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Exclusion of Linkage between Autosomal Dominant Split Hand/Split Foot and Markers from Chromosome 7q: Further Evidence for Genetic Heterogeneity

To the Editor:

The split hand/split foot anomaly (SHSF) is a developmental defect of the distal limbs, specifically involving the central digital rays. Such a defect is usually inherited as an autosomal dominant trait, although most cases occur sporadically.

Penetrance of SHSF is extremely variable, ranging from apparent excess of affected offspring in some families to very low penetrance in others. One explanation for this variability is that of locus heterogeneity. A putative locus for sporadic SHSF has been mapped to the 7q22.1 chromosomal region, because of the nonrandom occurrence of similar 7q22 rearrangements in isolated patients with SHSF (Tajara et al. 1989; Morey and Higgins 1990; Rivera et al. 1991; Akita et al. 1993). A familial variety of SHSF with low penetrance has also been mapped to 7q22.1, through the study of a family segregating the defect together with a 2;7 translocation (Genuardi et al. 1993).

More recently, we ascertained a family with normal chromosomes and a highly penetrant type of SHSF, segregating as an autosomal dominant trait, and investigated whether it could also be due to the putative limb-development mutant gene at the 7q locus. For this purpose, we studied linkage between the defect and highly polymorphic DNA markers from the 7q22 region.

The following microsatellites (Chi et al. 1992; Weissenbach et al. 1992; Grzeschik et al. 1994) were selected for segregation analysis (listed in proximal to distal order): COL1A2–D7S440–12 cM–D7S524–4 cM–D7S492–8 cM–D7S527–1 cM–D7S491–4 cM–D7S518–7 cM–D7S501–7 cM–D7S486. The genetic distance between COL1A2 and D7S440 is not known exactly, because of lack of an integrated linkage map including both loci. Physical mapping of the SHSF critical region on chromosome 7q21.3-q22.1 (Scherer et al. 1994) has placed the putative locus between D7S492 and COL1A2 proximally and D7S501 distally; therefore the nine markers used in the present study extensively span and in fact exceed the SHSF critical region.

For each microsatellite, primers were end-labeled with ^{35}S and were used to amplify DNA from the different family members. PCR products were run on polyacrylamide gel and were dried and autoradiographed.

Figure 1 shows the family pedigree and the haplotypes obtained for seven affected and one unaffected member. Haplotypes of individual III-2 were constructed in order to minimize the number of recombination events in her offspring.

Pairwise lod scores were calculated using the LIPED program (version 1991) (Ott 1976) and by assuming SHSF to be an autosomal dominant trait with a gene frequency of .001 and complete penetrance. Multipoint analysis was performed using the LINKMAP program of the LINKAGE package (version 5.1) (Lathrop and Lalouel 1984).

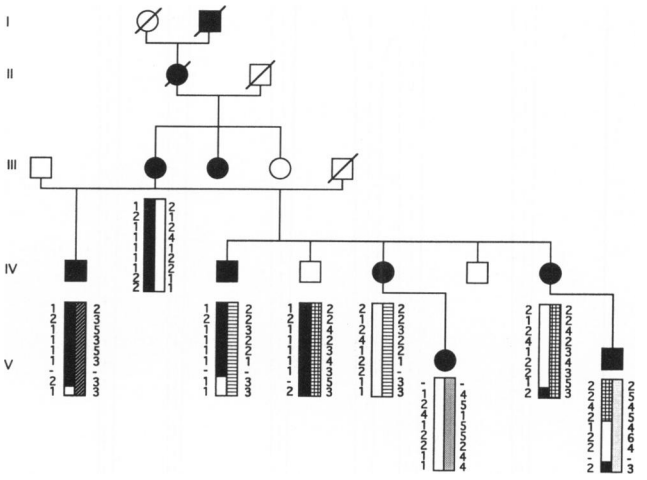

Figure 1 Pedigree and haplotypes of autosomal dominant SHSF family. Allele numbers are arranged from top to bottom in the proximal to distal order as specified in the text. Single recombination events are apparent in individuals IV-1, IV-2, IV-6, and V-2.

Table 1

Two-Point Lod Scores between SHSF and 7q Markers

	Lod Score at Recombination Fraction of								
SHFV vs.	0	.001	.01	.05	.1	.15	.2	.3	.4
C1A2	−.176	−.174	−.152	−.08	−.027	.001	.014	.015	.005
D7S440	∞	−5.068	−3.063	−1.649	−1.04	−.698	−.468	−.185	−.044
D7S524	∞	−7.195	−4.207	−2.164	−1.331	−.877	−.581	−.227	−.053
D7S492	∞	−7.195	−4.207	−2.164	−1.331	−.877	−.581	−.227	−.053
D7S527	.602	.601	.593	.558	.511	.461	.408	.292	.158
D7S491	∞	−4.196	−2.211	−.885	−.377	−.124	.021	.141	.123
D7S518	1.125	1.123	1.104	1.016	.902	.784	.661	.412	.179
D7S501	∞	−4.496	−2.508	−1.164	−.632	−.354	−.184	−.005	.044
D7S486	∞	−1.497	−.517	.093	.277	.331	.328	.241	.111

Recombinants were found between the putative SHSF locus and markers D7S440, D7S524, D7S492, D7S491, D7S501, and D7S486 (table 1), and, in addition, its presence was significantly excluded, by multipoint linkage analysis, from intervals D7S440–D7S524, D7S524–D7S492, D7S492–D7S491, and D7S491–D7S501 (fig. 2).

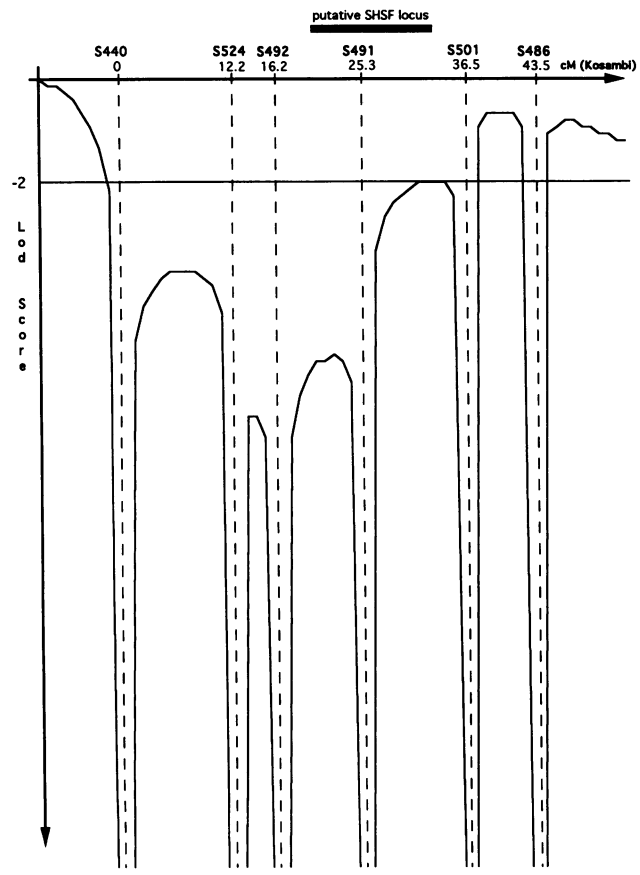

Figure 2 Multipoint linkage analysis. The putative SHSF locus is indicated by a black bar spanning marker locus D7S491, flanked by D7S492 and D7S501 marker loci.

These results demonstrate that, in this highly penetrant family, autosomal dominant SHSF is caused by a mutant gene not linked with the putative locus in 7q22.1. Our data are in agreement with the findings of other groups (Palmer et al. 1994) and provide further evidence for genetic heterogeneity of autosomal dominant SHSF.

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Two-Locus versus One-Locus Lods for Complex Traits

To the Editor:

In the detection of linkage for traits determined by multiple loci, one important methodological issue is the relative power of single-locus versus multilocus lods, under various conditions. If the advantage of multilocus lods over single-locus lods is substantial, then investigators must begin to consider calculating multilocus lods, even when the evidence for linkage from single-locus lods is slim. On the other hand, if the advantage is small, then multilocus lods should be used sparingly. Multilocus likelihood is not only difficult to compute, but also requires the specification of many genetic parameters, usually unknown.

In their recent simulation study, Schork et al. (1993) found a substantial gain (78%) of information by the two-locus lod, when the true model was "dominant or recessive." This is contrary to two other recent studies (Goldin and Weeks 1993; MacLean et al. 1993), which indicated that two-locus lod scores were usually only slightly more powerful than single-locus lod scores.

We suggest that part of the gain reported by Schork et al. (1993) was due to suboptimal specification of the single-locus models. It is well known that misspecification of genetic model reduces the power to detect linkage (Clerget-Darpoux et al. 1986; Greenberg 1989). When the true model contains two loci, then any single-locus model is a simplication, but for each locus there still exists some single-locus specification that maximizes the power to detect linkage to that locus (Greenberg 1990). In our simulations (MacLean et al. 1993), the optimal "marginal" penetrance of a particular genotype at one locus appears to be a weighted average of the "joint" penetrances of genotypes containing that genotype, over all possible genotypes at the other locus, the weights being the frequencies of the possible genotypes at the second locus, i.e., $P(A|g_1) = \sum P(A|g_1, g_2)P(g_2)$ where A is affection status, and g_1 and g_2 are the genotypes at the two loci, the summation being over all possible genotypes at the second locus. For high-density pedigrees, the appropriate genotype frequencies at the second locus are those specific to the sample, rather than those of the general population. Using single-locus penetrances determined by this method, we found by simulation that the sum of single-locus lods was usually only slightly less than the multilocus lod (MacLean et al. 1993). The average gain achieved by two-locus lods over the sum of single-locus lods ranged from −11% to +17%; the largest average gain occurred for a two-locus model in intense epistasis.

In contrast, Schork et al. (1993) concluded from their simulations that two-locus lods had a substantial gain over single-locus lods, for a "dominant-recessive" two-locus model. However, the single-locus parameters used in the lod calculations were derived by fitting a single-locus model to the simulated family data. Clearly, when the true model contains two loci, the parameters estimated by fitting a single-locus model may not be appropriate for both loci. The gene frequency tends to be overestimated, and the penetrances may or may not be appropriate, depending on the joint penetrance matrix. The "dominant-or-recessive" model is particularly problematic, since the joint-penetrance matrix is assymmetric, so that the no single-locus model can be appropriate for both loci. If a dominant-like model is obtained, this will be appropriate for the dominant but not for the recessive locus. On the other hand, if a recessive-like model is obtained, this will be appropriate for the recessive but not for the dominant locus. The best-fitting single-locus model for Schork et al.'s "dominant-recessive" two-locus model turned out to be nearly recessive. Not surprisingly, the single-locus lod for the second, dominant locus was very low. Schork et al. (1993) repeated the analysis by using a near-dominant model, which reduced the lod for the recessive locus but increased the lod for the dominant locus. Although the sum of their best single-locus lods was still substantially lower than their two-locus lod (4.74 vs. 6.41), these single-locus lods did not take into account possible locus heterogeneity, and it remained unclear whether the single-locus model parameters obtained by model fitting were optimal.