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Evidence for Locus Heterogeneity in Acrocephalosyndactyly: A Refined Localization for the Saethre-Chotzen Syndrome Locus on Distal Chromosome 7p—and Exclusion of Jackson-Weiss Syndrome from Craniosynostosis Loci on 7p and 5q

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Summary

Craniosynostosis (premature fusion of the skull sutures) occurs as a clinically heterogeneous group of disorders, frequently involving digital abnormalities. We have previously provisionally assigned the gene for one such condition, Saethre-Chotzen syndrome (ACS III), to chromosome 7p. Linkage analysis is now reported between ACS III and dinucleotide repeat loci on distal 7p. The maximum lod scores, Z_{max}, were 5.57 at a recombination fraction of .05, with D7S488, and 4.74 at a recombination fraction of .05, with D7S493. Only weak linkage, not reaching significance, was found with distal markers (D7S513 and afm281vc9) and a proximal marker (D7S516). Multipoint analysis shows that the disease locus lies between D7S513 and D7S516. Analysis of individual recombinants shows that the most likely position is between D7S493 and D7S516. Linkage data in regard of Jackson-Weiss syndrome demonstrate that this autosomal dominant form of acrocephalosyndactyly does not map to the ACS III region on 7p or to the acrocephalosyndactyly locus on 5q (Boston type). These findings underline the genetic heterogeneity among the different clinical conditions manifesting with acrocephalosyndactyly.

Introduction

Craniosynostosis, the premature closure of one or more of the cranial sutures, is a relatively common birth defect occurring at a frequency of approximately 1/2,500 persons. Craniosynostosis occurs frequently as an isolated abnormality but may also occur in association with other congenital anomalies. In 1986 Cohen (1986) identified more than 60 syndromes involving craniosynostosis, and the number has grown since (Winter and Baraitser 1993). Pedigrees manifesting autosomal dominant forms of craniosynostosis provide a means of identifying genes that are involved in premature fusion of the cranial sutures.

The acrocephalosyndactylies (ACSs) consist of a clinically similar group of autosomal dominant craniosynostosis syndromes, with characteristic features being craniosynostosis occurring in association with distal limb anomalies, in particular syndactyly. We have previously reported data suggesting the mapping of one of these conditions, Saethre-Chotzen syndrome (ACS III), to 7p, by using conventional biallelic markers (Brueton et al. 1992). We have now undertaken more detailed linkage studies of six pedigrees fulfilling strict diagnostic criteria for classical ACS III syndrome, using dinucleotide $(CA)_n$ repeats that map to 7p (the Généthon microsatellite map).

Jackson-Weiss syndrome is an autosomal dominant syndrome of craniosynostosis, midfacial hypoplasia, and abnormalities of the feet (Jackson et al. 1976). Penetrance is high, but there is great variability of expres-

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sion both within and between families. The disorder has been considered to be a possible allelic variant of one of the non-Apert dominant craniosynostosis syndromes, as there are clinical similarities to ACS III, Pfeiffer (ACS V), and Crouzon (craniofacial dysostosis) syndromes (Gorlin et al. 1990). To date, no linkage data have been published to substantiate or refute this possibility.

Apart from ACS III, none of the other major clinically defined forms of craniosynostosis have been mapped. A novel form of autosomal dominant craniosynostosis (Boston type) was recently described (Warman et al. 1993) and mapped to chromosome 5q (Muller et al. 1993). No significant hand or foot anomalies were observed in that family, apart from a short first metatarsal in three cases and a triphalangeal thumb in a single individual, but there were craniofacial similarities to ACS syndromes. We have undertaken linkage studies on a family with Jackson-Weiss syndrome, to see if this condition might be allelic with any of the mapped forms of ACS.

Patients, Material, and Methods

Patients

Fifty-one members of six families that satisfied strict criteria for classical ACS III were recruited, comprising 28 affected and 23 unaffected individuals (see fig. 1). Five of these families (fig. 1*A–E;* pedigrees 1, 4, 5, 11, and 13, respectively, in Brueton et al. 1992) had previously been studied. Pedigrees were chosen because they had classical ACS III and were of a suitable pedigree structure. The diagnosis of ACS III was made on the basis of physical examination and radiographic findings. There was no evidence of nonpenetrance in obligate gene carriers, although, as expected, clinical expression was variable (Gorlin et al. 1990).

A four-generation Jackson-Weiss kindred consisting of 8 affected and 13 normal individuals was also recruited (fig. 2). This family was originally reported as a possible example of Pfeiffer syndrome (Baraitser et al. 1980); however, Gorlin et al. (1990) consider the clinical features typical for Jackson-Weiss syndrome, and further clinical evaluation of the family supports this interpretation. This family (family 16 in Brueton et al. 1992) was included in our initial linkage investigations in ACS.

Primers

$(CA)_n$ repeat primers used at various loci on 7p are summarized in the Généthon report (Weissenbach 1992), except for afm281. Additional primers used were afm281vc9—(AC) AATTCTATCTTTCCAGGATTATCTG and (GT) GATCAGTGCTGGTATAAATAGTAGGT; afm290vg9—(AC)GGGGNCCTTGAGAAGT and (GT) TCCCAGTCCTGTGGCTAC; and D5S211 (Muller et al. 1993).

DNA Analysis

Genomic DNA was extracted from peripheral blood samples from all study subjects, by standard techniques (Aldridge et al. 1984). DNA was amplified in the PCR using primers flanking $(CA)_n$ repeat polymorphisms. Each PCR reaction contained 50 ng of genomic DNA; 10 mM Tris pH 8.3; 1.5 mM $MgCl_2$; 50 mM KCl; 200 μM each of dGTP, dATP, and dTTP and 20 μM dCTP; 0.7 μCi of alpha-^{32}P-dCTP; 25 pmol of each primer; and 0.5 units of *Taq* polymerase (Bioline) in a final volume of 20 μl. Amplification products were separated by denaturing PAGE (Weber et al. 1991). Autoradiography was performed at −70°C with radiographic film (X-Omat; Kodak) for 12–48 h.

Data Analysis

Pairwise linkage analysis between individual markers and the disease trait was carried out using LINKAGE, version 5.03 (Lathrop et al. 1985). Penetrance was assumed to be complete. The frequency of the mutant allele was set to 1/40,000.

Results

Pairwise lod score (Z) values for equal male and female recombination between ACS III and each of the chromosome 7 markers are shown in table 1. ACS III showed only weak linkage to markers proximal to D7S493 or distal to D7S488. However, results obtained with D7S493 and D7S488 show strong evidence for linkage to the disease locus. The highest two-point Z (Z_{max}) was for D7S488: $Z_{max} = 5.57$ at recombination fraction (θ) = .05. A significant Z value was also obtained at D7S493: $Z_{max} = 4.74$ at $\theta = .05$. Tight linkage to D7S513, afm281, and D7S516 was excluded.

Multipoint analysis, performed using LINKMAP, indicated that the disease locus is most likely in the interval between D7S493 and D7S516: $Z_{max} = 6.5$. The data do not rule out the possibility that the disease locus is in the interval between D7S488 and D7S493: $Z_{max} = 5.5$ in this region. All recombinant chromosomes in the families studied are schematically represented in figure 3. Comparing individual recombination events suggests that the smallest region likely to contain the disease

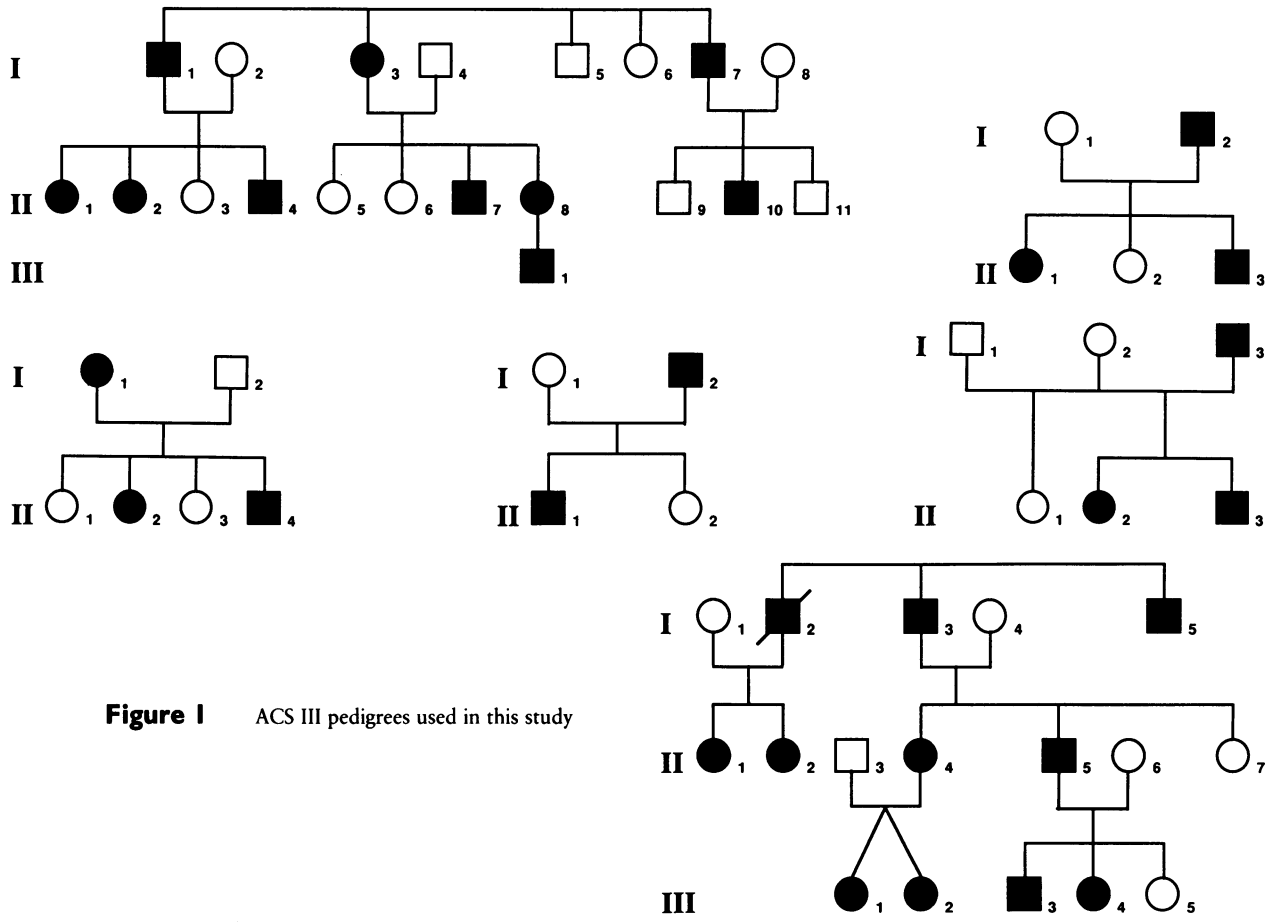

Figure 1 ACS III pedigrees used in this study

locus is between D7S493 and D7S516, which spans a genetic distance of 9 cM. However, the distal boundary of this region is defined by only one recombinant (individual II-3 in fig. 1*E*).

Table 2 presents pairwise *Z* values obtained between the 7p markers and the 5q marker for the Jackson-Weiss family. On 7p a genetic distance of 26 cM in the critical region for ACS III was covered (see map in fig. 3), flanked centromerically by D7S516 and telomerically by D7S513. The whole region is covered by a negative *Z* of ≤ -2, except for a 2-cM gap between afm281 and D7S488. It is extremely unlikely that the Jackson-Weiss locus is in this region with the disease conclusively excluded just 1 cM either side of this area (fig. 3 and table 2). The results with D5S211 exclude Jackson-Weiss syndrome to a distance of 10 cM on either side of this locus.

Discussion

The ACSs represent a group of conditions characterized by autosomal dominantly inherited craniosynostosis and digital abnormalities, especially syndactyly. On the basis of Blank's (1960) survey, the ACSs are classified into Apert and non-Apert types, the former being characterized by craniosynostosis, interdigital osseous fusion of the phalanges, and complete syndactyly and the latter being thought to comprise a heterogeneous group of disorders. McKusick (1992) recognizes four ACS syndromes—Apert syndrome (ACSI), ACS III, ACS V, and ACS type Robinow-Sorauf—although he doubts the separate delineation of the latter. Of these conditions, only ACS III has been provisionally mapped (Brueton et al. 1992). There is considerable clinical overlap between the non-Apert ACS syndromes, and the delineation of these syndromes from Jackson-Weiss syndrome and other autosomal dominant forms of craniosynostosis remains difficult. This degree of clinical overlap has led many authors to consider whether many or all of these conditions might not be allelic variants at the same locus.

The results of the current linkage analysis support localization of ACS III to the distal arm of chromosome

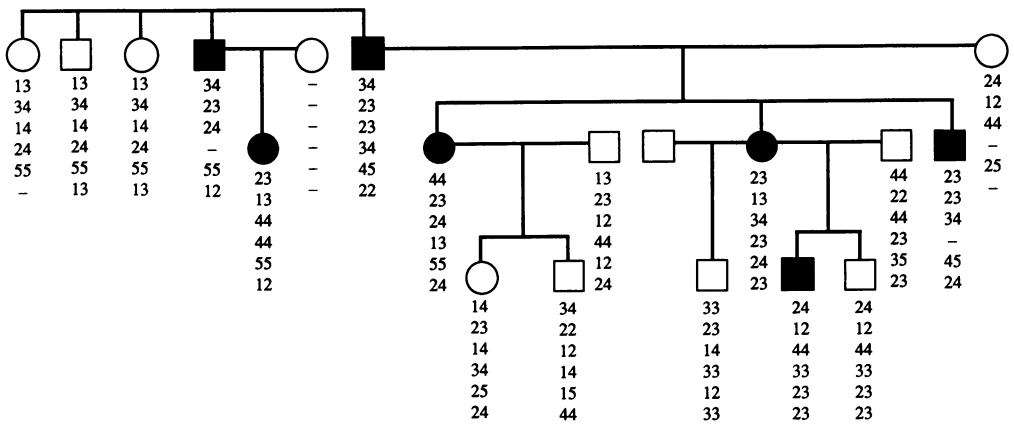

Figure 2 Jackson-Weiss syndrome pedigree used in this study. The genotype order, from top to bottom, is D7S513, afm281vc9, D7S488, D7S493, afm290vg9, and D7S516. A dash (-) indicates that the marker was not scored.

7p, with significant positive Z values for loci D7S488 and D7S493. Localization distal to D7S516 is supported by four crossovers—individual II-1 (fig. 1*F*), individual II-1 (fig. 1*B*), and individuals I-7 and II-5 (fig. 1*A*), and localization proximal to afm281 is supported by five crossovers—twins III-1 and III-2 (fig. 1*F*), individual II-2 (fig. 1*D*), individual II-1 (fig. 1*C*), and individuals II-5 and II-6 (fig. 1*A*). These two markers, 23 cM apart, are therefore definite flanking markers. A single affected individual, II-3 (fig. 1*E*), showed a recombination at D7S493, the disease segregating proximal to D7S493, making the D7S493-D7S516 interval the most likely to contain the ACS III locus.

The short arm of chromosome 7 appears to be rich in craniosynostosis-related loci. The gene for Greig cephalopolysyndactyly syndrome, which is sometimes associated with craniosynostosis, was mapped to 7p13 after this had been identified as a candidate region on the basis of two families showing cosegregation between the phenotype and cytogenetic translocations (Brueton et al. 1988). Several reports of patients with interstitial deletions more distal, mainly involving the 7p21/p22 region, have cited craniosynostosis as a clinical feature. These reports have recently been reviewed (Kikkawa et al. 1993). The case for a causative association between 7p21-p22 and ACS III has been further strengthened by the recent observation of de novo translocations of this region in two patients with the syndrome, although the breakpoints are slightly different in the two reports, with Reardon et al. (in press) citing the breakpoint as 7p21.2 and Reid et al. (1993) indicating 7p22 as the site of the breakpoint in their case. Whether, as discussed by Reid et al. (1993), this represents genetic heterogeneity for the ACS III or the limits of cytogenetic resolution remains unresolved.

For human craniodigital syndromes, a potential can-

Table 1

Pairwise Z Values, between ACS III and 5 7p$(CA)_n$ Repeat Markers

	Z AT θ =[a]						
PROBE	.00	.01	.05	.1	.2	.3	.4
D7S513	$-\infty$	−8.819	−3.61	−1.69	−.306	<u>.045</u>	.042
afm281	$-\infty$	−3.944	−1.369	−.435	.204	<u>.296</u>	.176
D7S488	$-\infty$	5.434	<u>5.574</u>	5.152	3.913	2.445	.941
D7S493	$-\infty$	4.544	<u>4.738</u>	4.386	3.302	2.015	.736
D7S516	$-\infty$	−4.186	−1.036	.026	<u>.606</u>	.523	.218

[a] Z_{max} values are underlined.

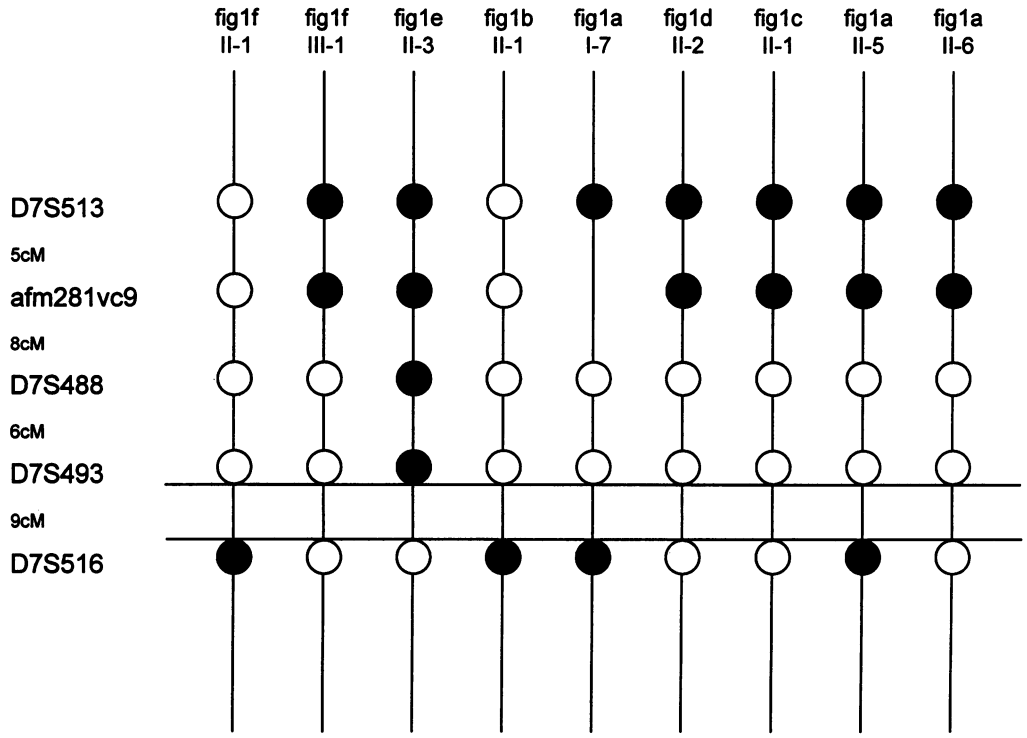

Figure 3 Schematic representation of nine recombinant chromosomes. Each vertical line represents a chromosome. Blackened circles indicate a crossover between the marker and the disease locus; and unblackened circles indicate no crossover. The two horizontal lines indicate the possible boundaries of the disease locus as indicated by these recombination events. The published distances (in cM) are shown between the marker loci.

didate gene that maps in the vicinity of the ACS III locus at 7p21 is ZNF12, a zinc finger protein encoding gene (Tsui and Farrall 1991). The Hox A cluster has been excluded as a candidate gene in the ACS III, by linkage analysis (Brueton et al. 1992).

Linkage analysis with the Jackson-Weiss syndrome pedigree indicates that the locus does not map close to the non-Apert ACS locus on 5q and that it is excluded from tight linkage with markers close to the ACS III locus on 7p, thereby providing evidence that the locus for Jackson-Weiss syndrome is not alellic to the ACS III locus. Crouzon syndrome (craniofacial dysostosis), a clinically separate form of autosomal dominant craniosynostosis, has also been shown not to be allelic at ei-

Table 2

Pairwise *Z* Values, between Jackson-Weiss Syndrome and Markers Used in This Study

	Z AT θ =						
PROBE	.0	.01	.05	.1	.2	.3	.4
D7s513	−∞	−6.99	−2.02	−1.23	−.52	−.19	−.06
afm281	−∞	−3.69	−.47	−.06	.19	.19	.09
D7S488	−∞	−8.39	−1.79	−.79	−.05	.15	.11
D7S493	−∞	−8.97	−2.35	−1.33	−.51	−.21	−.08
afm290	−∞	−6.27	−1.34	−.62	−.09	.06	.06
D7S516	−∞	−17.0	−5.26	−3.26	−1.46	−.62	−.19
D5S211	−∞	−12.59	−4.16	−2.73	−1.38	−.67	−.25

ther of these two craniosynostosis loci (Reardon et al. 1993). These findings underline the likelihood of several causative loci for craniosynostosis.

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