Am. J. Hum. Genet. 55:12–20, 1994

Fine Mapping of the Autosomal Dominant Split Hand/Split Foot Locus on Chromosome 7, Band q21.3-q22.1

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Summary

Split hand/split foot (SHFD) is a human developmental defect characterized by missing digits, fusion of remaining digits, and a deep median cleft in the hands and feet. Cytogenetic studies of deletions and translocations associated with this disorder have indicated that an autosomal dominant split hand/split foot locus (gene SHFD1) maps to 7q21-q22. To characterize the SHFD1 locus, somatic cell hybrid lines were constructed from cytogenetically abnormal individuals with SHFD. Molecular analysis resulted in the localization of 93 DNA markers to one of 10 intervals surrounding the SHFD1 locus. The translocation breakpoints in four SHFD patients were encompassed by the smallest region of overlap among the SHFD-associated deletions. The order of DNA markers in the SHFD1 critical region has been defined as PON–D7S812–SHFD1–D7S811–ASNS. One DNA marker, D7S811, detected altered restriction enzyme fragments in three patients with translocations when examined by pulsed-field gel electrophoresis (PFGE). These data map SHFD1, a gene that is crucial for human limb differentiation, to a small interval in the q21.3-q22.1 region of human chromosome 7.

Introduction

Split hand/split foot (SHFD; McKusick MIM 183600 [McKusick 1992]), is a human developmental defect that affects the central rays of the hands and feet, resulting in missing digits and clawlike extremities (fig. 1). SHFD (also known as ectrodactyly or lobster claw deformity) is usually inherited in an autosomal dominant fashion; however, autosomal recessive (Freire-Maia 1971; Verma et al. 1976) and X-linked forms (Ahmad et al. 1987; Faiyaz-ul-Haque et al. 1993) have been described. The incidence of SHFD is between 1/10,000 and 1/90,000 (Temtamy and McKusick 1978, pp. 53–71; Nelson and Holmes 1980).

SHFD demonstrates a number of distinctive genetic features that are not understood at the molecular level (Temtamy and Mckusick 1978, pp. 53–71). Approximately 30% of obligate carriers of autosomal dominant SHFD show no phenotypic abnormalities, the phenomenon of reduced penetrance. Variable expressivity is another striking characteristic of this disorder in which affected family members may display remarkably different phenotypes, ranging from mild abnormalities of a single limb to severe defects of all four limbs (fig. 1). Moreover, within a single individual one extremity may be more severely affected than another. Reduced penetrance and variable expressivity are problematic in the clinical setting, making it impossible to predict carrier status or severity of disease. Finally, another unusual genetic characteristic of autosomal dominant SHFD is segregation distortion, in which the mutant allele is transmitted to progeny in excess of the expected Mendelian proportion of 50% (McMullan and Pearson 1913; Stevenson and Jennings 1960). SHFD pedigrees that demonstrate segregation distortion (the first of which were reported by Pearson in 1908 [Pearson 1908]) were even used to argue against the applicability of Mendelian genetics to humans (McMullan and Pearson 1913)!

A consistent association between SHFD and deletions of chromosome 7 at bands q21-q22 provides compelling evidence for the location of an SHFD gene within this region (Del Porto et al. 1983; Pfeiffer 1984; Tajara et al. 1989; Morey and Higgins 1990; Rivera et al. 1991; Roberts et al. 1991), and the locus has been designated "SHFD1" (Tsui and Farrall 1991). Further support is provided by

Received January 19, 1994; accepted for publication March 2, 1994.

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0002-9297/94/5501-0003$02.00

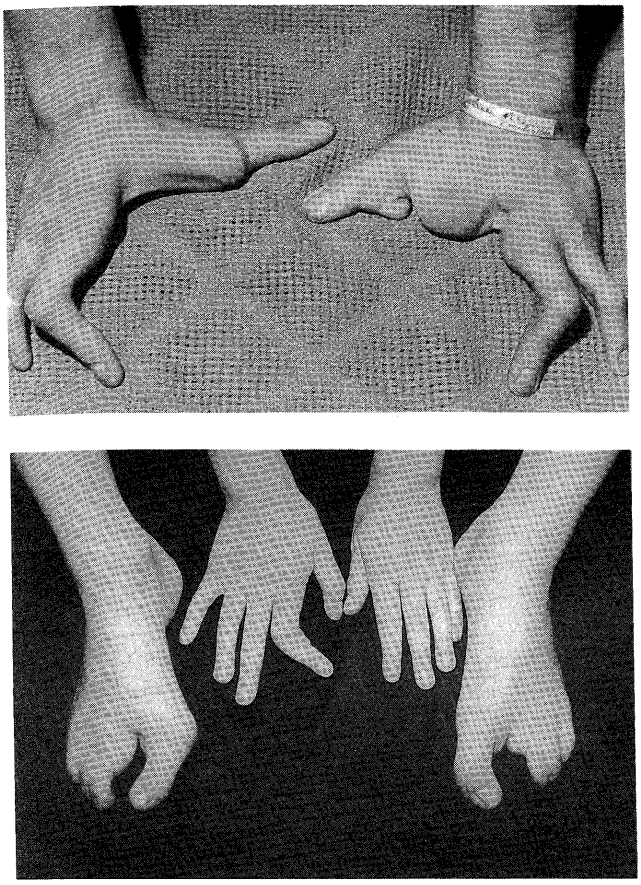

Figure 1 Phenotypic characteristics of typical SHFD in a father and daughter. The father's hands (*top*) show the characteristic median cleft and absence of digits. His feet (not shown) are also severely affected. The daughter's limbs (*bottom*) show only a mild abnormality of the right hand, consisting of a deviated index finger, but her feet are severely affected, with loss of the second toe, a deep median cleft, and syndactyly. The different phenotypic characteristics seen in this father and daughter illustrate the phenomenon of variable expressivity in which individuals who presumably carry the same mutation may have varying severity of involvement.

three published reports of SHFD patients with cytogenetically visible translocations involving breakpoints in the same area (Sharland et al. 1991; Genuardi et al. 1993; Naritomi et al. 1993). In the present study we report clinical, cytogenetic, and the first molecular analysis of published, as well as previously unpublished, SHFD patients with cytogenetic abnormalities in the 7q21-q22 region. These results refine the localization of the SHFD1 locus to 7q21.3-q22.1. Further physical delineation of this region and of SHFD-associated chromosomal rearrangements should allow isolation of the SHFD1 gene.

Subjects, Material, and Methods

SHFD Patients

Seven patients with SHFD and 7q cytogenetic abnormalities were included in this study (table 1). Three of the patients had apparently balanced translocations (T1, T2, and T3); one had a balanced inverted insertion (T4); and three had interstitial deletions (D1, D2, and D3). Patients T1 and D2 have not been previously reported and were identified through the clinical genetics services at Oregon Health Sciences University and Stanford Medical Center, respectively.

Transformation of Human Lymphocytes

Epstein-Barr virus (EBV) transformation of human peripheral B-lymphocytes was carried out within 48 h of phlebotomy (Neitzel 1986). Blood was collected in acid-citrate-dextrose preservative.

Establishment of Human-Hamster Somatic Cell Hybrids (SCHs)

Five of the SCHs in the mapping panel have been described elsewhere (Rommens et al. 1988; Scherer et al. 1993). Nine new human-hamster hybrid lines are described in the present report and were constructed according to a previously published method (Puck et al. 1989) with the following modifications. One 85-mm plate of hamster RJK cells that had almost reached confluence was washed three times with PBS and was overlaid with ~40 million exponentially growing lymphoblastoid cells that had been washed in PBS and suspended in serum-free medium. After a 30-min incubation at 37°C in 5% $C0_2$, the medium was carefully aspirated from the hamster cells, and 2 ml of 50% (w/v) polyethylene glycol (PEG; Gibco BRL) were added to the plate and incubated at room temperature for 1 min. PEG was aspirated, and the cells were washed four times in PBS. Serum-free medium was added, and the cells were incubated for 1 h at 37°C in 5% $C0_2$, after which Dulbecco's minimal essential medium supplemented with 10% FCS was added. The following day the cells were split 1:30 and were selected with hypoxanthine, aminopterin, thymidine, and ouabain (final concentration 2.5 mg/ml) for 2 wk.

Screening of Human-Hamster SCHs

Hybrids were screened for the presence of chromosome 7 derivatives by a PCR assay (Saiki et al. 1988). Each colony that survived selection was picked into a 24-well plate. On reaching confluence, the cells were trypsinized, and half were plated in an 85-mm plate, while the other half were solubilized in Triton X-100 and Tween 20 for analysis by PCR. To detect SCHs that retained chromosome 7 translocation and deletion derivatives, primers were used that were specific either for the T-cell receptor beta (TCRB) locus on the distal portion of 7q or for the T-cell receptor gamma (TCRG) locus on 7p. The sequences of the primers are as follows: TCRB, 5′ CTGCAGACATGCTGTCACTGC 3′ and 5′ GGTGAGAGTGGATGTAGACG 3′; and TCRG, 5′ GACAACTGGGGTAGGAATGG 3′ and 5′ GAGGCCTGCACATGGATCTG 3′. Two microliters of

Table 1

Cytogenetic and Clinical Characteristics of SHFD Patients Analyzed

Patient	Karyotype	Limb Anomalies	Other Anomalies	Reference
T1	46, XX t(7;12)(q22.1;q24.2)	Bilateral split hand/ split foot	Mild speech delay	Present study
T2	46, XY, t(5,9,7)(5pter-5q11.2::5q34-5qter; 9pter-9q22.1::7q31.3-7q21.2::5q34-5q11.2::7q31.3-7qter; 7pter-7q21.2::9q22.1-9qter)	Bilateral split hand/ split foot	Mildly dysmorphic, low-set ears, normal cognitive development	Sharland et al. 1991
T3	46, XX, t(2;7)(q21.1;q22.1)	Unilateral split hand Normal feet	Hearing loss Familial translocation, with 3 of 12 translocation carriers demonstrating abnormal phenotype (see text)	Genuardi et al. 1993
T4	46, XY, inv ins(3;7)(q21;q34q22)	Unilateral split hand, bilateral split foot	High arched palate, bifid uvula, normal cognitive development	Naritomi et al. 1993
D1	46, XX del 7(q11.2-q22.1)	Unilateral split hand, bilateral split foot	Severe mental retardation, microcephaly, cleft palate	Pfeiffer 1984
D2	46, XY del 7(q21.12-q22.1)	Normal hands, bilateral split foot	Mental retardation, microcephaly, coloboma, short stature	Present study
D3	46, XY, ins(7), del(7)(pter-p15.1::q11.21-q11.23::p15.1-q21.2::q22.1-qter)	Bilateral split hand/ split foot	Mildly dysmorphic, mildly delayed speech, full-scale IQ of 84	Roberts et al. 1991

each SCH preparation were subjected to duplex PCR. Cycling was for 30 s at 58°C and for 30 s at 95°C, for 42 cycles. Products were analyzed on a 2% NuSieve, 0.8% agarose gel. The presence of a single band at 380 bp was indicative of only the TCRB locus being retained in the SCH, thus indicating that only the distal chromosome 7 derivative was present. A single band at 427 bp was indicative of the presence of only the TCRG locus and was thus consistent with the presence of only the proximal chromosome 7 derivative. Standard Southern analysis was performed to confirm the PCR results in positive clones.

DNA Markers

The majority of the DNA markers used in this study have been described elsewhere (Rommens 1988; Scherer et al. 1993). The genes and DNA markers not derived from the two previous studies are shown in table 2. Additional DNA markers were isolated by Alu-PCR amplification (Nelson et al. 1989) of DNA either from the human-hamster SCH 1CF2/5/K016 (fig. 2; Scherer et al. 1993) or from YAC clones mapped to 7q21-q22 by FISH. Human phage clones corresponding to Alu-PCR fragments K16.A89 and K16.A37 were isolated from a *Sau*3A partial digest genomic phage library, to provide longer genomic fragments for hybridization analysis. The genomic clones isolated were K16.89 (*D7S812*), a 5.5-kb *Eco*RI fragment isolated from phage SS5, and K16.37 (*D7S811*), a 1.7-kb Xba1 fragment isolated from phage clone SS22. DNA sequence analysis was not performed on the markers used in this study; thus, Southern hybridization was employed instead of PCR analysis.

Gel Electrophoresis, Southern Blotting, and Hybridization Analysis

Agarose gel electrophoresis and PFGE of DNA were performed according to standard protocols (Scherer and Tsui 1991). For PFGE, agarose blocks were prepared from EBV-transformed lymphoblasts, and high-molecular-weight DNA fragments were separated using the CHEF DRII system (BioRad). Prehybridization and hybridization were performed as described elsewhere (Scherer and Tsui 1991). DNA probes were excised from plasmid vectors and labeled with [α^{32}P]-dCTP (Amersham or Dupont-

Table 2

List of DNA Markers Used in the Present Study

Locus	Marker Name	Location	Probe Name	Reference
ACHE	Acetylcholinesterase	7q22	ACHE	Ehrlich et al. 1992
ASL	Argininosuccinate lyase	7q11.23	AL3	O'Brien et al. 1986
ASNS	Asparagine synthetase	7q22.1	p131-1	Andrulis et al. 1987
COL1A2	Collagen, type 1, alpha 2	7q21.3-q22.1	NJ-1	Tsipouras et al. 1984
CUTL1	CCAAT displacement protein	7q22	Clone 6191	Neufeld et al. 1992
D7S13		7q22	B79a	Wainwright et al. 1987
D7S15		7q21.3	Lam4-917	Tsui et al. 1985
D7S811		7q21.3-q22.1	K16.89	Present study
D7S812		7q21.3-q22.1	K16.37	Present study
EPO	Erythropoietin	7q22	pCDM8-VAIIEPO	Law et al. 1986
GNB2	Guanine nucleotide-binding protein, β-2	7q22	Human GNB2 cDNA	Fong et al. 1987
GUSB	β-Glucuronidase	7q11.23	pHUG60.2	Guise et al. 1985
HGF	Hepatocyte growth factor	7q21.1	83-II	Fukuyama et al. 1991
LAMB1	Laminin, B1 polypeptide	7q22.3-q31.1	0.9-kb *Pst*I fragment from pHL-40	Pikkarainen et al. 1987
MUC3	Mucin	7q22	SIB124	Fox et al. 1992
PGY1	P glycoprotein 1	7q21	pHDR5A	Chen et al. 1986
PGY3	P glycoprotein 3	7q21	HepG2-16	Van der Bliek et al. 1987
PLANH1	Plasminogen activator inhibitor, type 1	7q22.1-q22.3	1.0-kb *Pst*I fragment from pPAI-E5.2	Bosma et al. 1988
PON	Paraoxonase	7q21.3-q22.1	Genomic clone 55-4	Hassett et al. 1991
PRKAR2B	cAMP-dependent protein kinase regulatory, type II, β	7q22	hRIIβ	Solberg et al. 1992

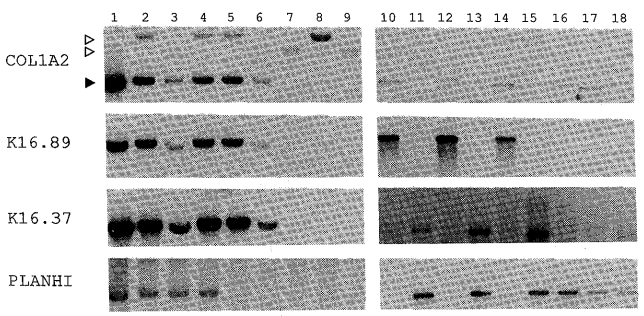

Figure 2 Hybridization analysis of DNA markers against SCH lines. DNA was isolated from the hybrid cell lines, digested with *Eco*RI, fractionated by gel electrophoresis on 0.8% agarose gels, and examined by Southern-blot hybridization with [α^{32}P]-labeled probe for each of the markers, as indicated. In the first panel of hybrids (derived from individuals without SHFD; lanes 2–9) lanes 2, 4, and 5 are human-hamster hybrids, and lanes 3, 6, and 7 are human-mouse hybrids. Lane 1, Human. Lane 2, 4AF1/106/K015. Lane 3, JSR-17S. Lane 4, 1CF2/5/K016. Lane 5, 1EF2/3/K017. Lane 6, GM1059Rag5. Lane 7, 2068Rag22-2. Lane 8, Chinese hamster ovary cell line. Lane 9, Mouse A9 fibroblast cell line. The second panel of human/hamster SCHs were derived from SHFD1 patients. Lane 10, T1.55, Lane 11, T1.8. Lane 12, T2.2. Lane 13, T2.41. Lane 14, T3.3. Lane 15, T3.43. Lane 16, D1. Lane 17, D2. Lane 18, D3. The proportion of the 7q11.23-q31 region of chromosome 7 maintained in these cell lines is schematically shown in fig. 3. In lanes where more than one band is visible, the blackened triangle indicates human-specific bands, and the unblackened triangles indicate cross-hybridizing rodent sequences.

NEN) to a specific activity of 4–10 × 10^8 cpm/μg DNA by the random priming method.

Results

Physical Mapping of the SHFD1 Locus at 7q21.2-q22.1

Regional localization of DNA markers was accomplished by Southern blot hybridization analysis of 14 SCHs that divide the 7q11.23-q31 region into at least 10 intervals (fig. 3). The SCH lines originated from two sources. Five were derived from individuals without SHFD and were previously found to be informative for mapping the 7q21-q22 region (Rommens et al. 1988; Scherer et al. 1993). Nine new SCH lines were established from six different SHFD patient cell lines. Three of the SHFD SCHs were derived from patients with deletions (patients D1, D2, and D3), while the remaining six cell lines contained a translocation derivative isolated from the three SHFD patients with balanced translocations (patients T1, T2, and T3). SCHs have not yet been established from patient T4, but this patient's DNA was analyzed by PFGE (see below).

In a previous study (Scherer et al. 1993), >150 genes and DNA segments were mapped to one of five intervals within 7q11.23-q32, on the basis of the breakpoints contained in six SCH lines containing various portions of chromosome 7. These DNA markers, as well as additional ones collected from the literature (table 2), were tested on the SHFD SCHs (figs. 2 and 3). The results indicated that the chromosomal location of 91 DNA markers could be fur-

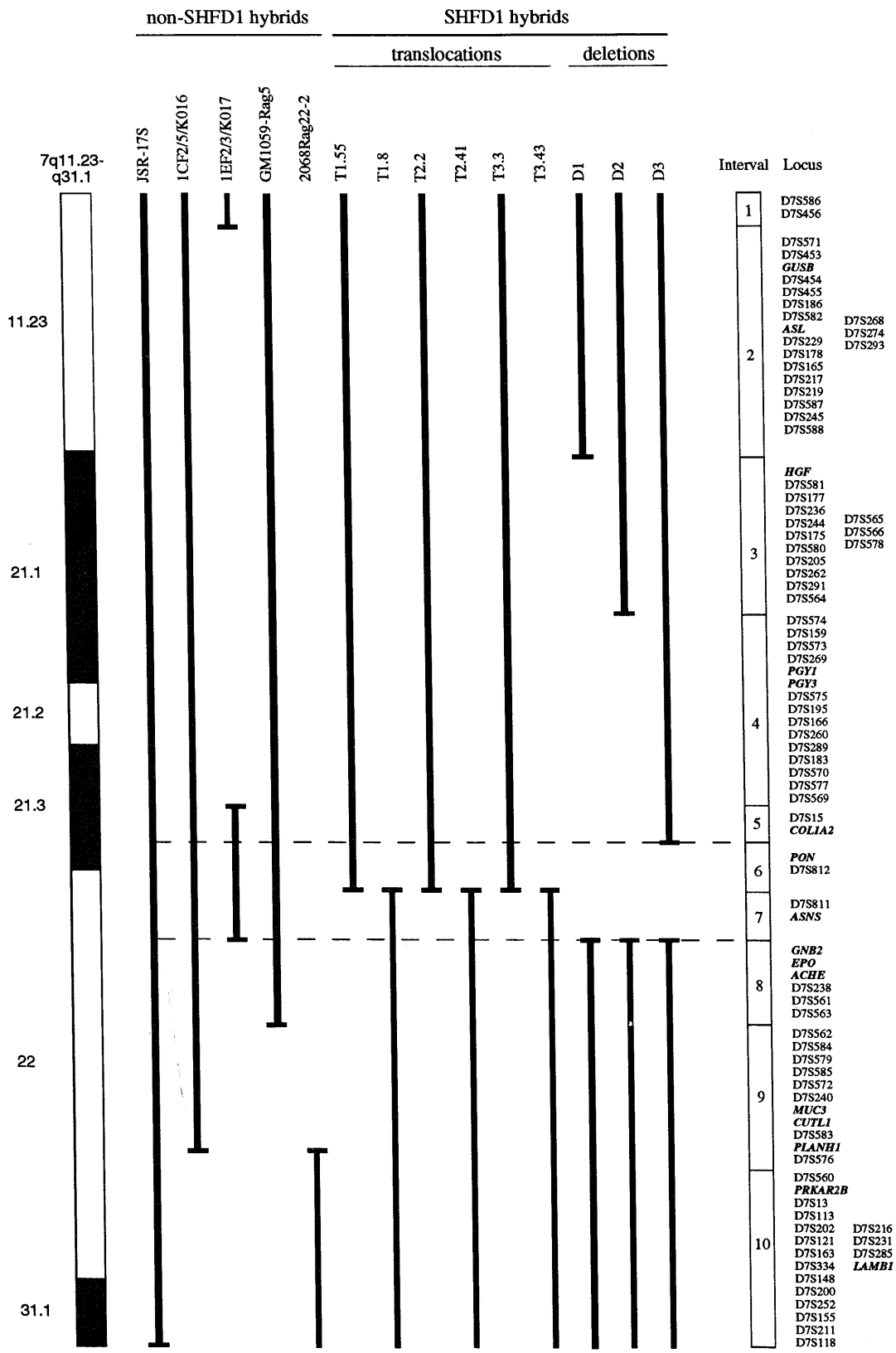

Figure 3 Summary of the regional localization of genes and DNA segments to 1 of 10 intervals in the 7q21-q22 region. The proportion of the 7q11.23-q31 region estimated, by molecular and cytogenetic analysis, to be contained in the SCHs is represented by the black vertical lines, and breakpoints are indicated by blackened horizontal bars. The hybrids derived from the SHFD-associated translocation cell lines are named for the translocation patient and clone number; for example, T1.55 contains the 7pter-q22 derivative, whereas T1.8 contains the 7q22-qter derivative from patient T1. The horizontal dashed lines delineate the SHFD1 critical region encompassing intervals 6 and 7.

ther refined to one of 10 intervals by using these hybrids (fig. 3). Two markers, *PON* and *ASNS*, were found to be deleted in all three deletion patients, and the D3 deletion cell line was shown to contain the smallest deletion. To obtain additional markers located within this deleted region, DNA probes were isolated by Alu-PCR amplification of an SCH that contains 7cen-q22 (1CF2/5/K016; Scherer et al. 1993) and of YAC clones mapped to 7q21-q22 by FISH (Grzeschik et al. 1993). These markers were tested for their absence in the D3 SCH line, and two DNA segments, K16.89 (*D7S812*) and K16.37 (*D7S811*), were identified (fig. 2). Of the four DNA markers (*PON*, K16.89, K16.37, and *ASNS*) absent in all three deletion SCH lines, *PON* and K16.89 were located proximal, and K16.37 and *ASNS* were located distal, to the three translocation breakpoints (T1, T2, and T3). Thus, these data suggest that the SHFD1-associated translocation breakpoints can be assigned to the 7q21.3-q22.1 region between the DNA markers defining interval 6 (*PON* and K16.89) and interval 7 (K16.37 and *ASNS*) (fig. 3).

Molecular Definition of the SHFD1 Locus by PFGE

PFGE was employed to define the relationship between the four DNA markers located within the SHFD1 critical interval and the translocation breakpoints. High-molecular-weight DNA was prepared from patients T1, T2, T3, and T4. DNA was digested with various rare-cutting restriction enzymes and was subjected to PFGE. Figure 4 shows the results of blot hybridization analysis with marker K16.37. A normal *Not*I fragment 670 kb in size was present in all lanes. In the T1, T2, and T4 cell lines, however, an altered *Not*I fragment—of 450 kb, 920 kb, and 1.5 Mb, respectively—was present, in addition to the normal 670-kb *Not*I fragment. It is worth noting that the mother and father of patient T2 (who has a de novo translocation) show only the normal, 670-kb *Not*I fragment, which argues against the possibility of a polymorphism causing the altered fragment sizes (fig. 4). These results suggest that K16.37 is both <670 kb from the breakpoint in T2 and T4 and <450 kb from the translocation breakpoint in patient T1. PFGE analysis of the same cell lines with *PON*, K16.89, and *ASNS* with several rare-cutting restriction enzymes (*Not*I, *Cla*I, *Bss*HII, *Nru*I, *Xho*I, and *Sal*I) failed to show altered restriction fragments (data not shown), suggesting that K16.37 is the marker closest to the breakpoints. Thus, the suggested order of DNA markers in the SHFD1 critical region is *PON*-K16.89-*SHFD1*-K16.37-*ASNS*.

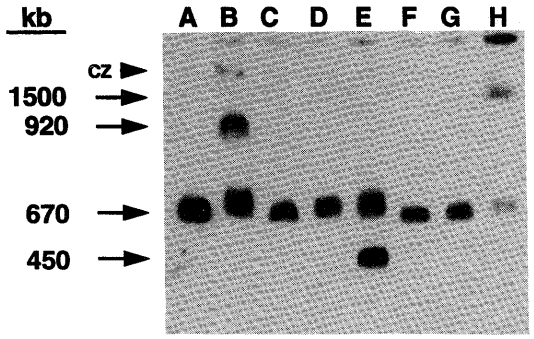

Figure 4 PFGE–Southern blot hybridization analysis of DNA from translocation patients and normal controls, with DNA probe K16.37 (D7S811). High-molecular-weight DNA was isolated from lymphoblastoid cell lines, digested with *Not*I, and, after separation by PFGE, examined by Southern-blot hybridization with α^{32}P-labeled K16.37. Lane A, Mother of patient T2. Lane B, Patient T2. Lane C, Father of patient T2. Lane D, Normal control, Lane E, Patient T1. Lane F, Patient T3. Lane G, Normal control. Lane H, Patient T4. *Not*I fragments of altered size are observed in lanes B, E, and H. The amount of DNA in lane H is markedly less than that in the other lanes, as determined by ethidium bromide staining, thus explaining the reduced signal density (not shown). The parents of patients T1, T3, and T4 were not available for study. The molecular weights were estimated by comparison with *Saccharomyces cerevisiae* molecular-weight markers. cz = Compression zone.

Discussion

In this study it has been demonstrated that four SHFD patients with balanced translocations have breakpoints that cluster within a discrete interval defined by the smallest SHFD-associated deletion. Moreover, PFGE analysis indicated that three of these breakpoints localize to within 670 kb of a specific DNA marker that lies within the critical region. The clustering of these translocation breakpoints among unrelated individuals provides compelling evidence that the SHFD1 gene is located in this interval.

Presumably, all of the patients deleted for this portion of chromosome 7 have null alleles, rendering them hemizygous for the wild-type SHFD1 gene. Thus, haploinsufficiency of the SHFD1 gene product results in the SHFD phenotype and is likely to be the mechanism responsible for the dominant mode of transmission typically observed in this disorder. Precedent exists for haplo-insufficiency as a cause of other human developmental malformations. For example, Waardenburg syndrome and aniridia have recently been found to result from mutations in PAX3 and PAX6, respectively (Jordan 1992; Tassabehji 1993).

The phenotypic variation between these patients is of interest. Of the seven patients analyzed, three had all four limbs severely affected. However, two deletion patients (D1 and D2) and two translocation patients (T3 and T4) each had at least one normal extremity. This variation in phenotype even among individuals who presumably have null mutations indicates that other factors (either stochastic or genetic) contribute to the ultimate expression of the SHFD phenotype and that they may be the same factors that are generally responsible for variable expressivity and reduced penetrance in this disorder. Finally, it should be noted that patient D1 has a cleft palate and that patient T4 has a bifid uvula (a mild form of cleft palate). Patient T4's

breakpoint lies <670 kb from the other patients' breakpoints, and patient D1's deletion encompasses the SHFD1 critical region. These findings suggest a possible association between SHFD and the EEC syndrome, an autosomal dominant condition that consists of ectrodactyly, ectodermal dysplasia, and cleft lip/cleft palate. Syndromes such as EEC, in which ectrodactyly is just one of several congenital abnormalities, may result from disruption of the same gene or contiguous genes. In support of this conjecture, two recent reports have described EEC patients with cytogenetic abnormalities of the long arm of chromosome 7 (Akita et al. 1993; Fukushima et al. 1993).

The translocation breakpoint in patient T3 is located within the same physically defined interval as are the other three patients' breakpoints, but it does not show an altered fragment by PFGE. It is possible that an altered fragment may be either so large or so small that it is not evident on pulsed-field gels under the conditions employed. Likewise, it may comigrate with the normal fragment and not be visible. However, these explanations seem unlikely, in light of the fact that (*a*) the DNA has been digested with numerous enzymes and (*b*) an altered fragment has yet to be detected. One plausible explanation for these findings is that the SHFD1 gene is relatively large, so that breakpoints that are physically distant from one another still disrupt the SHFD1 locus. Alternatively, it is possible that a cluster of genes exist in this region that are involved in various aspects of limb development—and that they result in similar phenotypes when mutated. Finally, there may be *cis*-acting sequences some distance from the SHFD1 gene, which, when disrupted by a translocation, alter expression of the gene and result in a weakly penetrant SHFD phenotype. This latter explanation is particularly attractive, since in the family of patient T3, 12 individuals have the translocation but only 3 show an SHFD phenotype (the other 9 are phenotypically normal). Intriguingly, another human developmental abnormality, Grieg cephalopolysyndactyly, has been shown to result from a translocation breakpoint >20 kb distant from the coding region of the causative gene, *GLI3*, and it presumably disrupts important regulatory sequences (Vortkamp et al. 1991).

Several of the chromosomal breakpoints in the five non-SHFD somatic cell lines have been defined by DNA markers contained within YAC contigs (Scherer et al. 1993). The results from the present study combined with the previous analysis suggest a physical order of the following reference markers for the 7cen-q31 region: cen–*D7S456*–*GUSB* – *D7S186* – *ASL* – *HGF* – (*PGY1* – *PGY3*) – *D7S15* – *COL 1A2* – *PON* – *D7S812* – *SHFD1* – *D7S811* – *ASNS* – (*ACHE* – *GNB2* – *EPO*) – *D7S238* – *D7S2 40* – (*MUC3* – *CUTL1*–*PLANHI*)–(*PRKAR2B*–*D7S13*)–*LAMB1*.

COL1A2 and *LAMB1* have been localized to the 7q21-q22 region and represent candidate SHFD1 genes. Both of these gene products play a role in structural integrity of connective tissue, are expressed early in development, and therefore could be implicated in a disorder of abnormal morphogenesis such as SHFD. However, the physical mapping data presented here argue strongly against either of these genes being responsible for this disorder, as both are located well outside the critical SHFD1 region.

A mouse model of human SHFD would be valuable in efforts to identify the causative gene and to study it once it has been isolated. However, no previously described mouse mutants with limb defects have been identified that map to the regions of either distal mouse chromosome 5 or proximal mouse chromosome 6 that are syntenic with the long arm of human chromosome 7.

The localization of 93 DNA segments into 10 intervals by using the SCHs described here will be useful in the analysis of 7q breakpoints associated with other disease conditions. For example, several patients with acute nonlymphocytic leukemia (ANLL) and acute myelogenous leukemia (AML) have interstitial 7q deletions with breakpoints between *COL1A2* and *EPO* (Kere 1989; Kere et al. 1989).

The physical mapping data presented in this study were obtained from hybrids derived from several independent sources and, besides being consistent internally, agree well with the proposed genetic order of several of the DNA markers (Helms et al. 1992). Moreover, many of these results have been confirmed using FISH mapping experiments (authors' unpublished data). Thus, these data provide valuable reference markers for physical mapping of the entire region and serve to clarify previously ambiguous mapping information. For example, RFLP and dosage analyses of granulocyte DNA derived from ANLL and AML patients with cytogenetic deletions have led to conflicting results regarding the order of *PGY1*, *COL1A2*, and *EPO* (Grzeschik et al. 1993). In light of the present data, it is likely that the rearrangements in these patients are more complex than cytogenetic analysis indicates. Likewise, our initial mapping of *COL1A2*, *PON*, and *D7S15* as proximal to *PGY1* (Scherer et al. 1993) was based on hybridization results from using the 1EF2/3/K017 cell line, which, in view of the present data, appears to contain a more complex rearrangement than had been suspected previously (fig. 3).

Interestingly, the distal breakpoints in all three of the SHFD deletion patients appear to be clustered, with no currently available DNA markers capable of distinguishing them. Through screening of a human chromosome 7–specific YAC library, clones have been identified for most of the DNA markers shown in figure 3 (Scherer et al. 1993). A detailed physical map of these YAC clones and the genomic region should form a basis for the analysis of various rearrangement breakpoints and further gene-mapping studies.

The localization of DNA markers within the SHFD1 critical interval at 7q21.3-q22.1 provides valuable entry points for a YAC-based positional cloning approach to analysis of the SHFD1 region. Such analysis should lead to

isolation and characterization of *SHFD1*, a human developmental gene involved in pattern formation of the limb, and will allow investigation of the molecular basis for its distinctive genetic characteristics.

Acknowledgments

The authors would like to thank Drs. David Cox and Anat Taraboulos for supplying the hamster RJK cell line and for helpful advice regarding the generation of SCHs. This work is supported by funds from the Lucille P. Markey Foundation, the Canadian Genome Analysis and Technology Program, and the Howard Hughes Medical Institute (International Scholarship to L.-C.T). S.W.S. is the recipient of a studentship from the Medical Research Council (MRC) of Canada. L.-C.T. is an MRC Scientist and Sellers Chair of Cystic Fibrosis Research. J.P.E., P.P., M.N., and D.G. were supported by the Lucille P. Markey Molecular Medicine Center at The University of Washington. F.G. is a fellow of the Associazione Studio Malformazioni, Milan.

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