Identification of a Yeast Artificial Chromosome Clone Spanning a Translocation Breakpoint at 7q32.1 in a Smith-Lemli-Opitz Syndrome Patient

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Summary

Smith-Lemli-Opitz syndrome (SLOS) is a mental retardation/multiple congenital anomaly syndrome. The gene(s) involved has not been mapped or cloned, but, recently, a biochemical abnormality in cholesterol biosynthesis has been shown to occur in most SLOS patients. The defect is suspected to occur in the penultimate step of the cholesterol pathway, involving the enzyme 7-dehydrocholesterol reductase, which has not been isolated. On the basis of the hypothesis that a de novo balanced translocation [t(7;20)(q32.1;q13.2)] in an SLOS patient directly interrupts the *SLOS* gene, positional cloning techniques are being employed to localize and identify the *SLOS* gene. We report the identification of a chromosome 7–specific YAC that spans the translocation breakpoint, as detected by FISH. This is the first study narrowing a candidate *SLOS* region and placing it on physical and genetic maps of the human genome.

Introduction

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder characterized by a constellation of multiple congenital anomalies, mental retardation, and failure to thrive (Smith et al. 1964; Cherstvoy et al. 1984). While there are few features specific to SLOS, some particular findings often seen include ambiguous genitalia in males, 2–3-toe syndactyly, microcephaly, thickened palatal ridges, and cleft palate. The condition may be classified as type I or II, representing mild and severe forms, respectively, possibly due to allelic variation (Curry et al. 1987). The incidence of SLOS is estimated to be in the range of 1/20,000–1/40,000 births, establishing it as one of the more frequent autosomal recessive disorders among Caucasians (Lowry and Yong 1980). Recently, it was discovered that a defect in the cholesterol biosynthetic pathway is present in SLOS patients (Irons et al. 1993; Tint et al. 1994). Patients' serum-cholesterol levels, as well as the cholesterol levels in all tissues studied, are significantly reduced, while an immediate cholesterol precursor, 7-dehydrocholesterol (7-DHC), is present at greatly elevated levels. It has been proposed that the accumulation of 7-DHC and the associated decrease in cholesterol is the result of a defect in the enzyme 7-dehydrocholesterol reductase (7-DHCR), which transforms 7-DHC to cholesterol (Tint et al. 1994). Since this protein has not been isolated or the corresponding gene cloned, the role of 7-DHCR in SLOS has yet to be determined.

We recently reported a patient (UF53) with the diagnosis of SLOS type II, having a de novo balanced translocation t(7;20)(q32.1;q13.2), and proposed that the translocation interrupts the *SLOS* gene on chromosome 7, while a subtle mutation disrupts the other allele (Wallace et al. 1994). Support for choosing to pursue the chromosome 7 breakpoint rather than chromosome 20 is based on other reports of SLOS patients with chromosomal aberrations involving distal 7q (Wallace et al. 1994); specifically, another SLOS type II patient exhibits a balanced translocation involving 7q32 (Curry et al. 1987). We report here confirmation of the biochemical defect in UF53, verifying the diagnosis of SLOS and strengthening the suggestion that cloning of the breakpoint region will reveal an *SLOS* gene. Because of a lack of large, well-documented SLOS families, no linkage data are available. Since neither genetic mapping data nor 7-DHCR protein sequence data are available, this translocation is invaluable in mapping this disorder and provides a resource for positional cloning of an *SLOS* gene. As an initial step, using FISH, we have identified a YAC clone that spans the 7q32.1 translocation

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Table 1

Summary of FISH Mapping Experiments Using YACs

YAC Clones	Map Location on chromosome 7[a]	Size (kb)	DNA Markers Contained within YAC	Location Relative to the Translocation Breakpoint[b]
HSC7E61	7q21.2–q21.3	460	D7S558, D7S646, D7S657, D7S689	Proximal
HSC7E67	7q32	360	D7S648	Proximal
HSC7E451	7q32	590	D7S487, D7S648	Proximal
HSC7E1351	7q32	1,200	D7S686, AFMa125wh1	Proximal
HSC7E1289	7q32	1,800	D7S686, AFMa125wh1, D7S680, D7S514	Crosses
HSC7E261	7q32–q33	580	D7S514, D7S635	Distal
HSC7E476	7q32–q33	700	D7S514, D7S635	Distal
HSC7E888	7q32–q33	410	D7S514	Distal
HSC7E244	7q32–q33	680	D7S680, D7S514, D7S635	Distal
HSC7E77	7q33	380	HBNF	Distal
HSC7E81	7q33	350	HBNF	Distal
HSC7E117	7q33–q34	480	TCRB	Distal
HSC7E591	7q33–q34	580	TCRB	Distal
HSC7E135	7q36	1,300	...	Distal
HSC7E5	7q36	300	D7S104	Distal

[a] The chromosome locations of the (HSC7E)-YACs were determined by FISH in this study or in another study (Kunz et al. 1994).
[b] Proximal = signals observed proximal to the breakpoint (retained on der7); distal = signals observed distal to breakpoint (transferred to the chromosome 20 translocation partner); and crosses = probe crosses the breakpoint (signals were observed on both translocation derivatives).

breakpoint in UF53. These results place an *SLOS* candidate gene region on a fine-level physical map, on the genetic map, and narrow this region to <2 Mb.

Methodology

Biochemical Assessment of Patient

The initial diagnosis of SLOS in patient UF53 was based solely on clinical presentation, since the defect in cholesterol biosynthesis had not been established before her death. Although the patient had low serum-cholesterol levels, her 7-DHC had not been measured. To confirm the diagnosis of SLOS, skin fibroblast cell cultures established from the patient at 3 mo of age were analyzed for cholesterol and 7-DHC levels by capillary-column gas chromatography (Tint et al. 1994).

YAC Clones and DNA Markers

The YAC clones were isolated from a chromosome 7–specific YAC library (Scherer et al. 1992) or from the CEPH-Généthon library by screening with the DNA markers listed in table 1. Information on the DNA markers can be found in the Genome Data Base. The unpublished Généthon microsatellite marker AFMa125wh1 was kindly provided by Dr. Jean Weissenbach. The sizes of the YACs were determined by pulsed-field gel electrophoresis of the yeast chromosomes followed by blot hybridization with vector-specific probe (pBR322) and comparison to YPH149 standards (Scherer and Tsui 1991). The sizes of the CEPH-Généthon YACs were found to be approximately the same size as is described in their database.

Alu PCR

Yeast containing chromosome 7 YACs were grown in yeast extract-peptone-dextrose medium to a density of 2×10^7 cells/ml (Scherer et al. 1993). The cells underwent spheroplasting using the Yeast Cell Lysis Preparation kit (BIO 101), and high-molecular-weight DNA was prepared using the G NOME™ DNA Kit (BIO 101). The isolated DNA was phenol-chloroform extracted, precipitated, and dissolved in Tris-EDTA. Approximately 20–30 ng of purified DNA from each YAC were subjected to inter-*Alu* PCR under conditions described by Tagle and Collins (1992) using combinations of *Alu*- and YAC-vector primers (Brooks-Wilson et al. 1990; Riley et al. 1990; Lengauer et al. 1992; Tagle and Collins 1992).

FISH

Five-hundred nanograms of combined *Alu* and *Alu*-vector PCR products were biotinylated by random priming using the BioPrime™ DNA Labeling System (Gibco BRL). Approximately 100–150 ng of biotinylated DNA were used as a probe for each slide. Peripheral lymphocyte and lymphoblastoid chromosome spreads were freshly prepared by standard cytogenetic procedures (Yunis 1974). The denatured biotinylated YAC probe was simultaneously hybridized to the slides with digoxygenin-labeled alpha-satellite centromeric probes for

chromosomes 7 and 20 (Oncor), using Cot-1 DNA competition (Gibco-BRL) (Pinkel et al. 1986). Slides were hybridized for ~16 h at 37°C. Post-hybridization washes consisted of one 1 × SSC wash at 72°C for 5 min followed by three consecutive washes in 1 × PBD for 3 min at room temperature. Signal detection was attained by incubation with fluorescein isothiocyanate-conjugated (FITC) avidin and antidigoxygenin (Oncor). One round of signal amplification, using antiavidin/FITC-avidin, was also carried out. Chromosomes were counterstained with propidium iodide (Oncor). The hybridization signals were analyzed and documented with an Olympus BHS fluorescence microscope system and the CytoVision/CytoProbe computer imaging device (Applied Imaging).

Results

Biochemical Diagnosis of SLOS

UF53 had serum-cholesterol levels of 45 and 55 mg/dL, at age 3 weeks and 5 weeks, respectively. In contrast, plasma cholesterol levels in three-month-old children fed low cholesterol corn-soybean oil–based diets are reported to be 109±9 mg/dL with a 95% confidence interval of 98–120 mg/dL, (Hayes et al. 1992). Recent studies of UF53 fibroblast cell cultures also displayed a significantly reduced cholesterol level, which is associated with a substantial increase in 7-DHC. The concentrations of cholesterol and 7-DHC in the fibroblast cultures were 48 and 4.0 mg/2 × 10^6 cells (respectively, in lipidated media). In normal fibroblasts, this assay shows cholesterol concentrations in the range 40–60 mg/2 × 10^6 cells, whereas 7-DHC was absent. 7-DHC constituted 10% of the total neutral sterols in UF53's fibroblast cell line. These results, as well as the clinical findings, confirm the diagnosis of SLOS in this patient.

Localization of the Translocation Breakpoint at 7q32.1 Using FISH

Alu PCR products from 11 YAC clones (HSC7E61, HSC7E67, HSC7E451, HSC7E1351, HSC7E888, HSC7E77, HSC7E81, HSC7E117, HSC7E591, HSC7E135, and HSC7E5), from locations throughout the long arm of chromosome 7, were initially used as probes in FISH mapping experiments to determine their location relative to the UF53 translocation breakpoint. Examples of the hybridization signals are shown in figure 1, and the results are summarized in table 1.

HSC7E1351 and HSC7E244, which were previously mapped to the 7q32 region (Kunz et al. 1994), were determined by FISH to be proximal (centromeric) and distal (telomeric), respectively, to the translocation breakpoint (table 1). These two clones were also already linked within a contig of 14 YACs anchored by the microsatellite markers AFM323wd5 (D7S686), AFMa125wh1, AFM309yf1 (D7S680), AFM218xf10 (D7S514), and AFM206xc1 (D7S635) (fig. 1). The contig spans ~3 Mb (fig. 1), and the genetic distance between D7S686-(D7S680-D7S514-D7S635) is 1 cM (Gyapay et al. 1994).

To refine the localization of the translocation breakpoint within the YAC contig, *Alu* PCR products from four additional YACs were tested by FISH. Three of the YACs (HSC7E888, HSC7E261, and HSC7E476) appear to be distal to the breakpoint (table 1). However, HSC7E1289 was found to span the breakpoint, since, in addition to showing a signal only on the normal chromosome 7 (and not the normal chromosome 20), it also showed signals on both translocation derivatives (fig. 1). The combination of the FISH results from the other YACs suggests that the *SLOS* breakpoint lies in the centromeric half of HSC7E1289 (fig. 2). The results unequivocally support the karyotyping results that placed the UF53 translocation breakpoint (and presumably *SLOS*) at 7q32.1.

Discussion

The existence of an SLOS patient with a de novo balanced translocation suggests a direct association between the chromosomal abnormality and the syndrome. The UF53 translocation has served as a landmark in our initial search for the *SLOS* gene. We have identified an 1,800-kb YAC, HSC7E1289, that spans the translocation breakpoint, moving the level of map resolution for this candidate gene region from the cytogenetic band to the megabase-level.

Because of the large size of HSC7E1289, steps were taken to narrow the region of the breakpoint. HSC7E1351 and HSC7E244, which overlap HSC7E1289, mapped proximal and distal, respectively, by FISH. These results tentatively place the breakpoint between markers AFMa125wh1 and AFM309yf1 (fig. 2). It should be noted, however, that the *Alu* PCR FISH approach yields results of a qualitative nature. Since *Alu* element distribution is likely nonuniform and unknown for all of these YACs, the *Alu* PCR probe fragments may not be representative across the entire YAC; thus, lack of signal on one or the other derivative chromosome may be a false negative signal. However, the probes included vector-*Alu* PCR products (present for most of the YACs) to help represent the YAC ends in the probe mixture. Other false negative signals may be the result of a microdeletion at the breakpoint, for which there is currently no evidence. Therefore, we cannot yet exclude the breakpoint from the other regions of HSC7E1289, and our conservative estimate of the *SLOS* breakpoint region is ~1.8 Mb. Efforts are continuing to further narrow and characterize this region by using FISH with additional YACs and cosmids. Ultimately, positional

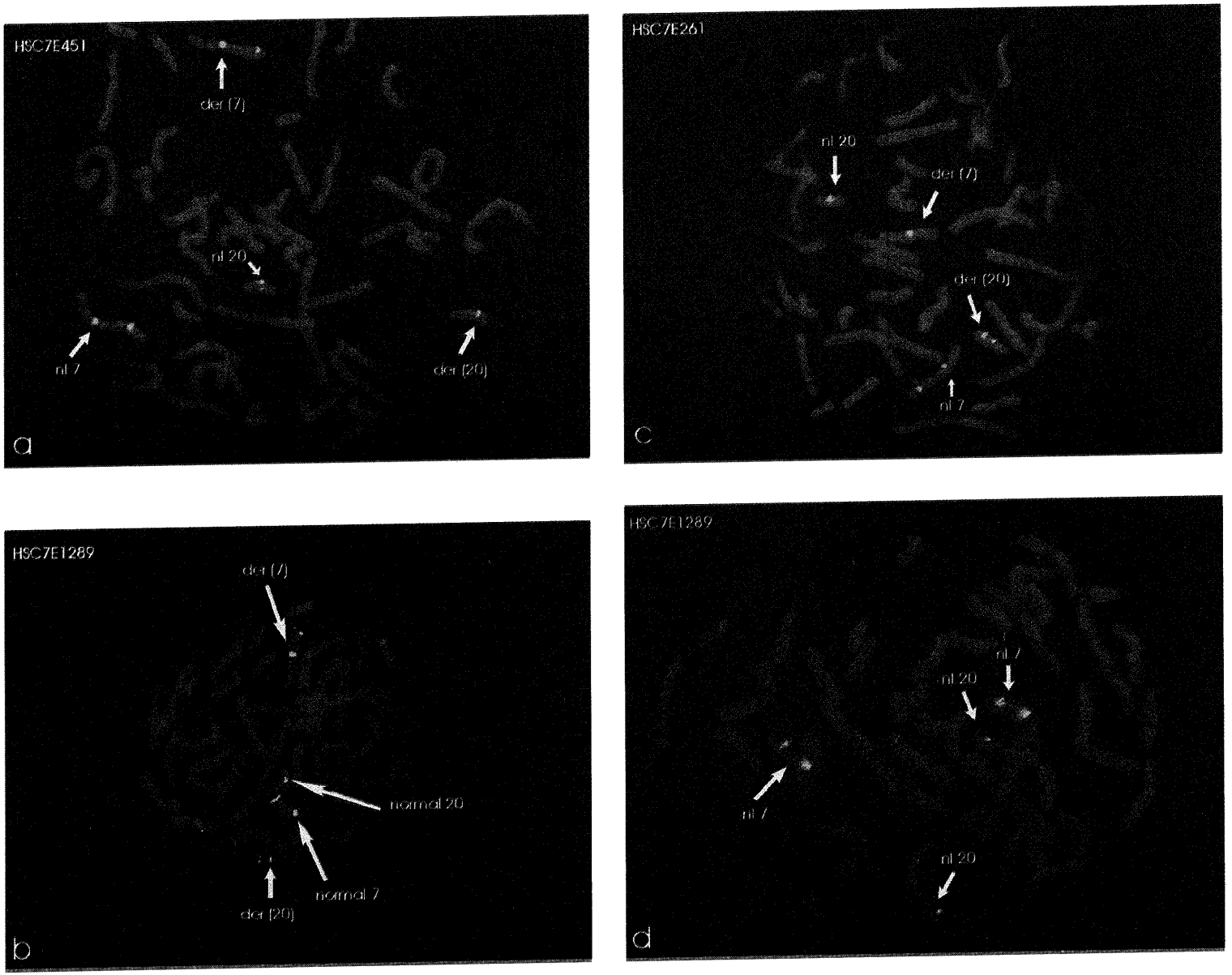

Figure 1 FISH analysis of the SLOS translocation breakpoint region using YAC clones from the 7q32 region. Biotin-labeled *Alu* PCR products from the YACs (yellow) were cohybridized with digoxygenin-labeled alpha-satellite centromeric probes for chromosome 7 and 20 (yellow) to metaphase chromosomes, under competitive conditions. Metaphase spreads of UF53 lymphocytes after hybridization with the YAC clones (*a*) HSC7E451, (*b*) HSC7E1289, and (*c*) HSC7E261 demonstrate proximal, spanning, and distal positioning of probes, respectively. Metaphase spread of an unaffected individual (*d*) hybridized with HSC7E1289 serves as a control. Chromosomes are counterstained with propidium iodide. Arrows point to the centromeric signals specific for chromosome 7 and 20.

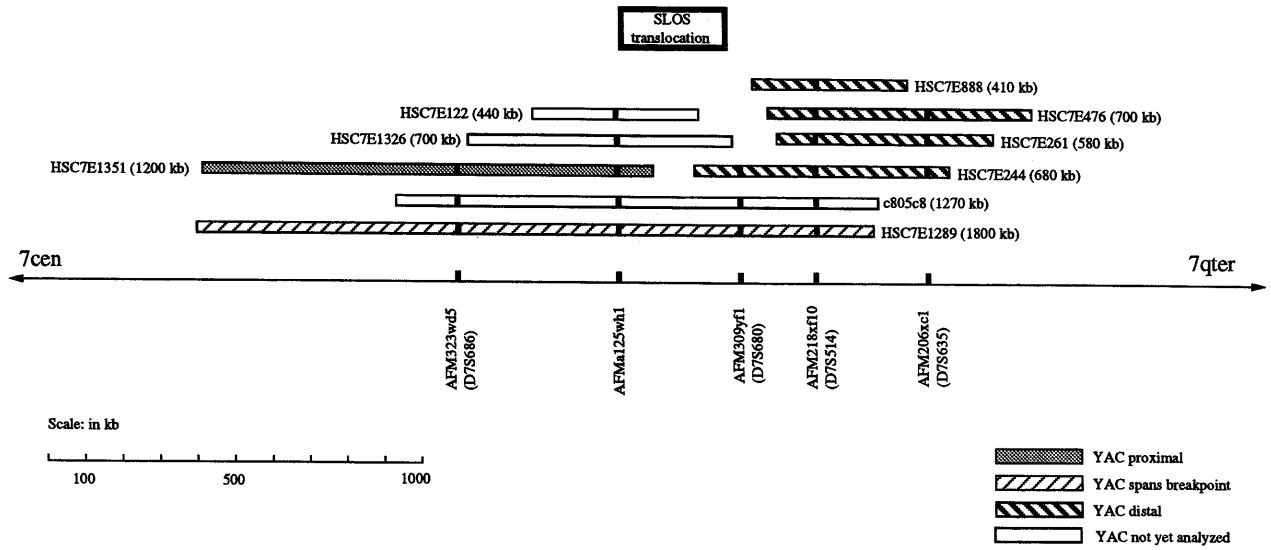

Figure 2 Tentative map of YACs overlapping HSC7E1289, which is known to span the translocation breakpoint. Only the shaded or striped YACs have been tested against the breakpoint, and their locations are indicated according to the key. The YACs represented are considered nonchimeric, since the marker data are consistent, and the YACs were derived from a chromosome 7 library in which chimerism is unusual (Kunz et al. 1994). Genetic markers used to determine overlaps are listed below the line. FISH results suggest that the translocation most likely falls between markers AFMa125wh1 and AFM309yf1, as indicated by the box.

cloning efforts will be directed at isolating cDNAs from the region and testing each of these candidate genes for disruption by the translocation.

The wide phenotypic spectrum of SLOS causes a dilemma in clinical diagnosis; it is thought that many cases of SLOS are not appropriately diagnosed and are categorized under the nonspecific label of multiple congenital anomalies/mental retardation syndrome. SLOS, previously viewed as an uncharacterized, difficult-to-diagnose, rare autosomal recessive disorder, has been seen in a new light since the recent discovery of the associated defect in cholesterol biosynthesis. However, it is possible that the biochemical findings may not be exclusive to SLOS and that there may be biochemical or genetic heterogeneity. It is clear that, for clinical purposes and development of effective therapies, correct diagnosis is crucial, and it will be very important to elucidate the gene(s) involved in SLOS. Identification of *SLOS* gene(s) will be essential in understanding the pathology of this disorder and the involvement of 7-DHCR. Mutation studies in SLOS may help elucidate the phenotypic diversity and genetic heterogeneity of this syndrome. Our localization of the *SLOS* candidate gene region in 7q32 is a significant step toward this goal.

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