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Evidence That the Saethre-Chotzen Syndrome Locus Lies between D7S664 and D7S507, by Genetic Analysis and Detection of a Microdeletion in a Patient

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Summary

The locus for Saethre-Chotzen syndrome, a common autosomal dominant disorder of craniosynostosis and digital anomalies, was previously mapped to chromosome 7p between D7S513 and D7S516. We used linkage and haplotype analyses to narrow the disease locus to an 8-cM region between D7S664 and D7S507. The tightest linkage was to locus D7S664 ($\hat{Z}$ = 7.16, θ = .00). Chromosomes from a Saethre-Chotzen syndrome patient with t(2;7)(p23;p22) were used for in situ hybridization with YAC clones containing D7S664 and D7S507. The D7S664 locus was found to lie distal to the 7p22 breakpoint, and the D7S507 locus was deleted from the translocation chromosomes. These genetic and physical mapping data independently show that the disease locus resides in this interval.

Introduction

Saethre-Chotzen syndrome is a common inherited craniosynostotic condition (Cohen 1986). In addition to craniosynostosis (the premature fusion of the calvarial sutures, which may lead to an abnormal head shape), patients with Saethre-Chotzen syndrome display a wide range of associated features. These features include facial asymmetry, a low frontal hairline, ptosis, deviated nasal septum, brachydactyly, and partial cutaneous syndactyly, most often of the second and third fingers and toes (Saethre 1931; Chotzen 1932). Saethre-Chotzen syndrome is inherited in an autosomal dominant fashion, with high penetrance and variable expressivity. The actual frequency of this condition remains unknown, because mild cases may go undetected, and sporadic cases, which feature only craniosynostosis or syndactyly (but not both), are often misdiagnosed (Cohen 1986). Saethre-Chotzen syndrome is believed to occur more frequently than Crouzon syndrome, a well-recognized craniosynostotic condition with an incidence of ~1/50,000 (Cohen and Kreiborg 1992).

The Saethre-Chotzen syndrome gene has been mapped to distal chromosome 7p (Brueton et al. 1992), specifically in the 7p21-p22 region. The tightest linkage was with two markers: pJ5.11, which maps in the 7p22-p15 region, and pRMU7.4, which maps in the 7p21-p15 region. Previously, the location of the Saethre-Chotzen syndrome gene was narrowed to a 27-cM region on distal 7p, between loci D7S513 and D7S516 (Lewanda et al. 1994).

Several chromosomal translocations involving chromosome 7p2 have been reported in patients with Saethre-Chotzen syndrome. Reardon et al. (1993) reported an affected father and daughter who shared an apparently balanced t(7;10)(p21.2;q21.2) translocation, implicating 7p21.2 as the causative locus. Three additional cases with apparently balanced translocations at 7p21.2 have recently been reported (Rose et al. 1994). Reid et al. (1993) presented a mother and son with Saethre-Chotzen syndrome and an apparently balanced t(2;7)(p23;p22) translocation. This finding points instead to the 7p22 region as the location for at least one of the genes involved in the Saethre-Chotzen syndrome.

Our goal was to further refine the location of the Saethre-Chotzen syndrome gene on chromosome 7p, by using both genetic data and physical data obtained by FISH. Chromosomes from the patient reported by Reid et al. (1993) were examined to investigate the relevance of this translocation with regard to the position of the Saethre-Chotzen syndrome locus.

Patients, Material, and Methods

Patients

Sixty-six individuals (30 males and 36 females) contributing to 44 total meioses in 10 Saethre-Chotzen syndrome

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families were clinically evaluated by one of the authors or had previously been reported by Lewanda et al. (1994), where they had been examined by one of the authors. The proband in each family was affected with at least four features of this condition. Because this is a highly penetrant disorder, family members with at least one of the craniofacial features (craniosynostosis, facial asymmetry, ptosis, or prominent ear crura) or the digital findings of brachydactyly with syndactyly were considered affected. Individuals with none of the characteristic features were considered unaffected. Lymphoblast cultures were established (Penno et al. 1993), and DNA samples were obtained (Sambrook et al. 1989) from all participants. In addition, high-resolution chromosome analysis with banding by Giemsa techniques (Yunis et al. 1978) was performed on peripheral blood from one affected member of each pedigree.

DNA Polymorphic Markers and Linkage Analysis

Polymorphisms in the length of dinucleotide repeats were detected using the PCR (Weber and May 1989). All oligonucleotide primers were synthesized by the Johns Hopkins University Genetic Resources Core Facility. Markers for loci D7S513 and D7S516, plus nine markers known to map between them, were used for analysis. Earlier data on markers AFM217yc5 (D7S513), AFM200we7 (D7S507), AFM162xa7 (D7S493), AFM248zd1 (D7S529), and AFM224xg5 (D7S516) have been published previously (Lewanda et al. 1994). Information on markers AFM199xc3 (D7S503) and AFM113xc11 (D7S488) was obtained from Weissenbach et al. (1992), while that for AFM281vc9 (D7S664), AFM217yb6 (D7S638), AFM102xg7 (D7S654), and AFM290vg9 (D7S673) was derived from Gyapay et al. (1994).

Four hundred nanograms of one primer of each oligonucleotide primer pair was end labeled at the 5′ end with (gamma $^{-32}$P) dATP (Sambrook et al. 1989). The PCRs were carried out in a volume of 15 μl containing 300 ng of genomic DNA template; 45 ng of each unlabeled oligonucleotide primer and 45 ng of the labeled primer; 200 μM each of dATP, dGTP, dCTP, and dTTP; 50 mM KCl; 10 mM Tris (pH 8.3); 1.5 mM $MgCl_2$; 0.001% gelatin; and 0.35 U of AmpliTaq polymerase (Perkin Elmer Cetus).

All PCR reactions were initially denatured at 94°C for 8 min. They were then processed through 30–35 temperature cycles consisting of 30 s at 94°C for denaturation, 30 s–1 min at a primer pair–specific annealing temperature (range 52°C–62°C) and 1 min at 72°C for extension. These cycles were followed by a single cycle of 10 min at 72°C.

Aliquots of amplified DNA were separated by electrophoresis through a 6% polyacrylamide DNA sequencing gel at 50–55 W for 1¾–3 h. Polymorphisms were scored from autoradiographs, and data were entered using linkage format. Linkage analyses were performed using the computer program LINKAGE (Lathrop et al. 1984). An autosomal dominant model was used, and 99% penetrance and a gene frequency of .001 were assumed.

FISH

Metaphase spreads from the Saethre-Chotzen syndrome patient described by Reid et al. (1993) were used for FISH. These cells have a t(2;7)(p23;p22) balanced translocation. YACs, each known to contain a marker locus of interest, were derived from one of two libraries—that prepared at CEPH (Albertsen et al. 1990; Bellanne-Chantelot et al. 1992) and that derived from hybrid cell line GM10791 (E. D. Green, unpublished data). The latter was constructed from a human-hamster hybrid cell line containing chromosome 7 as its only human DNA. Clones containing marker loci were identified by PCR. YAC yWSS2695 (CEPH 774G07) contained D7S493; yWSS1354 contained D7S513; yWSS3476, yWSS2968 (CEPH 802E11), and yWSS2805 (CEPH 784A04) contained D7S507; and yWSS4095 contained D7S664 (Green et al. 1994). These YACs were used as probes for in situ hybridization.

YAC clones were grown in uracil- and tryptophan-deficient AHC medium. DNA was isolated according to the method of Green and Olson (1990). Agarose plugs containing total yeast plus YAC DNA were prepared using protocols described by Southern et al. (1987) and Anand et al. (1989). Orthogonal field alternation gel electrophoresis (OFAGE), as described by Carle and Olson (1985) and Jabs et al. (1989), and low-melting-point agarose (BRL) were used to separate the YAC and yeast chromosomes. Electrophoresis was performed in 0.5 × TBE at 12°C, 240 V, and switch interval of 20–50 s for 20–25 h. The solitary band of YAC DNA was identified, excised, and heated to 65°C to purify the DNA from low-melting-point agarose (FMC Biochemical). Gel-purified YAC DNA was nick-translated with the BioNick Labeling System (GIBCO BRL) for 2 h. Probe mix (50% formamide, 10% dextran sulfate, 2 × SSCP [0.3 M sodium chloride/0.03 M sodium citrate containing 0.04 M sodium phosphate], 25 ng of biotinylated probe/μl, 200 × Cot DNA, and 300 × salmon sperm DNA) was denatured at 70°C for 5 min, followed by preannealing at 37°C for 30 min. One microliter of chromosome 7 alpha-satellite probe was added to 20 μl of Hybrisol VI (both from Oncor) and was denatured. One such alpha-satellite probe sample was added to each 20 μl of YAC DNA probe.

Slides with the metaphase chromosome spreads were treated with RNAse (Oncor) for 1 h at 37°C and denatured in 70% formamide/2 × SSC at 70°C for 2 1/2 min. Forty-one microliters of combined probe mix (YAC and alpha-satellite) were placed on a slide and hybridized at 37°C overnight. Slides were washed in 50% formamide/2 × SSC at 37°C for 20 min. This was followed by two washes of 2 × SSC at 37°C for 5 min each. Biotinylated probe was detected with FITC-avidin and was amplified with biotiny-

Table 1

Two-Point Linkage Analysis between Saethre-Chotzen Locus and 7p Loci

Genetic Distance (cM)	Locus	Z	$\hat{\theta}$
	Telomere		
5	D7S513	5.00	.08
8	D7S664	7.16	.00 ± .08
1	D7S507	4.83	.07
0	D7S503	5.30	.05
0	D7S488	4.47	.08
1	D7S638	3.51	.10
6	D7S654	4.56	.06
4	D7S493	4.98	.04 ± .12
2	D7S673	5.05	.06
2	D7S529	4.76	.06
	D7S516	2.54	.07
	Centromere		

NOTE.—Genetic distances between loci are based on the Genethon map from Gyapay et al. (1994).

lated anti-avidin, using reagents and instructions from an in situ hybridization kit (Oncor). Propidium iodide/antifade mix was placed on the slide, which was then examined under the fluorescence microscope. A signal was scored as positive only if there was hybridization to both chromatids of a chromosome at a single site.

Results

In order to refine the location of the disease gene between loci D7S513 and D7S516, two-point linkage analyses were performed with 10 Saethre-Chotzen syndrome families and 11 markers on chromosome 7p. Most recent data suggest these markers span a genetic distance of 29 cM (Gyapay et al. 1994). As shown in table 1, all loci tested gave positive lod scores, and all but one (D7S516) gave maximum lod scores ($\hat{Z}$) >3.5. The tightest linkage was to marker D7S664, which had $\hat{Z}$ = 7.16 and no recombinants. The confidence limits for linkage between locus D7S664 were 8 cM. The next smallest confidence interval was 12 cM, for marker D7S493. These confidence intervals overlap between D7S664 and D7S507, suggesting that the disease locus lies in this region.

Haplotype analysis was also used to define the location of the Saethre-Chotzen syndrome gene. The location could be determined by informative haplotypes of two affected individuals. Haplotypes of two unaffected individuals were used for confirmation, as accurate diagnosis can be difficult in such a variably expressed disorder. Both sets of haplotypes consistently indicated the same region as containing the Saethre-Chotzen syndrome gene. Figure 1 shows all five families that demonstrated recombination events between a marker locus and the disease gene. The affected grandson in family C served to eliminate all loci centromeric to D7S507 if only one recombination event occurred. Haplotype analysis of the unaffected grandson in family E confirmed the exclusion of those same loci. The affected granddaughter in family D showed that the gene is centromeric to D7S513, and the unaffected granddaughter in family C confirmed this. Thus, the location of the disease gene has been narrowed to the 13-cM region between D7S513 and D7S507. Although this location, based on haplotype analysis, did not further refine the critical region, it is consistent with and includes the region of overlap between confidence intervals for D7S664 (8 cM) and D7S493 (12 cM). This candidate region for the Saethre-Chotzen syndrome locus spans 8 cM and lies between D7S664 and D7S507. Although less likely, if more than a double-recombination event occurred in the affected grandson of family C, the region between D7S654 and D7S673 could not be excluded, and the unaffected grandson in family E could further refine the region to between D7S654 and D7S493. A composite of our two-point linkage and haplotype analysis is shown in figure 2.

FISH was used to obtain physical data on the position of these markers, with respect to the chromosomal breakpoint (7p22) of our Saethre-Chotzen syndrome patient. YACs containing the D7S513 and D7S493 loci mapped distal and proximal to the translocation breakpoint, respectively (data not shown). The location of the breakpoint was further refined by using YACs containing markers that lie between these two. Our results are shown in figure 3. The D7S664 locus in YAC yWSS4095 was found to lie distal to the translocation breakpoint, as the signal was found on the normal chromosome 7 and on the derivative chromosome 2 in 19/20 metaphase spreads. The D7S507 locus was deleted from the translocation chromosomes of our patient. The signal was clearly seen on the normal chromosome 7, but it was not seen on either the derivative 7 or on the derivative 2 in 33/34 metaphase spreads with YAC yWSS3476. This finding was confirmed with two additional YACs that contain the D7S507 locus: yWSS2968/CEPH 802E11 (21/23 spreads) and yWSS2805 /CEPH 784A04 (32/33 spreads). The largest deleted YAC is 1.4 Mb in length, demonstrating that the microdeletion is at least that size.

Marker loci D7S664 and D7S507 flank the Saethre-Chotzen syndrome gene by genetic analysis. D7S664 also flanks the translocation breakpoint distally by in situ–based physical analysis. The D7S507 locus was deleted, which suggests that the Saethre-Chotzen syndrome gene resides in this region and was deleted or disrupted by the translocation event in our patient.

Discussion

A patient's chromosomal translocation is a fortuitous and useful tool for gene mapping. A translocation may dis-

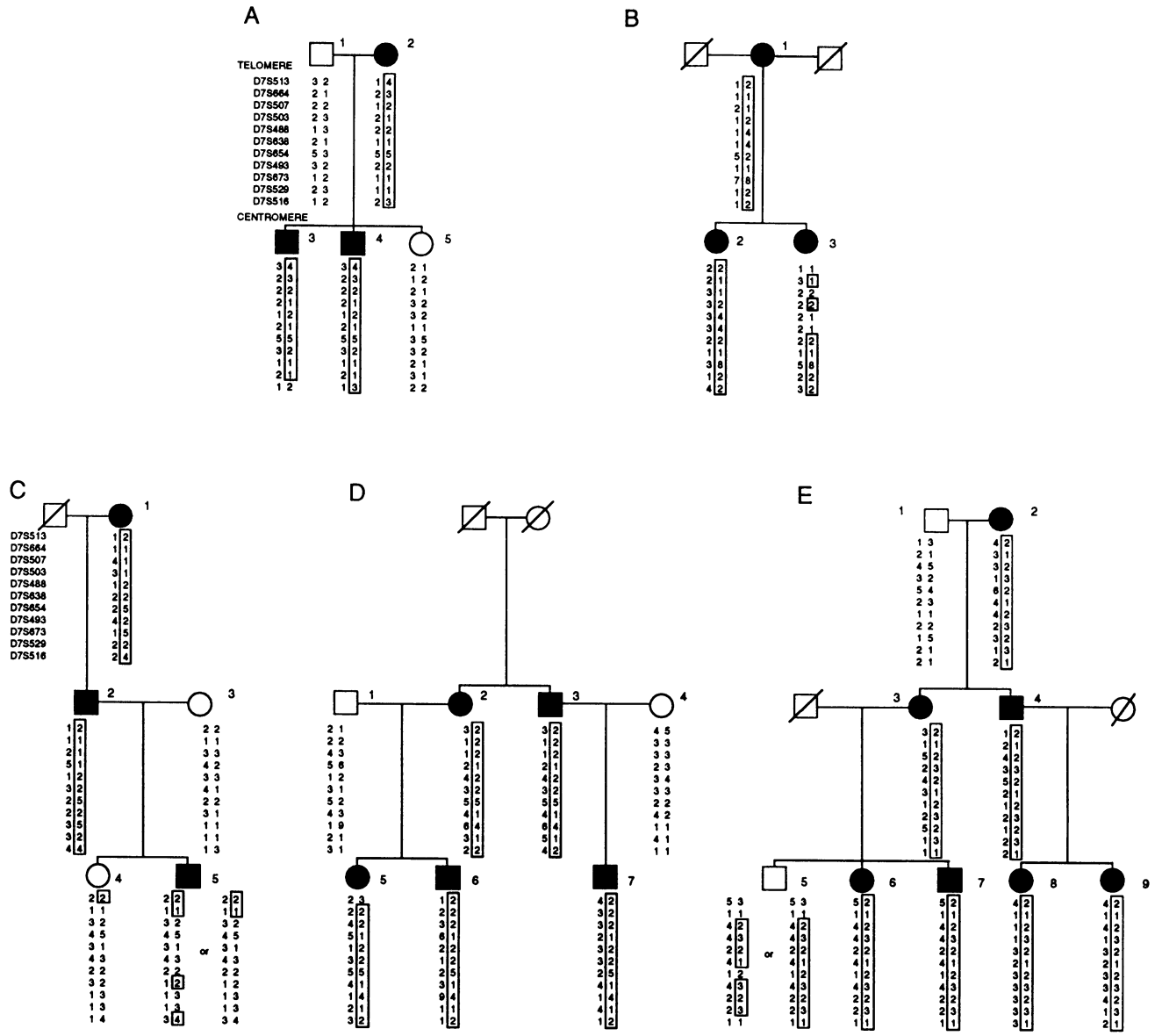

Figure 1 Haplotype analysis of Saethre-Chotzen syndrome families that had recombination events. For affected individuals, the boxed areas include alleles that are either certainly or possibly inherited from an affected parent and show the largest possible region in which the disease locus may lie. For unaffected individuals, the boxed areas include only alleles known to have been inherited from an affected parent, thereby showing the minimal region from which the disease locus can be excluded. The affected grandson in family C and the unaffected grandson in family E have two haplotypes; the one on the right assumes the fewest possible meiotic recombinations in the region. In family B, recombinations could have occurred in either individual 2 or individual 3 because loci D7S513, D7S507, D7S488, and D7S638, each recombined with the disease locus in only one of the two affected daughters, but we cannot determine in which daughter it occurred. Loci D7S664, D7S493, and D7S529 were uninformative. Therefore, it is difficult to determine a definitive haplotype, without the unaffected parents' genotype. For simplicity, we preserved the entire affected haplotype in individual 3 and assigned all the recombination events to the other.

rupt or delete a disease gene, thereby affecting its function and producing the condition. Several genes have been localized in this manner, including that for Greig cephalopolysyndactyly (Vortkamp et al. 1991). Our patient with Saethre-Chotzen syndrome has a chromosomal translocation at 7p22, and this location is consistent with previous genetic mapping data for this locus (Brueton et al. 1992; Lewanda et al. 1994). Another 7p location for the gene has been postulated by investigators who have identified translocations at 7p21.2 in Saethre-Chotzen syndrome patients (Reardon et al. 1993; Rose et al. 1994). These two sites are a chromosomal subband apart, a distance too great to encompass a single gene.

A study by van Herwerden et al. (1994) has placed the

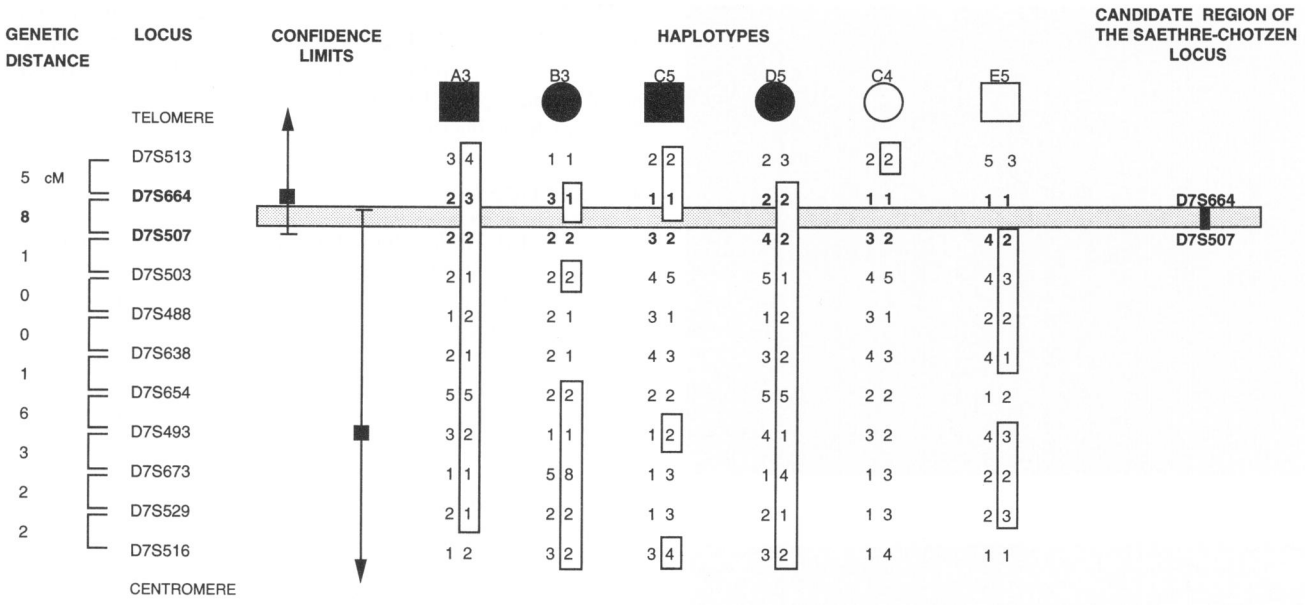

Figure 2 Compilation of linkage and haplotype analysis. Under the column designated "Confidence Limits," the vertical bars show the region where the gene is located, based on confidence intervals for marker D7S664 (*left*) and D7S493 (*right*). Listed under the "Haplotypes" heading are the analyses of specific informative individuals, with the letter of the family, identification number and pedigree symbol as assigned in fig. 1. The candidate region (*shaded*), as shown at the far right of the diagram, is not only consistent with the combined confidence interval but also is consistently transmitted only to affected (not to unaffected) offspring.

Saethre-Chotzen syndrome locus between markers D7S493 and D7S516. Even though this region is not completely excluded by our haplotype analysis if the possibility of double-recombination events is considered, it is excluded by our confidence limits for linkage between the disease and marker D7S664. Similarly, their data appear to exclude the D7S664-to-D7S507 region where we have placed the gene. However, 8/9 informative haplotypes presented by van Herwerden et al. (1994) are consistent with placement of the disease gene between D7S664 and D7S516, which overlaps and includes our critical region between D7S664 and D7S507. Exclusion of our region was based on one haplotype of an affected individual from a small family. It is possible that this family is genetically heterogeneous from the others examined, because the affected members are not entirely typical of Saethre-Chotzen syndrome (Robin Winter, personal communication). If this is the case, the data from their study and ours are not mutually exclusive. Nevertheless, our data are in conflict with the physical mapping results they have presented. Their FISH analysis of four apparently balanced translocations involving chromosome band 7p21.2 in Saethre-Chotzen syndrome patients shows that the breakpoints are flanked by markers D7S488 and D7S493. In one case, the location of the breakpoint is narrowed to between markers D7S488 and D7S654, a region excluded by both our linkage and haplotype data.

The most likely explanation for these findings is genetic heterogeneity, with at least two distinct genes causing phenotypically similar syndromes. Evidence for this is a slight phenotypic difference among the translocation patients. In this small data set, two patients whose breakpoints lie at 7p21.2 show either partial duplication of the great toes or broad great toes and several classic features of the Saethre-Chotzen syndrome (Reardon et al. 1993; Rose et al. 1994). One patient with a breakpoint in the same region has craniosynostosis and prominent helical crura, which are features of Saethre-Chotzen syndrome; another patient has craniosynostosis, abnormal external ears without prominent crura, bifid uvula, and low-normal intelligence, which are also features consistent with either Saethre-Chotzen syndrome or the chromosome 7p–deletion syndrome (Rose et al. 1994). The patient whose breakpoint is at 7p22 has syndactyly without other foot abnormalities, craniosynostosis, facial asymmetry, ptosis, and prominent ear crura, which are all characteristic of Saethre-Chotzen syndrome (Reid et al. 1993).

Other possible explanations for the discrepancy between the results are that two independent genes whose products must interact (e.g., dimerize) to be functional reside in this small region or that the translocations represent complex rearrangements. However, our FISH analysis of our translocation breakpoint at 7p22 excludes gross rearrangements in the location of markers D7S513 and D7S664, telomeric of markers D7S507 and D7S493. Our future efforts will be aimed at refining the breakpoint

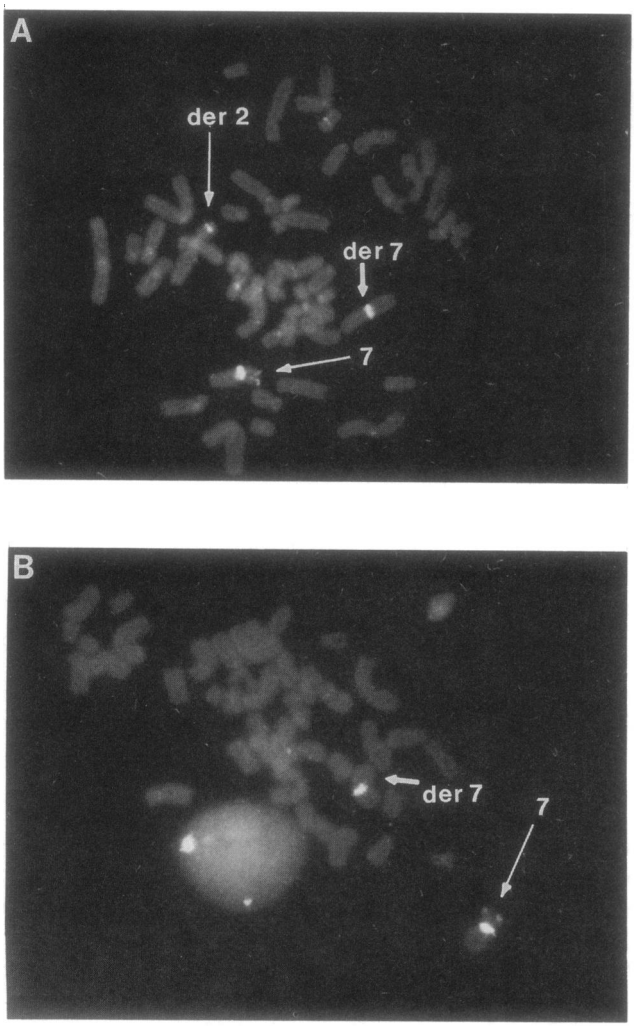

Figure 3 In situ hybridization analysis on t(2;7)(p23;p22) metaphase spreads. *A*, Location of YAC yWSS4095 containing D7S664. The 7-centromere probe on the derivative chromosome 7 (der 7) is indicated by a short arrow. YAC signal (indicated by a long arrow) is seen on both the normal chromosome 7 and the derivative chromosome 2 (der 2). *B*, Location of YAC yWSS3476, containing D7S507. Signal is seen only on the normal chromosome 7; there was no hybridization to either translocation chromosome.

within a YAC contig spanning the region surrounding locus D7S507, as well as cloning the translocation breakpoint in the patient described here. Analysis of the DNA at this site should allow us to identify a causative gene for Saethre-Chotzen syndrome. Similar analysis of the 7p21.2 breakpoint may uncover another gene defect with similar phenotypic effects.

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