4	D9S1796	148	148
2.8	D9S1842	147	147
0.1	D9S1781	146	146
0.0	D9S1841	206	206
1.2	D9S1815	228	228
0.6	D9S1803	164	164
0.1	D9S196	258	258
0.1	D9S1689	90	90
0.6	D9S197	211	211
0.1	D9S1816	151	151
1.4	D9S280	158	158
0.0	D9S1851	147	145
0.0	D9S287	174	170
1.1	D9S1809	137	125
	D9S1786	206	202

[a] Allele sizes of shared disease haplotype are underlined.

[b] Alleles for CHLC markers *D9S906* and *D9S318* are numbered arbitrarily. Recombination fractions are not given for these markers.

normality need to be considered. We entertain three scenarios: (1) premature truncation of limb outgrowth, (2) inappropriate apoptosis of cells within the digital rays, and (3) disruption caused by terminal blebs. Outgrowth of the limb is determined by epithelial-mesenchymal interactions, in which key roles have been demonstrated for the fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling pathways (Niswander and Martin 1993; Hogan 1996). Removal of the apical ectodermal ridge (a morphologically distinct epithelium at the limb tip) causes limb truncations that are restored by placement of FGF beads (reviewed by Martin 1998), and mutation of FGF receptor 3 (*FGFR3*) was demonstrated in craniosynostosis associated with brachydactyly (Glass et al. 1994; Moloney et al. 1997). Alteration of BMP signaling by overexpression of wild-type or dominant-negative BMP receptors can lead to digit truncation (Zou and Niswander 1996), as can implantation, at the tip of the digit, of a bead soaked in noggin protein, a BMP antagonist (Merino et al. 1998). Mutations in *Gdf5* have been described in brachypodism in mice, in which the length and number of bones in the feet are reduced (Storm et al. 1994). *Gdf5* is a member of the TGFβ superfamily and is the mouse orthologue of *CDMP1,* mutated in brachydactyly type C (Polinkovsky et al. 1997).

The brachydactyly phenotype could be caused by mutations in these or related genes involved in the final stages of digit formation, leading either to truncated outgrowth or to normal outgrowth followed by ectopic cell death. In support of the latter theory are observations of apparent amputation scars at the ends of the digits (MacArthur and McCulloch 1932; Battle et al. 1973). Alternatively, as discussed by Fitch (1979), the premature truncation of outgrowth could be caused by limb blebs. Various limb defects are observed in the blebs (my) and other blebbed mice (Carter 1959, Winter 1990), and one could imagine that the displaced terminal bone in digit 4 of the right hand of the patient illustrated in figure 2 might result from this mechanism. However, the usually symmetrical pattern of limb defects in BDB argues against defects arising by a stochastic process.

The disease interval that we have defined for *BDB1* maps to 9q22 and extends over a relatively wide region of 12.7 cM. No other limb anomalies map to this region. It seems unlikely that the disorder arises by a dosage effect, because brachydactyly is not a feature of either monosomy or trisomy of 9q22 (Farrell et al. 1991; Pfeiffer et al. 1993; Kroes et al. 1994; Lindgren et al. 1994). Three genes (*CTSL, FACC,* and *PTCH*) that have been localized to the disease interval and that also have been mapped in the mouse are all located on mouse chromosome 13, suggesting that this represents the major segment of conserved synteny (Stephenson and Lueders 1998); *TMOD* and *XPA* lie just distal of *D9S280* and have been mapped to mouse chromosome 4, which therefore may provide a small contribution (Mock and Hirano 1998). The only mouse-limb mutant gene that maps to either of the predicted homologous regions is *mdac,* a modifier of the dactylaplasia phenotype, on mouse chromosome 13 (Johnson et al. 1995).

Among potential candidate genes mapping to 9q22, the most obvious is *TGFBR1,* the TGFβ receptor type 1 (Pasche et al. 1998). However, radiation hybrid mapping places it between *D9S287* and *D9S277* (Unigene map; Schuler et al. 1996), >1.4 cM telomeric to the *BDB1* disease interval on the Généthon linkage map (Dib et al. 1996), indicating probable exclusion. Unigene has mapped >40 distinct transcripts, including 13 known genes, to the *BDB1* disease interval. One possible candidate is *osteoglycin,* a gene that is of unknown function and that contains leucine-rich repeats. Orthologous bovine, human, and mouse cDNAs were isolated from osteoblasts, osteosarcoma cell lines, and limb buds, respectively (Madisen et al. 1990; Ujita et al. 1995).

If such initial candidates are excluded, it will be necessary to refine the localization of *BDB1* before positional cloning can be attempted. It may be possible to

undertake this quite efficiently, by the ascertainment of other BDB families of English origin and the identification of a more narrow region that shows conservation of the disease haplotype. This should lead eventually to novel insights into normal acral-limb morphogenesis.

Acknowledgments

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Electronic-Database Information

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

Cooperative Human Linkage Center (CHLC) Genetic Mapping, http://www.chlc.org/ (for D9S906 and D9S318)
Généthon Human Genetic Linkage Map, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995 (for Généthon markers)
Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/ (for chromosomal location of markers)
Online Mendelian Inheritance in Man (OMIM), http://www.hgmp.mrc.ac.uk/Omim/ (for BDB [MIM 113000])
Unigene, http://www.ncbi.nlm.nih.gov/Schuler/UniGene/ (for transcript mapping)
Whitehead/MIT STS map, http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map (for radiation hybrid mapping)

References

Bass HN (1968) Familial absence of middle phalanges with nail dysplasia: a new syndrome. Pediatrics 42:318–323
Battle HI, Walker NF, Thompson MW (1973) Mackinder's hereditary brachydactyly: phenotypic, radiological, dermatoglyphic and genetic observations in an Ontario family. Ann Hum Genet 36:415–424
Bell J (1951) On brachydactyly and symphalangism. In: The treasury of human inheritance. Vol 5. Cambridge University Press, Cambridge, pp 1–31
Blair IP, Hulme D, Dawkins JL, Nicholson GA (1998) A YAC-based transcript map of human chromosome 9q22.1-q22.3 encompassing the loci for hereditary sensory neuropathy type I and multiple self-healing squamous epithelioma. Genomics 51:277–281
Carter TC (1959) Embryology of the Little and Bagg X-rayed mouse stock. J Genet 54:311–326
Collins A, Frezal J, Teague J, Morton NE (1996) A metric map of humans: 23,500 loci in 850 bands. Proc Natl Acad Sci USA 93:14771–14775
Cottingham RWJ Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263
Cragg E, Drinkwater H (1916) Hereditary absence of phalanges through five generations. J Genet 6:81–89
Cuevas-Sosa A, García-Segur F (1971) Brachydactyly with absence of middle phalanges and hypoplastic nails. A new hereditary syndrome. J Bone Joint Surg (Br) 53:101–105
Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, et al (1994) A genome-wide search for human type I diabetes susceptibility genes. Nature 371: 130–136
Degenhardt K-H, Geipel G (1954) Dominant erbliche Perodaktylien in 4 Generationen einer Sippe. Zeitschrift für menschliche Vererbungs- und Konstitutionslehre 32: 227–307
Dib C, Fauré 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
Farrell SA, Siegel-Bartelt J, Teshima I (1991) Patients with deletions of 9q22q34 do not define a syndrome: three case reports and a literature review. Clin Genet 40:207–214
Fitch N (1979) Classification and identification of inherited brachydactylies. J Med Genet 16:36–44
Glass IA, Chapman S, Hockley AD (1994) A distinct autosomal dominant craniosynostosis-brachydactyly syndrome. Clin Dysmorphol 3:215–223
Gong Y, Chitayat D, Kerr B, Chen T, Babul-Hirji R, Pal A, Reiss M, et al (1999) Brachydactyly type B: clinical description, genetic mapping to chromosome 9q, and evidence for a shared ancestral mutation. Am J Hum Genet 64:570–577 (in this issue)
Hogan BLM (1996) Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev 10: 1580–1594
Houlston RS, Temple IK (1994) Characteristic facies in type B brachydactyly. Clin Dysmorphol 3:224–227
Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, et al (1995) An STS-based map of the human genome. Science 270:1945–1954
Johnson KR, Lane PW, Ward-Bailey P, Davisson MT (1995) Mapping the mouse dactylaplasia mutation, *Dac*, and a gene that controls its expression, *mdac*. Genomics 29:457–464
Kellie (1808) Letter from Mr L. to Dr Kellie, Leith. Edinb Med Surg J 4:252–253
Kroes HY, Tuerlings JHAM, Hordijk R, Folkers NRP, ten Kate LP (1994) Another patient with an interstitial deletion of chromosome 9: case report and a review of six cases with del(9)(q22q32). J Med Genet 31:156–158
Lathrop GM, Lalouel J-M (1984) Easy calculations of LOD scores and genetic risks on small computers. Am J Hum Genet 36:460–465
Lindgren V, Rosinsky B, Chin J, Berry-Kravis E (1994) Two patients with overlapping de novo duplications of the long arm of chromosome 9, including one case with Di George sequence. Am J Med Genet 49:67–73
MacArthur JW, McCullough E (1932) Apical dystrophy: an inherited defect of hands and feet. Hum Biol 4:179–207
MacKinder D (1857) Deficiency of fingers transmitted through six generations. Br Med J 1:845–846

Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, Dasch J, Thompson A, et al (1990) Molecular cloning of a novel bone-forming compound: osteoinductive factor. DNA Cell Biol 9:303–309

Martin GR (1998) The roles of FGFs in the early development of vertebrate limbs. Genes Dev 12:1571–1586

Merino R, Gañan Y, Macias D, Economides AN, Sampath KT, Hurle JM (1998) Morphogenesis of digits in the avian limb is controlled by FGFs, TGFβs, and noggin through BMP signaling. Dev Biol 200:35–45

Mock BA, Hirano MC (1998) Mouse chromosome 4. Mamm Genome 8:S68–S90

Moloney DM, Wall SA, Ashworth GJ, Oldridge M, Glass IA, Francomano CA, Muenke M, et al (1997) Prevalence of Pro250Arg mutation of fibroblast growth factor receptor 3 in coronal craniosynostosis. Lancet 349:1059–1062

Niswander L, Martin GR (1993) Fgf-4 and Bmp-2 have opposite effects on limb growth. Nature 361:68–71

Pasche B, Luo Y, Rao PH, Nimer SD, Dmitrovsky E, Caron P, Luzzatto L, et al (1998) Type I transforming growth factor beta receptor maps to 9q22 and exhibits a polymorphism and a rare variant within a polyalanine tract. Cancer Res 58:2727–2732

Pfeiffer RA, Lachmann E, Schreyer W, Volleth M (1993) Deletion of 9q22: a new observation suggesting a specific phenotype. Ann Genet 36:167–170

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

Polymeropoulos MH, Ide SE, Magyari T, Francomano CA (1996) Brachydactyly type C gene maps to human chromosome 12q24. Genomics 38:45–50

Povey S, Attwood J, Chadwick B, Frezal J, Haines JL, Knowles M, Kwiatkowski DJ, et al (1997) Report on the Fifth International Workshop on Chromosome 9. Ann Hum Genet 61:183–206

Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SCL, et al (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7:390–395

Santos HG (1995) Characteristic facies in type B brachydactyly. Clin Dysmorphol 4:274–275

Santos HG, George FH, Ferreira JR (1981) Braquidactilia hereditaria com anoniquia. Acta Med Port 3:147–160

Schaffer AA, Gupta SK, Shriram K, RW Cottingham Jr (1994) Avoiding recomputation in linkage analysis. Hum Hered 44: 225–237

Schuler GD, Boguski MS, Stewart EA, Stein LD, Gyapay G, Rice K, White RE, et al (1996) A gene map of the human genome. Science 274:540–546

Schuster H, Wienker TF, Bahring S, Bilginturan N, Toka HR, Neitzel H, Jeschke E, et al (1996) Severe autosomal dominant hypertension in a unique Turkish kindred maps to human chromosome 12. Nat Genet 13:98–100

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

Stephenson DA, Lueders KK (1998) Mouse chromosome 13. Mamm Genome 8:S258–S274

Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee S-L (1994) Limb alterations in brachypodism mice due to mutations in a new member of the TGF-β superfamily. Nature 368:639–643

Terwilliger JD, Ott J (1994) Handbook of human genetic linkage. Johns Hopkins University Press, Baltimore

Thompson EM, Baraitser M (1988) Sorsby syndrome: a report on further generations of the original family. J Med Genet 25:313–321

Ujita M, Shinomura T, Kimata K (1995) Molecular cloning of the mouse osteoglycin-encoding gene. Gene 158:237–240

Wells NH, Platt M (1947) Hereditary phalangeal agenesis showing dominant mendelian characteristics. Arch Dis Child 22:251–260

Wilson LC, Leverton K, Luttikhuis MEMO, Oley CA, Flint J, Wolstenholme J, Duckett DP, et al (1995) Brachydactyly and mental retardation: an Albright hereditary osteodystrophy–like syndrome localized to 2q37. Am J Hum Genet 56: 400–407

Winter RM (1990) Fraser syndrome and mouse "bleb" mutants. Clin Genet 37:494–495

Xie J, Quinn A, Zhang X, Bare J, Rothman A, Collins C, Cutone S, et al (1997) Physical mapping of the 5 Mb D9S196-D9S180 interval harboring the basal cell nevus syndrome gene and localization of six genes in this region. Genes Chromosomes Cancer 18:305–309

Zou H, Niswander L (1996) Requirement for BMP signaling in interdigital apoptosis and scale formation. Science 272: 738–741