

Refined mapping of a gene for split hand-split foot malformation (SHFM3) on chromosome 10q25

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Abstract

Split hand-split foot malformation (SHFM) is a genetically heterogeneous limb developmental defect characterised by the absence of digital rays and syndactyly of the remaining digits. Three disease loci have recently been mapped to chromosomes 7q21 (SHFM1), Xq26 (SHFM2), and 10q25 respectively (SHFM3). We report the mapping of SHFM3 to chromosome 10q25 in two large SHFM families of French ancestry (Z_{\max} for the combined families = 6.62 at $\theta = 0$ for marker AFM249wc5 at locus D10S222). Two recombinant events reduced the critical region to a 9 cM interval (D10S1709-D10S1663) encompassing several candidate genes including a paired box gene PAX2 ($Z_{\max} = 5.35$ at $\theta = 0$). The fibroblast growth factor 8 (FGF 8), the retinol binding protein (RBP4), the zinc finger protein (ZNF32), and the homeobox genes HMX2 and HOX11 are also good candidates by both their position and their function.

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Key words: split hand-split foot; ectrodactyly; SHFM3; chromosome 10q25.

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Split hand-split foot malformation (SHFM) is a rare developmental defect (1/90 000 live births) characterised by the absence of central rays (lobster claw variety) or deficient radial rays with no cleft (monodactyly) and syndactyly of the remaining digits.¹ Autosomal dominant, autosomal recessive,² or X linked recessive inheritance have been reported in the disease.³ On the basis of cytogenetic abnormalities,⁴⁻⁶ the first locus was mapped to chromosome 7q21.3-q22.1 (SHFM1^{7,8}). A second locus has been mapped to chromosome Xq26,⁹ and, recently, a third locus has been mapped to chromosome 10q24-q25 (SHFM3^{10,11}). By studying two large unrelated SHFM families, we support the mapping of SHFM3 to chromosome 10q24-q25 and reduce the genetic interval encompassing the disease gene to a region (9 cM) where several genes, expressed during early development, have recently been localised.

Families

Eighteen affected subjects (eight males, 10 females) and 10 unaffected relatives belonging to two unrelated multigenerational SHFM families were recruited from the Service de

Pédiatrie, Hôpital Cl Huriez, Lille, France (family 1, fig 1) and the Laboratoire d'Embryologie Pathologique, Hôpital Saint-Antoine, Paris, France (family 2, fig 1). Inclusion criteria were: (1) bilateral clefts of hands/feet, (2) absence of one or more digits with syndactyly of the remaining digits, and (3) no other associated osseous or visceral anomalies (fig 2). High resolution chromosomal analyses of the probands were normal (II.4, family 1, III.6, family 2).

Transmission of SHFM was consistent with a fully penetrant autosomal dominant mode of inheritance, with several instances of male to male transmission and no skipping of generations.

Methods

For each person, an EDTA blood sample was collected and DNA was prepared from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. For genotyping with the polymerase chain reaction (PCR) based hypervariable microsatellite technique,^{12,13} genomic DNA (100 ng) was submitted to PCR amplification using *Taq* polymerase (1 unit) in a buffer (25 μ l) containing 50 pmol of each primer, 6.25 nmol of each deoxynucleotide, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8, 1.5 mmol/l MgCl₂, and 0.1% gelatin.

PCR was performed in a thermocycler (Perkin Elmer 480) with a 10 minute initial denaturation and 30 cycles of denaturation (94°C, 40 seconds), annealing (55°C, 40 seconds), and elongation (72°C, 40 seconds), and a final elongation (72°C, 10 minutes). An aliquot of amplified DNA was mixed with the loading buffer. The samples were denatured for 10 minutes at 94°C and loaded on a 6% polyacrylamide denaturing gel. After blotting, nylon membranes (Hybond N⁺, Amersham) were fixed in 0.4 N sodium hydroxide, hybridised with a (CA)₁₂ peroxidase glutaraldehyde labelled probe (Amersham ECL kit) for at least eight hours at 42°C. Blots were washed once in a 0.3 \times SSC, 0.1% SDS solution for 15 minutes, then twice in a 0.2 \times SSC solution for 20 minutes each time and visualised using a detection solution (ECL Amersham kit) before autoradiography.

We used the Linkage package (version 5.1^{14,15}) to calculate lod scores (M-Link program). Two point lod scores were calculated for linkage between the disease locus and each

Table 1 Pairwise linkage data between the SHFM3 locus and 14 polymorphic markers on chromosome 10q

Locus	Families	Recombination fraction							
		0	0.01	0.05	0.10	0.20	0.30	Zmax	θ
D10S583 (AFM289zh5)	1	−∞	−2.13	−0.23	0.41	0.73	0.64	0.75	0.2
	2	1.28	1.26	1.16	1.04	0.77	0.49	1.28	0
	Total	−∞	−0.87	0.93	1.45	1.50	1.13	1.58	0.16
D10S574 (AFM269zd9)	1	−∞	−1.93	−0.67	−0.25	−0.01	0.01	0.01	0.25
	2	0.96	0.95	0.88	0.78	0.55	0.31	0.96	0
	Total	−∞	−0.98	0.21	0.53	0.54	0.32	0.59	0.15
D10S1758 (AFM311ze9)	1	−∞	0.45	1.60	1.89	1.76	1.31	1.88	0.15
	2	1.5	1.48	1.39	1.28	1.02	0.73	1.5	0
	Total	−∞	1.93	2.99	3.17	2.78	2.04	3.17	0.10
D10S1709 (AFMb050zc5)	1	−∞	2.45	2.88	2.83	2.36	1.68	2.88	0.05
	2	−0.72	0.73	0.74	0.73	0.61	0.42	0.74	0.05
	Total	−∞	3.18	3.62	3.56	2.97	2.10	3.62	0.05
D10S198 (AFM126yd6)	1	4.51	4.45	4.16	3.78	2.96	2.04	4.51	0
	2	0.22	0.21	0.19	0.15	0.09	0.04	0.22	0
	Total	4.73	4.66	4.35	3.93	3.05	2.08	4.73	0
D10S192 (AFM094tc9)	1	3.31	3.26	3.04	2.76	2.15	1.46	3.31	0
	2	2.11	2.07	1.93	1.74	1.33	0.88	2.11	0
	Total	5.42	5.33	4.97	4.50	3.48	2.34	5.42	0
PAX2	1	3.91	3.85	3.60	3.27	2.56	1.76	3.91	0
	2	1.43	1.41	1.31	1.27	0.88	0.56	1.43	0
	Total	5.34	5.26	4.91	4.44	3.43	2.31	5.34	0
D10S222 (AFM249wc5)	1	4.51	4.45	4.16	3.78	2.96	2.04	4.51	0
	2	2.1	2.07	1.91	1.70	1.24	0.74	2.1	0
	Total	6.62	6.52	6.07	5.48	4.20	2.78	6.62	0
D10S534 (AFM198yh8)	1	4.51	4.45	4.16	3.78	2.96	2.04	4.51	0
	2	−0.44	−0.41	−0.31	−0.23	−0.11	−0.05	−0.05	0.3
	Total	4.08	4.04	3.84	3.55	2.85	1.99	4.08	0
D10S1663 (AFMa205wc9)	1	3.31	3.26	3.04	2.76	2.15	1.46	3.31	0
	2	−∞	0.12	0.67	0.79	0.69	0.43	0.788	0.10
	Total	−∞	3.38	3.71	3.55	2.84	1.89	3.72	0.05
D10S597 (AFM331xa9)	1	−0.08	−0.06	−0.02	0.003	0.02	0.02	0.02	0.2
	2	−∞	−0.52	0.07	0.23	0.24	0.13	0.24	0.2
	Total	−∞	−0.58	0.05	0.23	0.26	0.14	0.26	0.2
D10S562 (AFM259xg5)	1	−0.09	−0.08	−0.03	0.004	0.03	0.02	0.03	0.2
	2								
D10S221 (AFM249vf1)	1	−∞	−2.37	−1.03	−0.52	−0.12	0.01	0.03	0.4
	2								
D10S190 (AFM065yh11)	1	−∞	−0.81	0.48	0.9	1.07	0.89	1.07	0.2
	2								

Table 2 Summary of key recombinant events involving the SHFM3 locus and polymorphic markers on chromosome 10q

Families	Clinical status	D10S583	D10S574	D10S1758	D10S1709	D10S198	D10S222	D10S1663	D10S562
1	NA	+	+	+	−	−	−	−	−
1	NA	+	−	−	−	−	−	−	−
1	A	+	+	+	−	−	−	−	−
2	A	−	−	−	−	−	−	+	+

NA=not affected. A=affected. +=recombination event. −=no recombination.

microsatellite marker. SHFM was tested assuming that the disease is transmitted as an autosomal dominant trait (gene frequency $f = 1/10\ 000$ with a 70%–100% penetrance).¹⁶

Results

As SHFM1 has been assigned to chromosome 7q21–q22, the D7S527–D7S554 interval (2 cM) encompassing the SHFM1 locus was first tested and excluded in our two families ($Z = -2.9$ at $\theta = 0.02$, and $Z = -5.7$ at $\theta = 0.05$ for loci D7S527 and D7S554 respectively, not shown). We observed linkage between the disease gene and probe AFM126yd6 at the D10S198 locus on chromosome 10q25 ($Z_{max} = 4.73$ at $\theta = 0$ for the combined families 1–2) (table 1). Further pairwise linkage analyses using additional markers mapping to the same region are shown in table 1. Assuming full penetrance the maximum lod score values for the combined families were above 3 for four markers and the highest pairwise lod score value was obtained with probe AFM249wc5 at

the D10S222 locus ($Z_{max} = 6.62$ at $\theta = 0$ for the combined families 1 and 2). No significantly different results were obtained when the penetrance of the trait was allowed to vary between 70% and 100% ($Z_{max} = 5.71$ and $Z_{max} = 6.62$ at $\theta = 0$ at locus D10S222 for a penetrance of 70% and 100%, respectively).

Results of haplotype analyses provided direct evidence of four recombination events in the two families (table 2). All recombinant meioses were informative with D10S1709. One recombinant event was observed between this locus and the disease gene (family 1, III.5) and placed the SHFM3 gene distal to the D10S1709 locus. One recombinant event was observed between the D10S1663 locus and the disease gene (family 2, III.2) placing the SHFM3 gene proximal to D10S1663. With respect to the marker loci, all haplotypes could be resolved with a maximum of one recombination event (fig 1, table 1). These recombination events allowed us to assign the SHFM3 locus to the 9 cM interval defined by loci D10S1709 and D10S1663 and encompassing loci D10S198, D10S192,

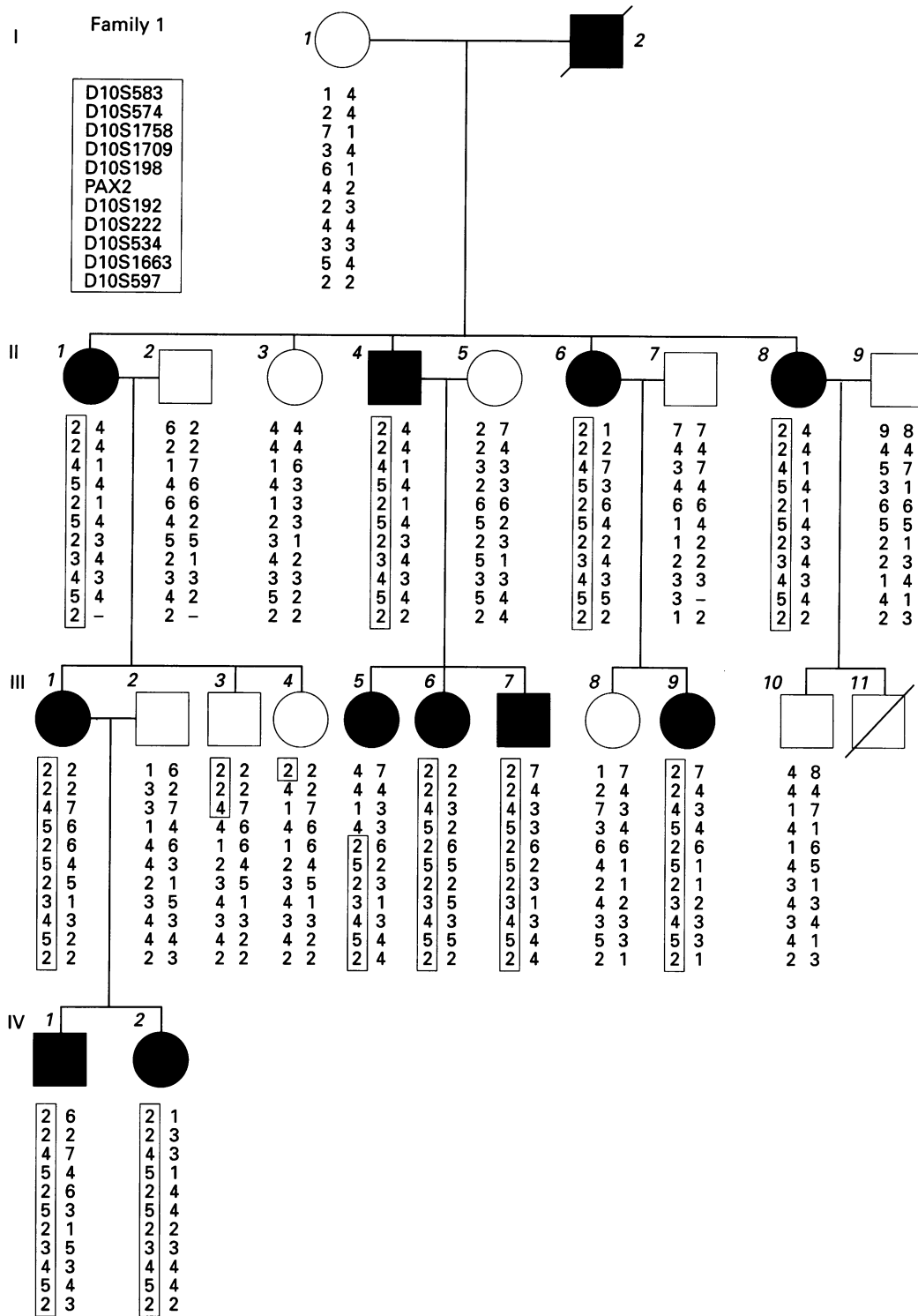


Figure 1 Pedigrees and haplotypes at the SHFM3 locus. Boxes outline common haplotypes. The Pax2 gene is located between D10S198 and D10S192 according to Gurrieri et al.¹¹

D10S222, and D10S534: pter-D10S583-(2)-D10S574-(1)-D10S1758-(2)-D10S1709-(3)-D10S198-(2)-D10S192-(1)-D10S222-(2)-D10S534-(1)-D10S1663-(2)-D10S597-(6)-D10S562-(2)-D10S221-(2)-D10S190-cen (with genetic distance estimate in cM shown in parentheses).¹³

Interestingly, PAX2, a human paired box protein gene mapped to chromosome 10q25 by both in situ hybridisation¹⁷ and linkage analysis in our families showed no recombination with several polymorphic markers mapping to

the same interval ($Z_{max}=6.45$, $Z_{max}=7.58$, $Z_{max}=7.88$, and $Z_{max}=5.71$ at $\theta=0$ at loci D10S198, D10S192, D10S222, and D10S534 respectively; data not shown, available on request). A yeast artificial chromosome (YAC) positive for D10S198 and D10S192 was also consistently positive for PAX2.¹⁸ These features, along with the absence of recombinant events between PAX2 and the disease locus ($Z_{max}=5.35$ at $\theta=0$, table 1), suggest that PAX2 should be considered as a candidate gene for SHFM3.

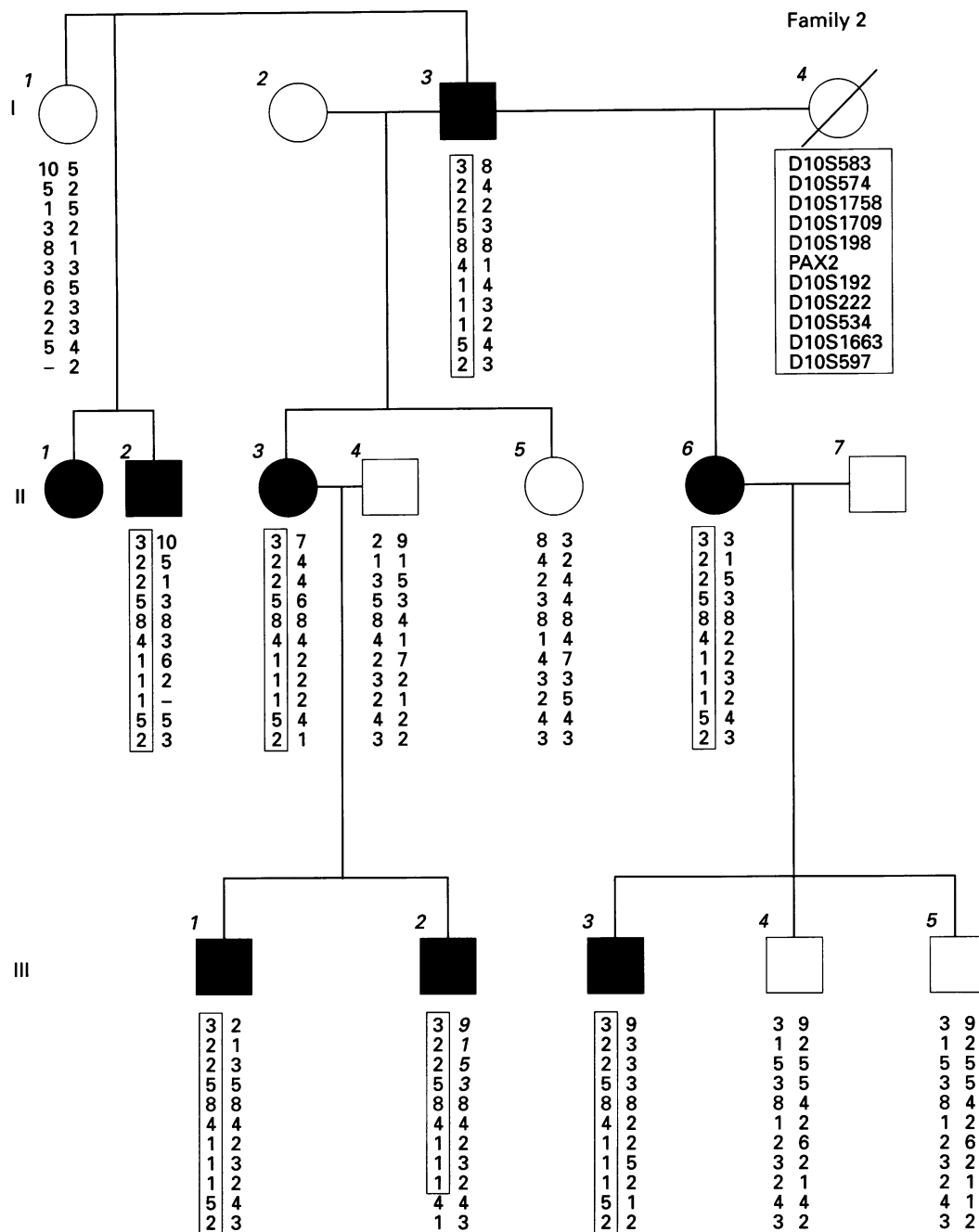


Figure 1—contd

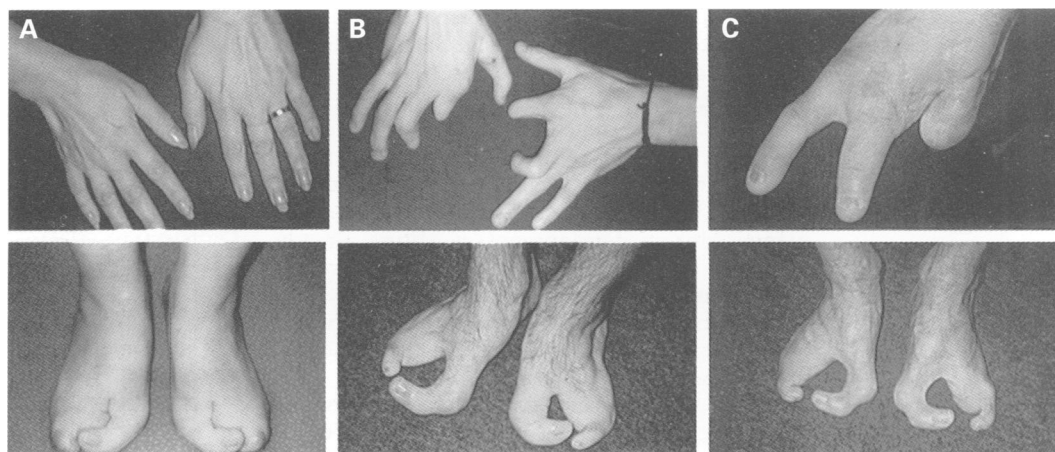


Figure 2 Intrafamilial variability of the SHFM3 phenotype in family 2. (A) Hands and feet of II.3. (B) Hands and feet of III.2. (C) Hand and feet of III.1.

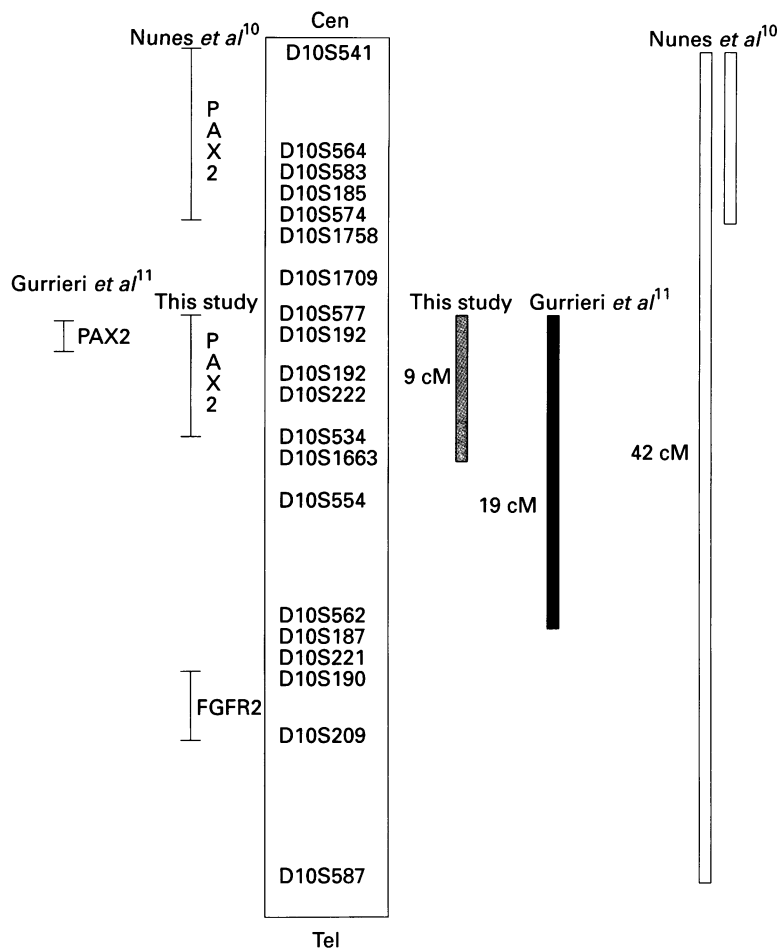


Figure 3 Delineation of the SHFM3 critical region on chromosome 10q.

Discussion

The present study supports the mapping of a gene for split hand-split foot deformity (SHFM3) to chromosome 10q24-25 in two large unrelated families of French origin. Taking advantage of two key recombinants, we were able to map the SHFM3 gene to the D10S1709-D10S1663 interval and therefore to reduce the interval encompassing the disease gene from 19 cM¹¹ to only 9 cM. This interval is included both in the D10S541-D10S587 maximal interval (42 cM) and the D10S198-D10S187 interval (19 cM) reported by Nunes *et al.*¹⁰ and Gurrieri *et al.*¹¹ respectively as the most likely genetic intervals in SHFM3 (fig 3).

This study does not rule out, however, locus heterogeneity on chromosome 10q25, a possibility which might account for the inter-familial variability of the malformation (fig 2). Alternatively, the variable expression of SHFM3 might involve unknown modifying genes during development, a feature already reported in the dactylaplasia mouse where the dominant mutation *Dac* has been mapped to a region syntenic to chromosome 10q25 and whose expression is modified by another locus, called *mdac*.^{19,20}

Interestingly, several candidate genes involved in limb development map to chromosome 10q24-25, namely the fibroblast growth factor receptor 2 (FGFR2²¹), the fibroblast growth factor 8 (FGF8²²), the paired

box PAX2, the homeoboxes HOX11²³ and HMX2,²⁴ the retinol binding protein RBP4,²⁵ and the zinc finger protein genes (ZNF32²⁶). FGFR2 which accounts for several acrocephalosyndactyly syndromes²¹ could be excluded as it maps outside the genetic interval reported here (fig 3). FGF8 is an interesting candidate gene in both its position and its function, as it is expressed in the apical ectodermal ridge during the early formation of the limb bud. Hitherto, however, no mutation in the coding sequence of the gene has been found in our probands (not shown).

PAX2 also should be regarded as a candidate gene by both position and function. Indeed, this transcription factor is active during early embryogenesis and no recombination with SHFM3 has been found in our two families nor in the previous reports.^{10,11} Hitherto, however, PAX2 mutations have been associated with retinal and kidney defects, but no limb malformations have been observed in patients harbouring these particular PAX2 mutations.²⁷ Similarly, HOX11, a homeobox gene (mapping 500 kb away from PAX2) involved in segmentation and branching of limb elements, and HMX2 (also mapped to 10q25) should be regarded as candidate genes for SHFM3. The characterisation of the SHFM3 genes will hopefully shed light on the molecular mechanisms that govern hand and foot development in the early stages of embryogenesis.

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