The gene for triphalangeal thumb maps to the subtelomeric region of chromosome 7q

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Triphalangeal thumb is a developmental anomaly, sometimes dominantly transmitted, characterized by a long, finger-like thumb with three phalanges instead of two. The underlying genetic defect is unknown, but presumably involves genes that regulate the differentiation of the developing forelimb. In two large kindreds with triphalangeal thumb, evidence for linkage to the long arm of chromosome 7 was obtained with a maximum lod score of 12.61. Multipoint linkage and haplotype analysis placed the gene close to the telomere of the long arm. To our knowledge this is the first time that a human gene involved solely in the pathologic morphogenesis of the hand and feet has been localized.

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Pre-axial polydactyly or congenital deformities of the first digital ray of the hand can occur as an isolated anomaly, in association with other abnormalities of the hand or as a component of complex developmental disorders[1]. Tentamy and McKusick classified isolated, non-syndromic polydactyly on a anatomical basis into five separate entities[2]: postaxial polydactyly, thumb polydactyly (type I), polydactyly of a triphalangeal thumb (type II), polydactyly of the index finger (type III) and polysyndactyly (type IV).

Other morphologically based classifications have been proposed[2,3], but a classification based on the genetic components determining the different phenotypes is as yet impossible.

A triphalangeal thumb (TPT) is a long, finger-like thumb with three phalanges instead of two. Familial occurrence has been described, with an autosomal dominant mode of inheritance. Prevalence has been estimated to be 1 in 25,000 (ref. 4). The clinical presentation of TPT can vary from an opposable thumb with a delta-shaped extra phalanx to an non-opposable index-like digit instead of a thumb. TPT can occur as an isolated congenital defect in association with other anomalities of the hand and/or feet, or as part of a syndrome[1].

The underlying developmental defect for TPT is unknown but must involve disturbance of the formation of the anterior-posterior axis of the developing forelimb. Embryological studies on the development of the forelimb bud in vertebrates indicate that regulation of several homeobox genes or genes regulating programmed cell death are involved in the shaping of the final hand and foot[5–10].

Treatment varies from excision of the extra (delta) phalanx in the simple cases of triphalangeal thumbs to "double osteotomy" which is a pollicitation-type procedure, for the most extensive cases (J. Zguricas *et al.*, manuscript in preparation).

We now report the results of a linkage study in two large pedigrees where TPT segregates as an autosomal dominant disorder with apparently complete penetrance. Our findings indicate that the TPT locus maps near the telomere of chromosome 7q, in the vicinity of a known homeobox gene.

Family studies

To localize the gene for TPT, we ascertained two Dutch Caucasian kindreds with TPT from a relatively isolated population. This population has an estimated prevalence for TPT of 1 in 1000. Within these families the expression of thumb anomalies in different family members is highly variable and ranges from an opposable thumb with a delta phalanx, to an extreme form of pre-axial polydactyly with a triphalangeal index-digit instead of a thumb (Fig. 1), two extra hypoplastic rays radial to the "thumb" (septadactyly), hypoplastic thenar muscles and, occasionally, syndactyly between the fourth and the fifth ray. Following the classification of Tentamy and McKusick[2], the anomalies in all affected individuals could be diagnosed as polydactyly of a triphalangeal thumb (type II) and/or polydactyly of the index finger (type III).

Blood samples from 70 family members including 34 affected persons were obtained with informed consent (Fig. 2). The trait was never transmitted by unaffected family members. Male to male transmission was observed in seven cases ruling out X-linked inheritance. Transmission of TPT in these families was consistent with

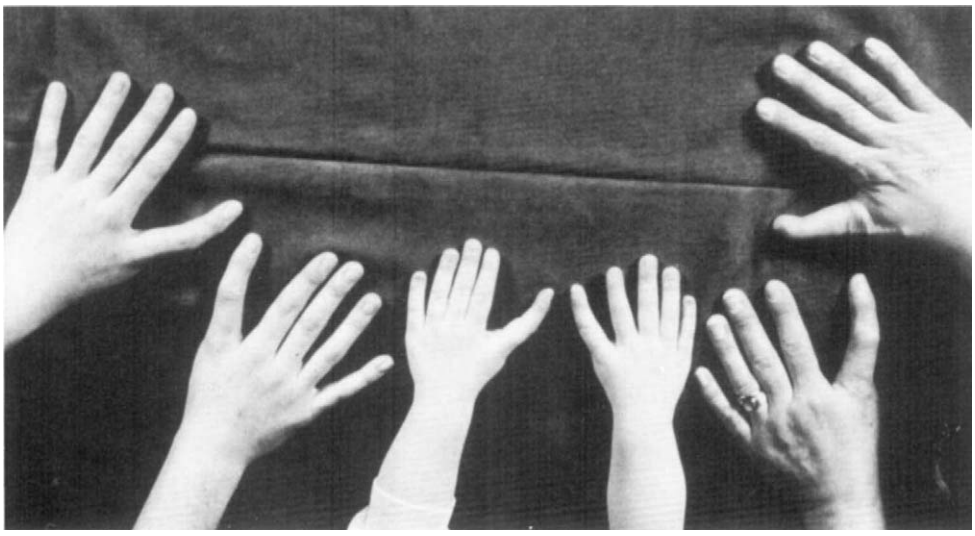


Fig. 1 Cases with triphalangeal index-digit instead of a thumb in a three generation family. Hands of grandmother on the right, mother on the left and child in between.

an autosomal dominant mode of inheritance with apparently complete penetrance. As yet, no common ancestor of the two families has been identified. Detailed information on these families will be given elsewhere.

Linkage studies

Linkage studies were initiated with family 1, which was in itself informative enough to demonstrate linkage. When lod scores greater than one were found in two-point linkage analyses, neighbouring markers were added to the analysis.

Our initial focus was on possible candidate genes. A number of genes have been implicated in the formation of the forelimb. Several homeobox genes are expressed in the developing limb bud. *Hox-4* has been proposed as a gene involved in patterning of the anterior-posterior axis of the developing forelimb. An intragenic marker for *Hox-4* (ref. 11) yielded strong evidence against linkage ($z = -17.63$ at $\theta = 0.0$). Markers located close to seven other homeobox genes were also tested but none of them suggested linkage. Subsequently, we began a systematic genome search with polymorphic microsatellite markers evenly distributed over the human autosomes. In all, 126 microsatellite DNA polymorphisms were analysed on family 1. Evidence of significant linkage was obtained with several markers on the most distal part of chromosome 7q (Table 1). These positive findings were confirmed by testing chromosome 7 markers in a second family with TPT (Fig. 2). The combined lod score for both families reached a value of $z_{max} = 12.61$ at $\theta=0.0$ with marker *D7S559*. Table 1 summarizes the pair wise lod scores, for both families, of chromosome 7q markers and TPT at various recombination distances.

Multipoint linkage and haplotype analysis

In order to determine the most likely position of the TPT gene relative to the chromosome 7q markers, we ordered the markers listed in Table 1 on the basis of information obtained from several sources. The positions of markers *D7S495, D7S498, D7S505, D7S483* and *D7S550* were obtained from the Genethon map[12]. The relative positions of AFM205va3, AFM211xc3 and *D7S559* within the Genethon map were obtained from unpublished genotypings on CEPH reference panels performed by Genethon and J.L. Weber (personal communication).

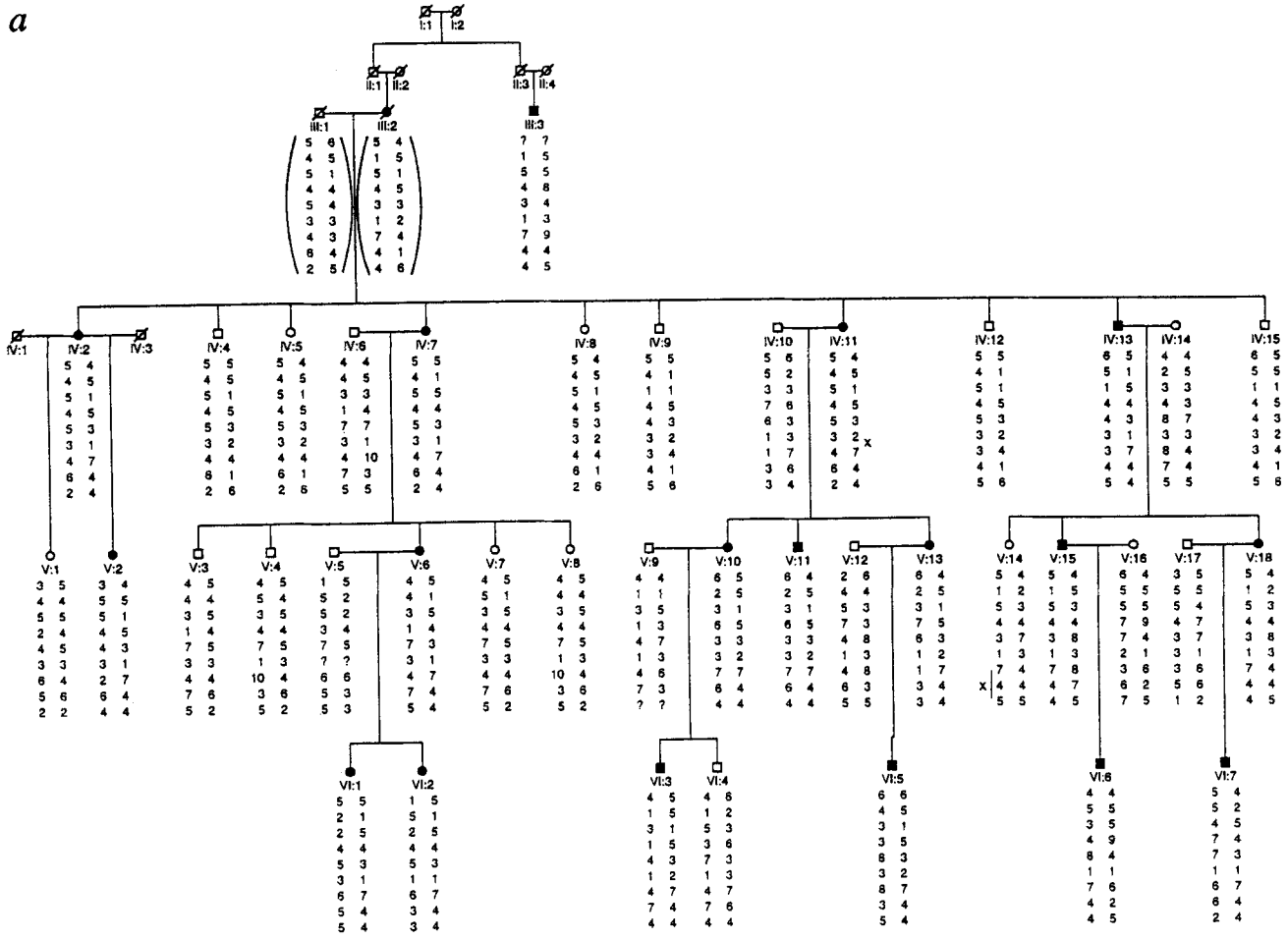
The position of marker *D7S594* in this map has not yet been determined. Its physical localization is about 40 kilobases (kb) from the telomere repeat of chromosome 7q and no other microsatellite markers could be isolated from the remaining sequences towards the telomere[13]. To construct a preliminary map of the 7q region including *D7S594*, *D7S594* was mapped against *D7S550* and *D7S559*. We were able to place *D7S594* between these two markers and 2 centiMorgans (cM) distal from *D7S559*.

Multipoint analysis was performed with *D7S550, D7S559* and *D7S594* (Fig. 3). This yielded a maximum lod score of 15.65 at *D7S550*. In the multipoint analysis, the candidate region for the TPT locus could not be determined. This is possibly caused by the fact that no genotypings are available from a number of individuals in the first generations of the pedigrees. We therefore performed haplotype analysis with the markers listed in Table 1. This analysis revealed several recombination events. One recombination event between marker AFM211xc3 and *D7S550* in family 1 (individual IV:11) and one event in family 2 (individual IV:5) place the TPT locus distal to AFM211xc3. A recombination event in family 2 between *D7S550* and *D7S559* places the TPT locus distal to *D7S550* (individual III:3). The unaffected individual V:14 in family 1 receives the haplotype associated with TPT except for *D7S559* and *D7S594*, also suggesting a localization distal to *D7S550*. A recombination event in individual III:14 of family 2 places TPT proximal to *D7S594*.

Discussion

Triphalangeal thumb as an isolated feature or in association with other anomalies of the hand and/or feet has been reported to segregate in an autosomal dominant fashion[14–16]. This was confirmed in our two families. Strong evidence for linkage of TPT with markers at the subtelomeric region of chromosome 7q was found with a maximum lod score of 12.61 at *D7S559*. This lod score was raised to 15.65 in the multi point analysis. Based on haplotype analysis of chromosome 7q35–qter markers,

Fig. 2▶ Pedigrees of two TPT families. *a*, Family 1. *b*, Family 2. Roman numbers represent the generations. Arabic numbers identify the individuals. The affected individuals are indicated by a filled symbol. Haplotypes are presented for 9 chromosome 7q markers ordered according to their chromosomal localization. pter–*D7S495–D7S498–D7S505–D7S483*–AFM205va3–AFM211xc3–*D7S550–D7S559–D7S594*–qter. Key recombination events are indicated by x. | indicates that the exact position of the recombination event could not be determined.



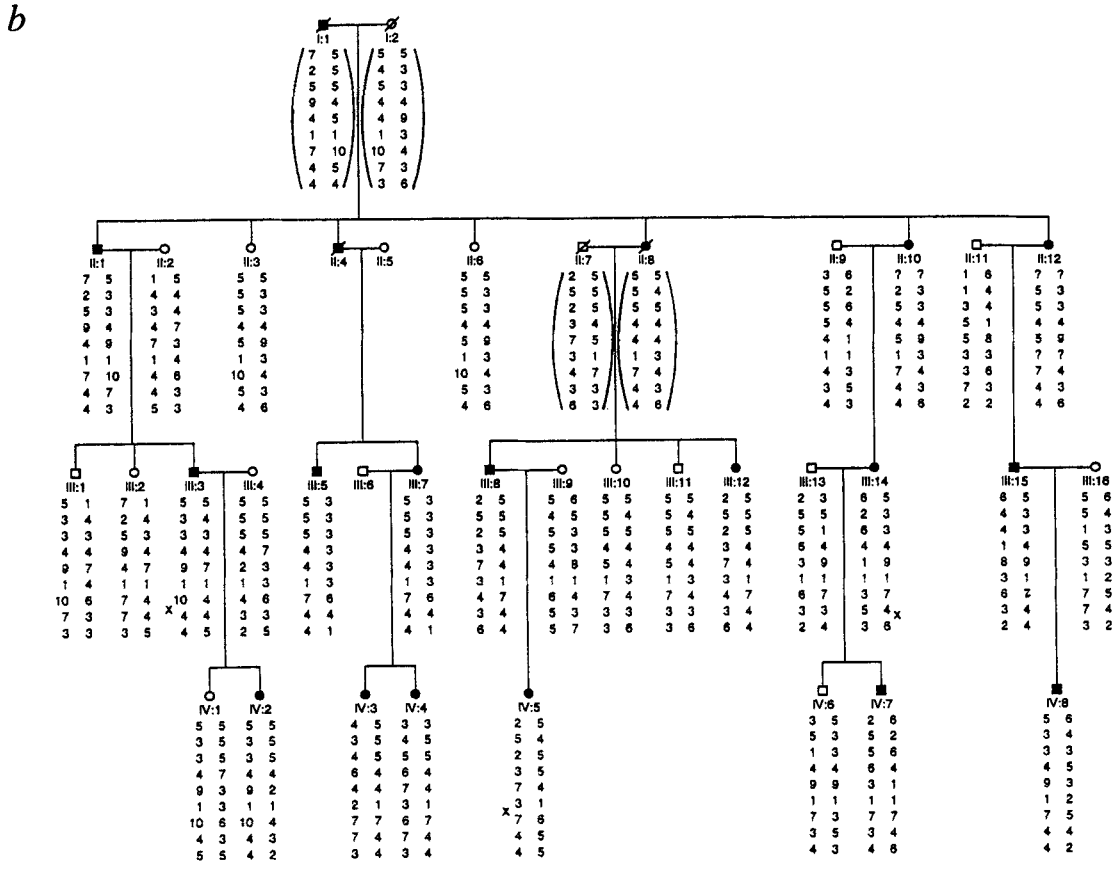


Table 1 Pairwise lod score of chromosome 7q markers at various recombination distances

		Recombination fraction (cM)						
Marker		0.00	0.01	0.05	0.10	0.20	0.30	0.40
D7S495	Family 1	-17.82	-10.11	-5.14	-2.94	-1.03	-0.29	-0.03
	Family 2	-1.80	-1.71	-1.44	-1.19	-0.79	-0.48	-0.22
	Total	-19.62	-11.82	-6.58	-4.12	-1.82	-0.76	-0.25
D7S498	Family 1	-11.65	-4.59	-1.36	-0.01	0.94	1.00	0.58
	Family 2	-2.63	-2.44	-1.87	-1.39	-0.73	-0.32	-0.10
	Total	-14.27	-7.03	-3.23	-1.39	0.21	0.68	0.47
D7S505	Family 1	-7.75	-1.20	0.40	0.97	1.17	0.93	0.51
	Family 2	-1.67	-1.56	-1.21	-0.90	-0.49	-0.24	-0.10
	Total	-9.42	-2.75	-0.80	0.07	0.68	0.70	0.42
D7S483	Family 1	-2.40	1.24	2.28	2.43	2.09	1.42	0.64
	Family 2	-0.36	-0.30	-0.15	-0.06	0.00	0.03	0.05
	Total	-2.75	0.94	2.13	2.38	2.09	1.39	0.69
205va3	Family 1	0.05	2.05	2.63	2.68	2.31	1.66	0.82
	Family 2	-2.96	-2.75	-2.01	-1.36	-0.63	-0.25	-0.08
	Total	-2.91	-0.70	0.62	1.32	1.68	1.49	0.74
211xc3	Family 1	-5.30	2.90	3.46	3.46	2.94	2.05	0.92
	Family 2	0.09	0.10	0.14	0.15	0.14	0.09	0.04
	Total	-5.21	3.01	3.60	3.61	3.07	2.15	0.96
D7S550	Family 1	6.57	6.52	6.23	5.76	4.58	3.17	1.52
	Family 2	-0.46	-0.08	0.59	0.89	0.94	0.67	0.29
	Total	6.10	6.44	6.83	6.65	5.52	3.84	1.81
D7S559	Family 1	7.30	7.18	6.69	6.04	4.67	3.17	1.50
	Family 2	5.31	5.22	4.84	4.35	3.30	2.13	0.86
	Total	12.61	12.40	11.53	10.40	7.97	5.30	2.37
D7S594	Family 1	8.04	7.91	7.37	6.67	5.18	3.53	1.68
	Family 2	2.18	2.70	3.00	2.91	2.38	1.63	0.75
	Total	10.23	10.61	10.37	9.58	7.56	5.17	2.44

Markers are arranged according to their chromosomal localization from 7pter to 7qter.

the TPT locus could be placed between *D7S550* and *D7S594*. The exact size of the candidate region could not be determined since *D7S594* has not been incorporated into the linkage maps that are available from Genethon or the Cooperative Human Linkage Center. Individual V:14 from family 1 shows no clinical signs of TPT, even after X-ray examination of the hands and feet, but has inherited the disease haplotype between marker *D7S495* and *D7S559*. This individual could therefore represent a case of non-penetrance. Penetrance for TPT is usually regarded as complete, therefore there is a high probability that individual V:14 has a recombination between *D7S559* and *D7S594*. For the linkage analysis we used a conservative penetrance value of 0.95. This is why this individual was not regarded as a recombinant in the analysis. In order to reduce the candidate region for the TPT gene, we are ascertaining more families with TPT so that positional cloning of the gene involved can be undertaken.

Other hereditary hand malformations that have been mapped include Greig cephalopolysyndactyly syndrome (GCPS) on chromosome 7p13 (ref. 17), Fanconi anaemia (FA) of which one gene is localized on chromosome 20q (ref. 18), and Holt Oram syndrome (HOS) for which loci on chromosomes 20 and 14q23–24.2 (ref. 19) have been suggested based on *de novo* structural rearrangements, and linkage mapping has suggested a chromosome 12q locus[20]. In all these syndromes preaxial hand malformations may occur, but as part of a complex malformation syndrome (GPCS, HOS) or as part of a multisystem disorder (FA). The gene for GCPS was recently identified as a zinc-finger gene[17]. To our knowledge, the TPT in our two families represents the first localization of a gene for isolated hand malformations including preaxial polydactyly. Identification and characterization of the gene defect and studies of the expression pattern during embryonic development may help clarify questions about the role of this gene in other developmental processes.

The regulation of differentiation of the developing forelimb is a complex process[9,10]. One of the key elements involved in the formation of the separate digits is the apical ectodermal ridge (AER). Numerous transplantation experiments in vertebrates indicate that disturbance of the AER can lead to the formation of an abnormal number of digits. In the AER and in the adjacent mesoderm, homeobox genes as well as genes regulating programmed cell death, growth factors or receptors are expressed. Disruption of any of these genes might potentially interfere with normal differentiation. *Hox-4*, a homeobox gene complex is involved in the regulation of differentiation along the anterior-posterior axis of the developing limb[9,10]. The more 3' genes in this complex are expressed earlier than the 5' genes and also have a more proximal expression boundary. *Hox-4* is therefore a good candidate for regulation of the development of the five digits. Although we have excluded linkage of TPT and the *Hox-4* complex it is possible that the TPT gene product interacts with *Hox-4* and/or other genes by regulating their expression, resulting in a fine tuning of the differentiation process in the developing limb. Interestingly, the human homologue of the engrailed-2 gene (*EN2*) is localized in the candidate region[21,22]. This is a homeobox gene found to be expressed in a band of the early neural plate around the mid/hindbrain junction. Expression continues in this region throughout development[23]. Whether or not this gene is also involved in the development of the limb remains to be investigated. *In vitro* studies show that the *Drosophila En* protein can act as a specific repressor of activated transcription[24]. Disruption of such a gene could lead to overgrowth of the AER or mis-regulation of programmed cell death resulting in an abnormal number of digits in the hand or foot.

En-2 maps very close to the *hemimelic extra-toes (Hx)* gene and the *hammer toe (Hm)* gene on mouse

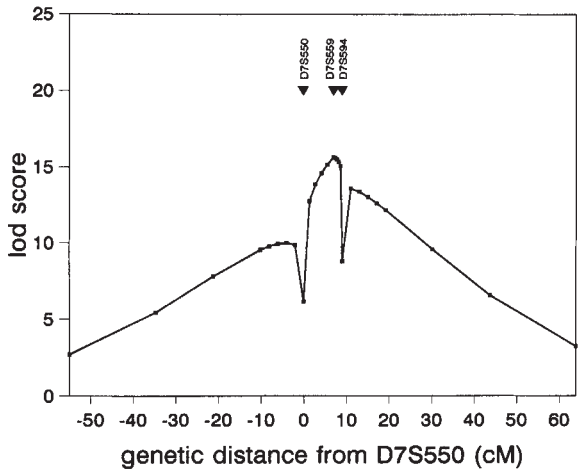


Fig. 3 Multipoint analysis for TPT versus three markers of chromosome 7. Relative position of the markers is indicated by their D numbers. Recombination fractions were converted to centiMorgans using Kosambi's map function.

chromosome 5 (refs 25,26). *Hx* and *Hm* mutations cause skeleton defects of all four limbs. The dominant mutation, *Hx*, causes abnormalities that include preaxial polydactyly and hemimelia[27], whereas the semidominant mutation, *Hm*, causes the failure of the webbing between the toes to undergo normal regression during development, and is characterized by syndactyly between digits 2 to 5, resulting in the strong flexion of the second phalanx of digits on all four feet[28]. These mouse mutants are not allelic but are located very close to each other as only one recombinant has been observed in 3,664 offspring of two crosses[29]. *En-2* and the human homologous of the *Hx* and the *Hm* genes are strong candidate genes in these two families.

Between affected individuals within a single family there are large differences in expression of the phenotype. There are also differences in the phenotypes of some of the other families that have been reported. In our two families, pre-axial polydactyly is a predominant feature, in contrast with one[14] where despite the presence of triphalangeal thumbs, post-axial polydactyly and syndactyly were the most prominent features. The complex nature of limb differentiation makes it likley that this variation is the result of modifying genes.

Cloning the TPT gene and its functional characterization will help us to understand the underlying aetiology of congenital malformations of the hand and the processes that are involved in the development. The localization of the TPT locus also marks the first step in the classification of polydactylies on a genetic basis. An intriguing question is whether other sporadic and familial forms of polydactyly and other hand and foot malformations are variations in the expression of the same gene defect, the result of different mutations in the same gene, or due to defects in genes localized elsewhere in the genome.

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Methodology

Family studies. Blood was obtained from 70 members of two large TPT kindreds. Diagnosis was made by means of physical examination. 34 family members were affected (20 females and 15 males). The defect was bilateral in all affected individuals. Between family members the large variability in expression of the disorder could not be related to the gender of a patient or the sex of the affected parent. Expression varied from ulnar deviation in the interphalangeal joint of the thumb based on an delta-shaped extra phalanx, to triphalangeal index like digits instead of the thumbs, associated with thenar hypoplasia, narrow first web, additional hypoplastic digits radial to the thumb, soft tissue syndactyly between the fourth and the fifth digit and polydactyly of the fifth toe.

DNA studies. Genomic DNA was isolated from peripheral blood as described[30]. 315 microsatellite markers evenly distributed on the human chromosomes were selected. Microsatellite markers were amplified in multiplex reactions and analyzed essentially as described[31]. Additional oligonucleotide primers were labeled during synthesis with Fluorescein Amidite (FluorePrime, Pharmacia). PCR products were resolved according to size by denaturing gel electrophoresis (5,5 % Hydrolink, 40 W) using an A.L.F. automated sequencer (Pharmacia LKB Biotechnology AB). Data were analysed with the Fragment Manager software package version 1.00 (Pharmacia LKB Biotechnology AB).

Linkage analysis. Pairwise lod scores were calculated for each family using the MLINK program of the LINKAGE package (Ver. 5.1)[32] assuming TPT to be an autosomal dominant disease with a gene frequency of 0.001 and a conservative penetrance estimate of 95%. Mutation rate was set at zero and equal recombination rates between males and females were assumed. Marker allele frequencies were kept equal. Calculation of pair-wise lod scores with allele frequencies calculated from individuals marrying in into the TPT kindreds did not substantially alter results (<10%).

Multipoint analysis was performed between the TPT locus and three loci mapping to the subtelomeric region of chromosome 7q (cen-*D7S550-D7S559-D7S594*-qter) using the LINKMAP program with sex-average recombination fractions of 0.070 and 0.020 in the respective intervals. In the analysis, Kosambi's map function was used.

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