

## Fine Mapping of the Split-Hand/Split-Foot Locus (*SHFM3*) at 10q24: Evidence for Anticipation and Segregation Distortion

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### Summary

Split-hand/split-foot malformation (SHFM, ectrodactyly, or lobster-claw deformity) is a human limb malformation characterized by aberrant development of central digital rays with absence of fingers and toes, a deep median cleft, and fusion of remaining digits. SHFM is clinically heterogeneous, presenting both in an isolated form and in combination with additional abnormalities affecting the tibia and/or other organ systems, including the genitourinary, craniofacial, and ectodermal structures. Three SHFM disease loci have been genetically mapped to chromosomes 7q21 (*SHFM1*), Xq26 (*SHFM2*), and 10q24 (*SHFM3*). We mapped data from a large Turkish family with isolated SHFM to chromosome 10q24 and have narrowed the *SHFM3* region from 9 cM to an ~2-cM critical interval between genetic markers D10S1147 and D10S1240. In several instances we found evidence for a more severe phenotype in offspring of a mildly affected parent, suggesting anticipation. Finally, data from this family, combined with those from six other pedigrees, mapped to 10q24, demonstrate biased transmission of *SHFM3* alleles from affected fathers to offspring. The degree of this segregation distortion is obvious in male offspring and is possibly of the same magnitude for female offspring.

### Introduction

Split-hand/split-foot malformation (SHFM, ectrodactyly, or lobster-claw deformity) is a human limb mal-

formation characterized by aberrant development of central digital rays with absence of fingers and toes, a deep median cleft, and fusion of the remaining digits. SHFM is clinically heterogeneous, presenting both in an isolated form and in combination with additional abnormalities affecting the tibia and/or other organ systems, including the genitourinary, craniofacial, and ectodermal structures (syndromic ectrodactyly). Three SHFM disease loci have been genetically mapped to chromosomes 7q21 (*SHFM1*, MIM 183600) (Scherer et al. 1994a, 1994b; Crackower et al. 1996), Xq26 (*SHFM2*, MIM 313350) (Faiyaz ul Haque et al. 1993) and 10q24 (*SHFM3*, MIM 600095) (Nunes et al. 1995; Gurrieri et al. 1996). One other autosomal dominant-appearing SHFM family fails to show linkage to 10q, 7q, 6q, 2q, or X (Gurrieri et al. 1996).

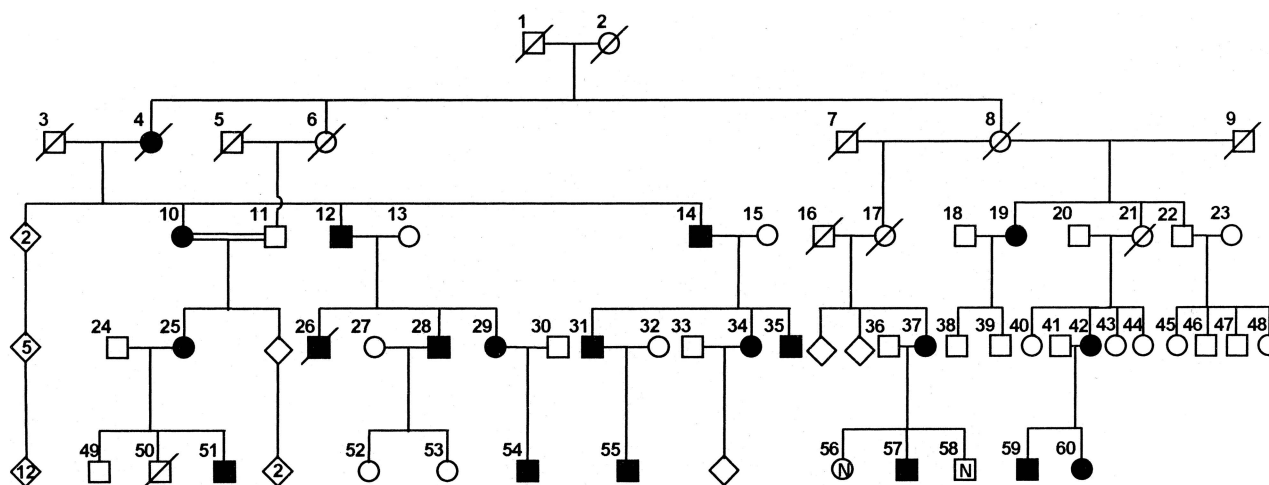
Whereas the *SHFM1* locus on chromosome 7 is characterized by isolated forms with cytogenetically visible deletions and chromosomal translocations, the *SHFM3* locus is typically associated with a normal karyotype. Nunes et al. (1995) first mapped *SHFM3* to a 26-cM region on chromosome 10q24 in one large family. Raas-Rothschild et al. (1996) further reduced the *SHFM3* critical interval from the 19-cM interval defined by Gurrieri et al. (1996) to a 9-cM interval between loci D10S1709 and D10S1663.

Idiosyncratic patterns of transmission have been reported for many SHFM pedigrees. Nonsyndromic ectrodactyly (and without other limb defects) was characterized by Zlotogora (1994) as having dominant transmission with almost complete penetrance. Other investigators, however, have reported variable expressivity between and within kindreds. Spranger and Schapera (1988) described a South African family in which three unaffected sibs with normal parents had each produced affected offspring. An unaffected offspring of one of the unaffected sibs produced affected children. Once established in the family, the disorder was transmitted in two different branches through three generations as a regular dominant mutation. Premutation of an autosomal dominant gene or cosegregation of an epistatic gene linked to the gene for SHFM was postulated. Zlo-

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**Figure 1** Turkish SHFM pedigree. Blackened symbols represent clinically affected individuals, and unblackened symbols represent unaffected individuals. Numbers within symbols represent the number of unaffected offspring and their unaffected descendants, collapsed for clarity and space limitations.

togora (1994) also suggested the possibility that SHFM is a premutation disorder caused by trinucleotide-repeat instability.

In addition, segregation distortion with excessive transmission from affected fathers to affected sons has been described in some SHFM pedigrees. A disturbed segregation ratio was found in the English SHFM family first reported by McMullen and Pearson (1913), and this information was brought up to date by Stevenson and Jennings (1960). Jarvik et al. (1994) studied new SHFM pedigrees with defined ascertainment and analyzed them in conjunction with previously published pedigrees, which included most of those summarized in 1960 by Stevenson and Jennings. They concluded that the new pedigrees confirmed Pearson’s initial observation of non-Mendelian transmission characterized by overtransmission of SHFM from affected fathers to sons. The study, however, drew no distinction between SHFM1, SHFM2, and SHFM3 (Jarvik et al. 1994).

We mapped data from a large Turkish family with isolated SHFM to chromosomal region 10q24 and have narrowed the *SHFM3* region from a previously reported 9-cM interval to an ~2-cM critical interval. In several instances, we found evidence for a more severe phenotype in the offspring of a mildly affected parent, raising the possibility of genetic anticipation. Finally, by combining the findings from the family in the present study with those from six other pedigrees mapped to 10q24, we observed biased transmission of *SHFM3* alleles from

affected fathers to offspring, obvious for male offspring and possibly of the same magnitude for female offspring.

## Subjects and Methods

### *SHFM Family*

We ascertained an extended Turkish pedigree with 18 affected individuals (16 living) in four generations (fig. 1). Subjects were classified as affected after careful physical examination of both upper and lower extremities. X rays were obtained from severely affected, mildly affected, and key unaffected individuals, when possible. Clinical characteristics of affected individuals within this family are presented in figure 2.

### *Marker Genotyping and Genetic Linkage Analysis*

DNA extraction, genotyping, and two-point linkage analysis were performed as described elsewhere (Baysal et al. 1997). In brief, we genotyped a total of 55 samples from the Turkish SHFM family (including 14 affecteds), using published chromosome 10 polymorphic STRP markers, according to standard PCR and PAGE techniques. A subset of 12 individuals were further genotyped to fully resolve the location of key genetic recombinants.

SHFM3 ID No	Left hand	Right hand	Left foot	Right foot
10 (Mother)	-TPT with posture deformity -Split tip of second finger	-TPT with posture deformity	-Cutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> , and 3 <sup>rd</sup> and 4 <sup>th</sup> toes -Fusion of distal and intermediate bones of 2 <sup>nd</sup> toe -Clinodactyly of 5 <sup>th</sup> toe	-Cutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> toes -Fusion of distal and intermediate bones of 2 <sup>nd</sup> toe -Duplication of proximal phalanx of 3 <sup>rd</sup> toe -Clinodactyly of 5 <sup>th</sup> toe
25 (Mother)	-TPT with posture deformity	-TPT with posture deformity	-Hallux Valgus -Clinodactyly of 5 <sup>th</sup> toe	-Hallux Valgus -Clinodactyly of 5 <sup>th</sup> toe
51	-TPT with posture deformity -Cutaneous syndactyly of 3 <sup>rd</sup> and 4 <sup>th</sup> fingers with flexion contracture -Fused duplication of intermediate phalanx of 2 <sup>nd</sup> finger -an extra bone at prox. Interphalangeal joint of 2 <sup>nd</sup> finger -Clinodactyly of 5 <sup>th</sup> finger	-TPT with posture deformity -Flexion contracture of 3 <sup>rd</sup> and 4 <sup>th</sup> fingers -Radial deviation of 2 <sup>nd</sup> finger -Clinodactyly of 5 <sup>th</sup> finger	-Cutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> toes -Absent 3 <sup>rd</sup> toe -Hypoplastic 3 <sup>rd</sup> metatarsal bone -Duplication of 2 <sup>nd</sup> phalanx of 2 <sup>nd</sup> toe -Loss of distal phalanx of 2 <sup>nd</sup> toe -Clinodactyly of 5 <sup>th</sup> toe	-Cutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> toe -Absent 3 <sup>rd</sup> toe -Duplication of 2 <sup>nd</sup> phalanx of 2 <sup>nd</sup> toe -Loss of distal phalanx of 2 <sup>nd</sup> toe -Clinodactyly of 5 <sup>th</sup> toe
12 (Father)	-TPT with posture deformity -Ungual agenesis of 2 <sup>nd</sup> finger	-TPT -Split tip of 2 <sup>nd</sup> finger -Ungual agenesis of 2 <sup>nd</sup> finger	-Loss of distal phalanx of 2 <sup>nd</sup> toe -Clinodactyly of 5 <sup>th</sup> toe	-Cutaneous syndactyly of 1 <sup>st</sup> , 2 <sup>nd</sup> and 3 <sup>rd</sup> toes -Hypoplastic intermediate phalanx of 2 <sup>nd</sup> toe -Duplication of proximal phalanx of 3 <sup>rd</sup> toe -Clinodactyly of 5 <sup>th</sup> toe
28	-Split tip of 2 <sup>nd</sup> finger -Ungual agenesis of 2 <sup>nd</sup> finger -Malformed 5 <sup>th</sup> metacarpal bone	-TPT -Split tip of 2 <sup>nd</sup> finger -Ungual agenesis of 2 <sup>nd</sup> finger -Malformed 5 <sup>th</sup> metacarpal bone	-Loss of distal phalanx of 5 <sup>th</sup> toe	-Loss of intermediate and distal phalanxes of 3 <sup>rd</sup> toe -Loss of distal phalanxes of 2 <sup>nd</sup> , 4 <sup>th</sup> and 5 <sup>th</sup> toes
29 (Mother)	-TPT with posture deformity -Split tip of 2 <sup>nd</sup> finger	-TPT with posture deformity -Ulnar deviation of distal phalanx of 2 <sup>nd</sup> toe	-Cutaneous syndactyly of 3 <sup>rd</sup> and 5 <sup>th</sup> toes -Complete loss of 2 <sup>nd</sup> toe -Loss of intermediate and distal phalanxes of 3 <sup>rd</sup> toe -Loss of distal phalanx of 4 <sup>th</sup> toe	-Cutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> toes -Duplication of proximal phalanx of 3 <sup>rd</sup> toe -Loss of intermediate and distal phalanxes of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes -Flexion deformity of 4 <sup>th</sup> and 5 <sup>th</sup> toes
54	-TPT with posture deformity -Split tip of 2 <sup>nd</sup> finger -Radial deviation of distal phalanx of 2 <sup>nd</sup> finger	-TPT with posture deformity -Complete loss of 3 <sup>rd</sup> finger and metacarpal bone -Flexion deformity of intermediate phalanx of 2 <sup>nd</sup> finger	-Cutaneous syndactyly of 4 <sup>th</sup> and 5 <sup>th</sup> toes -Complete loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes and 3 <sup>rd</sup> metacarpal bone -Mixed position of phalangeal bones of 4 <sup>th</sup> and 5 <sup>th</sup> toes	-Cutaneous syndactyly of 4 <sup>th</sup> and 5 <sup>th</sup> toes -Complete loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes and 3 <sup>rd</sup> metacarpal bone -Loss of distal phalanxes of 4 <sup>th</sup> and 5 <sup>th</sup> toes
37 (Mother)	-Malpositioned 3 <sup>rd</sup> -Malformed proximal phalanx of 4 <sup>th</sup> finger -Loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> fingers -Two long bones belonging to thumb	-Loss of one metacarpal -Hypoplastic thumb -Loss of two fingers	-Cutaneous syndactyly of 4 <sup>th</sup> and 5 <sup>th</sup> toes including 3 <sup>rd</sup> metacarpal bone -Complete loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes -Malformed distal phalanx of 4 <sup>th</sup> toe	-Osseocutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> toes -Cutaneous syndactyly of 4 <sup>th</sup> and 5 <sup>th</sup> toes -Complete loss of 3 <sup>rd</sup> toe -Flexion deformity of 4 <sup>th</sup> and 5 <sup>th</sup> toes
57	-TPT with posture deformity -Cutaneous syndactyly of 3 <sup>rd</sup> and 4 <sup>th</sup> fingers -Flexion deformity of 2 <sup>nd</sup> finger -End-to-end fusion of intermediate phalanx of 4 <sup>th</sup> finger and distal phalanx of 3 <sup>rd</sup> finger	-TPT with posture deformity -Cutaneous syndactyly of 3 <sup>rd</sup> and 4 <sup>th</sup> fingers	-Complete loss of 2 <sup>nd</sup> , 3 <sup>rd</sup> and 4 <sup>th</sup> toes -Clinodactyly of 5 <sup>th</sup> toe	-Complete loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes -Loss of intermediate and distal phalanxes of 4 <sup>th</sup> toe -Cutaneous syndactyly of proximal phalanx of 4 <sup>th</sup> and 5 <sup>th</sup> finger Clinodactyly of 5 <sup>th</sup> toe
42 (Mother)	-Median ungual aplasia of 2 <sup>nd</sup> finger (no x-ray)	-Median ungual aplasia of 2 <sup>nd</sup> finger (no x-ray)	(no x-ray)	(no x-ray)
59	(no x-ray)	-Median ungual aplasia of 2 <sup>nd</sup> finger (no x-ray)	(no x-ray)	(no x-ray)
60	Median ungual aplasia of 2 <sup>nd</sup> finger (no x-ray)	-Median ungual aplasia of 1 <sup>st</sup> and 2 <sup>nd</sup> fingers -Cutaneous syndactyly of 3 <sup>rd</sup> and 4 <sup>th</sup> fingers (no x-ray)	(no x-ray)	-Complete loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes (no x-ray)
14	-Normal by physical exam (no x-ray)	-Normal by physical exam (no x-ray)	-Normal by physical exam (no x-ray)	-Cutaneous syndactyly of 1 <sup>st</sup> and 2 <sup>nd</sup> toes (no x-ray)
35	-Normal by physical exam (no x-ray)	-Normal by physical exam (no x-ray)	-Normal by physical exam (no x-ray)	-Loss of 2 <sup>nd</sup> and 3 <sup>rd</sup> toes (no x-ray)
31	-Severely affected by family history (no x-ray)	-Severely affected by family history (no x-ray)	-Split foot by family history (no x-ray)	-Split foot by family history (no x-ray)
55	-Severely affected by family history (no x-ray)	-Severely affected by family history (no x-ray)	-Split foot by family history (no x-ray)	-Split foot by family history (no x-ray)

**Figure 2** Summary of clinical findings in affected relatives with SHFM

### Mutation Analysis of FGF8 and HOX11

Two genes that map within the 10q24 critical region, fibroblast growth factor 8 (*FGF8*) and *HOX11*, were treated as candidate genes for SHFM3. All three exons of *FGF8* from two affected and two unaffected individuals were amplified by use of PCR primers designed from surrounding intronic regions (Gemel et al. 1996). Direct sequencing of PCR products was performed with dye-

terminator technology on an ABI 373 sequencing machine.

To amplify DNA in the GC-rich region of the *HOX11* gene containing the CGG<sub>n</sub> repeat, we modified standard PCR conditions. PCR amplifications were performed in a Techne PHC3 thermal cycler with 100 ng of total genomic DNA in a 25- $\mu$ l PCR reaction volume and with PCR primers 5'-cctccaggacggagaatagc-3' and 5'-aggccagtggagttgtgacg-3'. The 1  $\times$  reaction mixture concen-

trations were as follows: 50 mM KCl; 1.5 mM MgCl<sub>2</sub>; 1 mM PCR primers; 0.20 mM each dATP, dCTP, dTTP, and de-aza dGTP; 5% dimethyl sulfoxide; 5% glycerol, and 1.0 U AmpliTaq DNA Polymerase. The mixture was initially denatured for 10 min at 94°C; cycled for 35 cycles of 94°C × 30 s, 55°C × 60 s, and 72°C × 180 s; with a final extension of 72°C for 10 min. Formal exclusion of all potential coding region mutations of *HOX11* was not performed.

## Results

### *Phenotype, Karyotype, and Possible Anticipation in SHFM3*

Careful clinical examination of the extended pedigree was undertaken in hopes of a better understanding of unresolved issues concerning penetrance and variable expressivity (fig. 2). The karyotypes of several affected individuals were normal and did not show gross chromosomal rearrangements, translocations, or obvious deletions (data not shown). Several individuals initially reported to be normal carriers were in fact found to have mild ungual dysplasia of the index finger and/or split distal phalangeal bone of the index finger and/or in-line duplication of a small phalangeal bone of the thumb (triphangeal thumb) that was readily apparent on subsequent x ray (fig. 2). Although genitourinary abnormalities have been reported in conjunction with various limb-deformity syndromes (MIM 176305, MIM 140000, MIM 186000, MIM 600460), no genital abnormalities were noted in patients at physical examination. To assess the presence of anticipation in SHFM3, we evaluated all affected parent-child pairs for evidence of worsening of hand and foot malformation in successive generations. We found seven parent-child pairs with evidence of worsening of limb-deformity phenotype (fig. 2, column 1). The SHFM3 phenotype appeared equally mild in one parent-child pair and equally severe in one other parent-child pair. In one affected parent-child pair, the mother's phenotype may have been slightly more severe. Two different three-generation vertical transmissions from grandparent to grandchild were particularly dramatic (see fig. 3, III-12; IV-29; V-54), suggesting, but not proving, anticipation. Because the determination of phenotype severity is somewhat subjective, we do not attach statements of statistical significance to these data.

### *Transmission Distortion in SHFM3*

Although previous studies of SHFM pedigrees show an excess number of affected male infants born to affected men, it is not clear at which SHFM loci this segregation distortion occurs. We compared the sex and number of affected and unaffected offspring born to affected fathers and affected mothers in seven SHFM3 families (table 1). Table 1 includes our own data; data

from the entire Pearson pedigree, as updated and expanded by Stevenson and Jennings (1960); and data from five other SHFM families for whom linkage to 10q24 had been reported (Nunes et al. 1995; Gurrieri et al. 1996; Raas-Rothschild et al. 1996). We include the Pearson pedigree because it is phenotypically similar to published SHFM3 families and because one of its descendant branches has recently shown linkage to 10q24 (P. Tsipouras, personal communication).

Under an assumed genetic model—namely, that SHFM3 is due to a rare, autosomal dominant disease allele—50% of the progeny from an affected parent should be affected, and the remainder should be unaffected. Visual inspection of the composite data from the families with linkage to 10q24 (table 1) suggests transmission bias from father to son, with more affected sons than would be expected by chance (exact binomial test,  $P = .0013$ ). No other transmission provides compelling evidence of bias, although it is noteworthy that affected fathers also tend to produce more affected female offspring than expected by chance. If one examines the  $\chi^2$  residuals from the null model of independence, only the deviations for transmission from fathers to sons are large.

A logit model provides a fuller characterization of these data. In this model, the log odds of being affected is the dependent variable and parent sex, progeny sex, and their interaction are the independent variables. Again significant transmission bias emerges from the model, with male parents producing a twofold excess of affected offspring ( $P = .034$ ). The interaction between parent and offspring, which is expected to be significant if the transmission bias is only from fathers to sons, is only marginally significant ( $P = .061$ ). This result raises a cautionary note regarding interpretation of these data: it is possible that segregation distortion occurs by excess transmission of the SHFM3 mutation from male parents to any offspring, not just to male offspring.

Another way to evaluate the transmission data from affected fathers to their offspring is by analysis of likelihood models. We considered three such models: the null model of 50:50 transmission of mutant and wild-type alleles from affected fathers to their sons and daughters, a common transmission distortion parameter for both sons and daughters (TSDS model), and transmission distortion for sons but 50:50 transmission for daughters (TDS model). Note that the models are binomial (conditional on margin totals of sons and daughters separately). The null model is nested in either the TDS and TSDS models, so there is standard theory for the distribution of the likelihood ratios. In fact, both models are highly favored over the null model, with likelihood ratios of 61.1 for the TSDS ( $P = .004$ ) and 203.0 for the TDS ( $P = .001$ ) models. Although the TDS and TSDS models are not nested, and thus no distri-



**Figure 3** Anticipation in SHFM3. Three-generational worsening of SHFM phenotype. Numbers refer to pedigree member numbering shown in figure 1.



**Table 1**  
**Transmission Distortion in SHFM3**

Parent	Male Offspring		Female Offspring	
	SHFM	Unaffected	SHFM	Unaffected
Father	19 (5)	4 (0)	19 (2)	13 (2)
Mother	38 (6)	37 (7)	34 (3)	28 (4)

NOTE.—Data are from Turkish SHFM3 family and six other published SHFM3 families. Numbers in parentheses refer to Turkish SHFM3 family only (fig. 1).

bution for their likelihood ratio is available, evaluation of the ratio is still valid. In this case, the TDS model is favored only 3.3 times over the TSD model. Thus the models are almost indistinguishable for these data.

*Mapping and Localization of SHFM3*

Using a full penetrance model, we obtained significant two-point LOD scores >3 for six 10q22-q24 markers (maximum LOD score of 8.6 for D10S1265 at recombination fraction [ $\theta$ ] of 0), identifying our Turkish family as the sixth such pedigree mapping to the SHFM3 locus. Analysis of inherited disease and nondisease haplotypes revealed two key crossover events at 10q24 in two affected and one unaffected individuals (fig. 4). The telomeric recombinant is defined by a crossover in an unaffected individual—information that is sometimes unreliable in diseases that are not fully penetrant. However, the individual defining our telomeric recombinant appears normal on physical examination and hand x ray, despite having a severely affected mother. Specifically, she does not have triphalangeal thumb, split distal index finger, or unguis dysplasia of the index finger, three clinical features that can be missed on casual examination. Thus, full resolution of the telomeric and centromeric recombinants reduces the SHFM3 critical region to an ~2-cM interval between loci D10S1147 and D10S1240 (fig. 4).

*Mutation Analysis of FGF8 and HOX11 Genes*

Because of chromosomal location and accumulated evidence that FGF8 plays an important role in the outgrowth and patterning of the vertebrate limb in embryonic development (Johnson and Tabin 1997), we proceeded to screen FGF8 exons for mutations by direct DNA sequencing (Gemel et al. 1996). No DNA changes between affected and unaffected individuals were uncovered (data not shown), in agreement with published data on human SHFM3 (Raas-Rothschild et al. 1996) and mouse dactylaplasia (Seto et al. 1997).

The HOX11 gene also maps to the 10q24 region, but functional studies in mice argue against a causative role in limb-deformity syndromes. Tlx1 (mouse homologue of HOX11) is not expressed in developing mouse limb buds (Raju et al. 1993), and knockout of the Tlx1 gene

causes only isolated asplenia (Roberts et al. 1995). However, because limb-deformity mutations in human and mouse polysyndactyly are associated with alterations in the repetitive CGG<sub>n</sub> trinucleotide repeats in the polyalanine tracts of two different HOX genes (Scott 1997), we amplified 400 bp of DNA that included GC-rich regions of human HOX11. No evidence for expansion or contraction of the polyglycine/polyalanine tract was detected (data not shown).

**Discussion**

The present study supports the mapping of SHFM3 to chromosome 10q24. Two key recombinants narrow

Chromosome 10 Markers	Genetic Distance (cM)	SHFM3 Disease Chromosome		Pedigree Individual #		
				28	60	56
				A	A	U
D10S577	126	1	1	8	2	
D10S1709	127	1	4	1	4	
D10S1726	130	9	9	2	3	
D10S198	130	1	1	1	1	
D10S1147*		2	2	8	4	
AFM277xe1		2	2	2	1	
D10S603	131	5	5	5	5	
PAX-2		1	1	1	5	
D10S1710	131	2	2	2	2	
D10S1778		2	2	2	2	
D10S192	131	4	4	4	8	
D10S1265	131	3	3	3	4	
D10S1266	131	2	2	2	4	
D10S278		1	1	1	1	
D10S1267	132	7	7	7	1	
D10S1697	132	1	1	1	2	
AFM301wf1		1	1	1	2	
D10S1240		2	2	2	2	
D10S1167		2	2	2	2	
AFM183xb12		2	2	2	2	
D10S1268	132	4	4	4	4	
D10S1668	132	1	1	1	1	
D10S222	132	2	2	2	2	
D10S1738	132	2	2	2	2	
D10S205	132	9	9	9	9	
D10S1692	132	2	2	2	2	
D10S534	135	2	2	2	2	
D10S1663	136	4	4	4	4	

**Figure 4** Genetic crossover events in the Turkish SHFM family and a genetic map of selected 10q23.1-10q24.3 STRP markers are shown. Genetic map distances are taken from the Whitehead/MIT Center for Genome Research. The dark gray bars represent the SHFM3 disease haplotype. Above the bar, the pedigree number and affection status are shown. A = affected, and U = unaffected. Alleles within the lightly shaded bar of individual 58 were uninformative.

the critical region to an ~2-cM interval from the 19-cM region reported by Gurrieri et al. (1996) and from the 9-cM region reported by Raas-Rothschild et al. (1996). The *SHFM3* locus maps to a syntenic region on mouse chromosome 19D, containing the mouse dactylaplasia (*dac*) locus (Johnson et al. 1995). Phenotypically similar to human *SHFM3*, mouse *dac* is caused by a dramatic cell death of the apical ectodermal ridge (Seto et al. 1997).

Several plausible candidate genes map to this general region by radiation hybrid or partial clone contig, including *FGF8*, *PAX2*, and *ZNF32*, and several genes with homeodomain motifs: *HOX11*, *HMX2*, *WNT8b*, *LDB1*, *LBX1*, and *PITX3*. Direct sequencing has excluded exonic regions of *FGF8*, and haplotype analysis allows exclusion of the *FGFR2* gene. Functional studies argue against *PAX2* as a causative *SHFM* gene, as *PAX2* mutations are associated with optic nerve colobomas, renal anomalies, and vesicoureteral reflux (Sanyanusin et al. 1995), and *PAX2* expression is restricted in the developing embryo to the urogenital and central nervous systems and is not detected in limb buds (Seto et al. 1997). Although the human homeobox gene *HMX2* maps to 10q25.2-q26.3 (Stadler et al. 1995), the mouse homologue *Hmx2* gene maps to mouse chromosome 7 (Wang and Lufkin 1997). Since *Hmx2* does not map to the mouse chromosome 19D *dac* region, *HMX2* is unlikely to be a candidate for *SHFM3*. *WNT* genes encode intercellular signaling molecules that play important roles in key processes of embryonic development, including limb development (Kengaku et al. 1998). *WNT8b*, a novel human homologue of the *Wnt* gene, maps to 10q24 (Lako et al. 1996), on the same YAC as *PAX2* (Gray et al. 1997), but mRNA expression studies suggest a greater biological role for *WNT8b* in developing neural-specific structures. Mouse Lim domain-binding factor, *Ldb1*, maps 1.7 cM centromeric to *PAX2*, but its mRNA expression in the nuclei of diverse neuronal cell types argues against a causative role in *dac* or *SHFM3* (Yamashita et al. 1998). *LBX1*, a human homeobox homologue of *Drosophila* ladybird (*Lbe*), maps to human chromosome 10q24, whereas mouse *Lbx1* maps to syntenic chromosome 19D (Jagla et al. 1995). *Lbx1* mRNA expression is restricted to developing central nervous system and to presumptive muscle cells, also making *LBX1* an unlikely candidate for *dac* or *SHFM3*. Finally, the *Pitx3* homeobox gene maps to mouse chromosome 19D near the *dac* and *aphakia* locus, whereas the human homologue *PITX3* maps to syntenic chromosome 10q24 (Semina et al. 1997). *Pitx3* mRNA expression is limited to the developing lens, making *Pitx3* a better candidate gene for mouse *aphakia* than for *dac* or *SHFM3*.

In several parent-child dyads, our Turkish *SHFM3* family demonstrates progression of disease from a mild

phenotype in an affected parent to a more severe phenotype in an affected offspring, suggesting anticipation. Emery (1977) first raised the question of minor hand anomalies such as split nail or extra joints as partial expression of *SHFM*. Two or more affected sibs with two normal parents have been noted by several authors (MacKenzie and Penrose 1951; Graham and Badgley 1955; Ray 1960; Verma et al. 1976; Zlotogora and Nubani 1989; Majewski et al. 1996), and this has sometimes been used as evidence for recessive inheritance (Zlotogora and Nubani 1989). Premutation of an autosomal dominant *SHFM* gene provides an alternative explanation. It is interesting to compare *SHFM3* inheritance patterns to another limb malformation syndrome, synpolydactyly, for which the causative gene has been cloned. Synpolydactyly is caused by an imperfect trinucleotide-repeat sequence encoding a 15-residue polyalanine tract in the homeobox gene, *HOXD13* (Akarsu et al. 1996; Goodman et al. 1997). Although there is no evidence of change in expansion size in *HOXD13* within families with synpolydactyly, or even over six generations, there is a highly significant correlation between the severity of the limb phenotype and larger expansion sizes (Muragaki et al. 1996). Polyalanine tracts are found in many other homeodomain proteins, and it is tempting to speculate whether intergeneration expansion of polyalanine tracts in an unidentified homeodomain gene could account for both the ectrodactyly limb-deformity phenotype and the anticipation phenomenon.

Well-understood examples of segregation distortion in human disease are rare, but reported examples include cone-rod retinal dystrophy caused by mutations in a photoreceptor-specific homeobox gene *CRX* (Evans et al. 1994; Freund et al. 1997), macular dystrophy mapped to 6q (Small 1993), retinoblastoma (Munier et al. 1994), Alport syndrome (Shaw and Glover 1961), and postaxial polydactyly (Orioli 1995). Segregation distortion of excess affected offspring born to affected parents has recently been shown in several trinucleotide repeat-expansion disorders, including spinocerebellar ataxia (Riess et al. 1997; Takiyama et al. 1997) and myotonic dystrophy (Carey et al. 1994; Gennarelli et al. 1994; Shaw et al. 1995; Chakraborty et al. 1996; Lee-flang et al. 1996; Inglehearn and Gregory 1997; Zatz et al. 1997). Cloning of the *SHFM3* gene will help to determine whether a trinucleotide repeat expansion disorder could account for both the apparent anticipation and the segregation distortion observed in *SHFM3*.

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## Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for *SHFM1* [MIM 183600], *SHFM2* [MIM 313350], *SHFM3* [MIM 600095], and various other limb-deformity syndromes (MIM 176305, 140000, 186000, and 600460))

Whitehead/MIT Center for Genome Research, release 12, <http://www.genome.wi.mit.edu> (for genetic map distances)

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