a single base substitution of the intron 5, resulting in two alternatively spliced mRNAs in a patient with maple syrup urine disease. Biochim Biophys Acta 1225:317–325

Lau KS, Herring WJ, Chuang JL, McKean M, Danner DJ, Cox RP, Chuang DT (1992) Structure of the gene encoding dihydrolipoyl transacylase (E2) component of human branched-chain α-keto acid dehydrogenase complex and characterization of an E2 pseudogene. J Biol Chem 267: 24090–24096

Lau KS, Lee J, Fisher CW, Cox RP, Chuang DT (1991) Premature termination of transcription and alternative splicing in the human tranacylase (E2) gene of the branched-chain α-ketoacid dehydrogenase complex. FEBS Lett 279:229–232

Mitsubuchi H, Nobukuni Y, Endo F, Matsuda I (1991) Structural organization and chromosomal localization of the gene for the E1β subunit of human branched chain α-keto acid dehydrogenase. J Biol Chem 266:14686–14691

Nobukuni Y, Mitsubuchi H, Endo F, Akaboshi I, Asaka J, Matsuda I (1990) Complete primary structure of the E1β subunit of human branched chain α-ketoacid dehydrogenase complex deduced from the nucleotide sequence and a gene analysis of patients with this disease. J Clin Invest 86: 242–247

Nobukuni Y, Mitsubuchi H, Endo F, Asaka J, Oyama R, Titani K, Matsuda I (1990) Isolation and characterization of a complementary DNA clone coding for the E1β subunit of the bovine branched-chain α-ketoacid dehydrogenase complex: complete amino acid sequence of the precursor protein and its proteolytic processing. Biochemistry 29:1154–1160

Nobukuni Y, Mitsubuchi H, Hayashida Y, Ohta K, Indo Y, Ichiba Y, Endo F, Matsuda I (1993) Heterogeneity of mutations in maple syrup urine disease (MSUD): screening and identification of affected E1α and E1β subunits of the branched-chain α-keto-acid dehydrogenase multienzyme complex. Biochim Biophys Acta 1225:64–70

Parrella T, Surrey S, Iolascon A, Sartore M, Heidenreich R, Diamond G, Ponzone A, et al (1994) Maple syrup urine disease (MSUD): screening for known mutations in Italian patients. J Inherit Metab Dis 17:652–660

Tantravahi J, Alvira M, Falck-Pedersen E (1993) Characterization of the mouse b^{maj} globin transcription termination region: a spacing sequence is required between the poly (A) signal sequence and multiple downstream termination elements. Mol Cell Biol 13:578–587

Wahle E, Keller W (1992) The biochemistry of 3′-end cleavage and polyadenylation of messenger RNA precursors. Annu Rev Biochem 61:419–440

Wynn RM, Chuang JL, Davie JR, Fisher CW, Hale MA, Cox RP, Chuang DT (1992) Cloning and expression in *Escherichia coli* of mature E1β subunit of bovine mitochondrial branched-chain α-keto acid dehydrogenase complex. J Biol Chem 267:1881–1887

Ye RD, Wun TC, Sadler JE (1987) cDNA cloning and expression in *Escherichia coli* of a plasminogen activator inhibitor from human placenta. J Biol Chem 262:3718–3725

Yeaman SJ (1989) The 2-oxo acid dehydrogenase complexes: recent advances. Biochem J 257:625–632

Zhao Y, Kuntz MJ, Harris RA, Crabb DW (1992) Molecular cloning of the E1β subunit of the rat branched chain alpha-ketoacid dehydrogenase. Biochim Biophys Acta 1132:207–10

Zneimer SM, Lau KS, Eddy RL, Shows TB, Chuang DT, Cox RP (1991) Regional assignment of two genes of the human branched-chain α-keto acid dehydrogenase complex: the E1β gene (BCKDHB) to chromosome 6p21-22 and the E2 gene (DBT) to chromosome 1p31. Genomics 10:740–747

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Deletion Mapping of 22q11 in CATCH22 Syndrome: Identification of a Second Critical Region

To the Editor:

The deletion at 22q11.2 implicates a variety of congenital anomaly syndromes, for which the acronym CATCH22 has been proposed (Wilson et al. 1993). Most patients with these syndromes share the common large deletion spanning 1–2 Mb, while the phenotypic variability of the patients does not seem to correlate with the extent of the deletions (Lindsay et al. 1995). On the basis of the deletions of rare cases with unbalanced translocation, the shortest region of overlap (SRO) had been identified in the most-centromeric region of the common large deletion (Halford et al. 1993*a*). One patient (ADU) has been reported to carry a balanced translocation with the breakpoint located in the SRO (Augusseau et al. 1986). Recently, three transcripts were identified at or very close to the ADU breakpoint (ADUBP), making them strong candidates for CATCH22 syndrome (Budarf et al. 1995). Here, we describe one patient with a unique deletion at 22q11.2 revealed by quantitative hybridization and/or FISH with six DNA markers in the common large deletion. The patient was dizygous at loci within the SRO and hemizygous only at the most-telomeric locus in the common large deletion. This finding suggests that there must be another critical region in the common large deletion besides the breakpoint of the ADU and that haploinsufficiency of genes in this deletion may also play a major role in CATCH22 pathogenesis.

To construct a detailed deletion map of 22q11 in patients with CATCH22, six DNA markers locating within the common large deletion were used to determine locus copy numbers. cHKAD26 is a cosmid clone isolated from chromosome 22–specific cosmid library, and cos77 a cosmid clone corresponding to mc512 isolated by 22q11 microdissection and microcloning

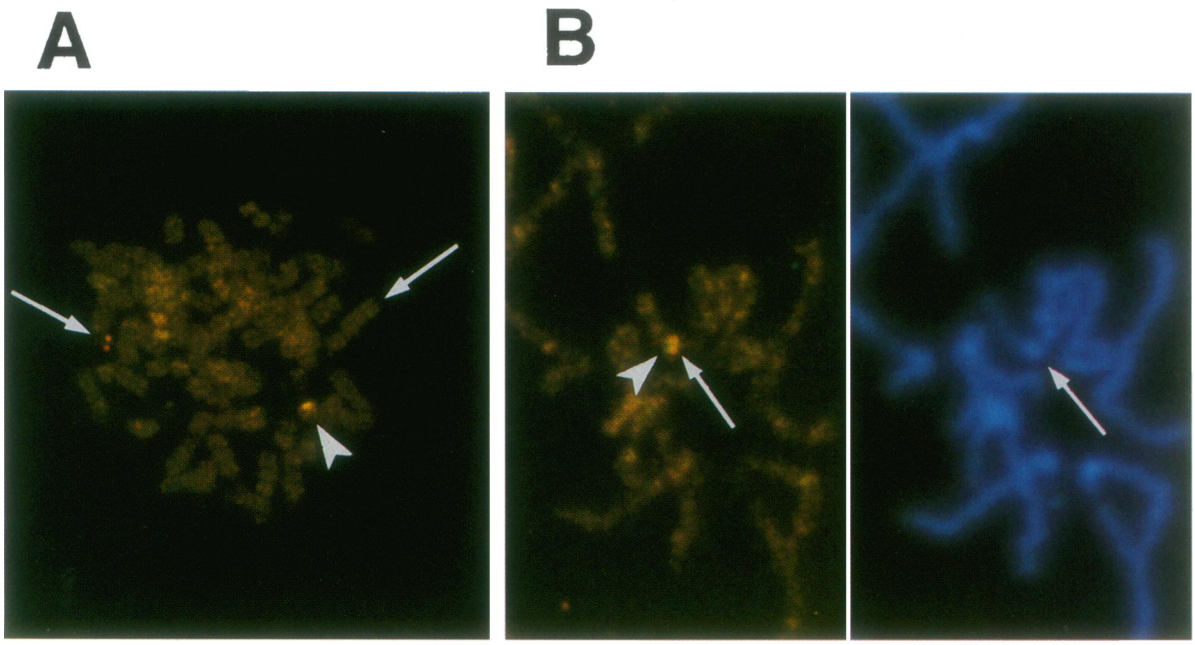

Figure 1 Mapping of the probes in 22q11 common large deletion by FISH. *A*, FISH analysis on metaphase chromosomes of GM5878. The signals of cos77 (red) were detected on der(22) (*arrow, left*), while those of cHKAD26 (green) were detected on der(10) (*arrow, right*), indicating that cos77 is centromeric and cHKAD26 is telomeric to the breakpoint of GM5878. Both signals were also detected on normal chromosome 22 of this line (*arrowhead*). *B*, High-resolution ordering on elongated prophase chromosomes. On the *left*, an *arrow* indicates the rhodamine-labeled D0832, while an *arrowhead* indicates the FITC-labeled cHKAD26. The arrangement of the red and green signals indicates that the order on 22q is centromere–D0832 (red)–cHKAD26 (green)–telomere. On the *right*, an *arrow* indicates the centromere of chromosome 22 in DAPI-stained prophase chromosome.

method (Kurahashi et al. 1994, 1995). D0832 was kindly provided by Dr. S. Halford (Halford et al. 1993*b*). The N25 (*D22S75*) probe was purchased from Oncor, Inc. The TUPLE1 cDNA probe was generated by PCR amplification from a fetal brain cDNA library, with primers generated by the *TUPLE1* cDNA sequence reported elsewhere (Halford et al. 1993*a*; Lamour et al. 1995). The IDD probe was also generated by PCR amplification from a fetal brain cDNA library, with primers generated with a *IDD* cDNA sequence reported elsewhere (Demczuk et al. 1995; Wadey et al. 1995). Cosmids corresponding to the TUPLE1 and IDD loci were isolated by screening of total human genomic cosmid library probed, with either one of the two PCR products. Cosmid clones obtained with the IDD probe were identified to contain the ADU breakpoint and were confirmed by comparison with the *Eco*RI restriction map in the original report (Demczuk et al. 1995).

The order of the six loci was at first determined by FISH on metaphase chromosomes of two cell lines, GM00980 and GM5878 (Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research). Both contained reciprocal translocation with the breakpoint in the common large deletion, and the breakpoint of GM00980 demarcates the telomeric border of the SRO (Fu et al. 1976; Kelley et al. 1982). FISH analyses on GM5878 revealed that four markers, ADUBP, TUPLE1, N25 (*D22S75*), and cos77, were located on der(22) and the remaining two, D0832 and cHKAD26, were not, which implies that the former four loci are centromeric from the GM5878 breakpoint (fig. 1*A*). Analyses on GM00980 identified three loci, ADUBP, TUPLE1, and N25, as hemizygous because of the unbalanced translocation with a deletion at 22pter-22q11, which suggests that the order of these six loci is centromere–(ADUBP, TUPLE1, N25)–GM00980 breakpoint–(cos77)–GM5878 breakpoint–(D0832, cHKAD26)–telomere. The order of two loci, D0832 and cHKAD26, was determined by two-color FISH on elongated prophase chromosomes (Inazawa et al. 1994). A simultaneous delineation of two-color signals, with a linear arrangement of centromere–D0832–cHKAD26–telomere, was seen in almost all prophase chromosomes examined (fig. 1*B*). Since the ADUBP is reported to be proximal to both the TUPLE1 and N25 loci, the order of the six loci on 22q was determined as centromere–ADUBP–(TUPLE1, N25)–cos77–D0832–cHKAD26–telomere.

The patients with CATCH22 phenotype were examined for 22q11 deletion by quantitative hybridization and/or FISH methods for the six loci. Eighty patients were included in this study because they had one or more

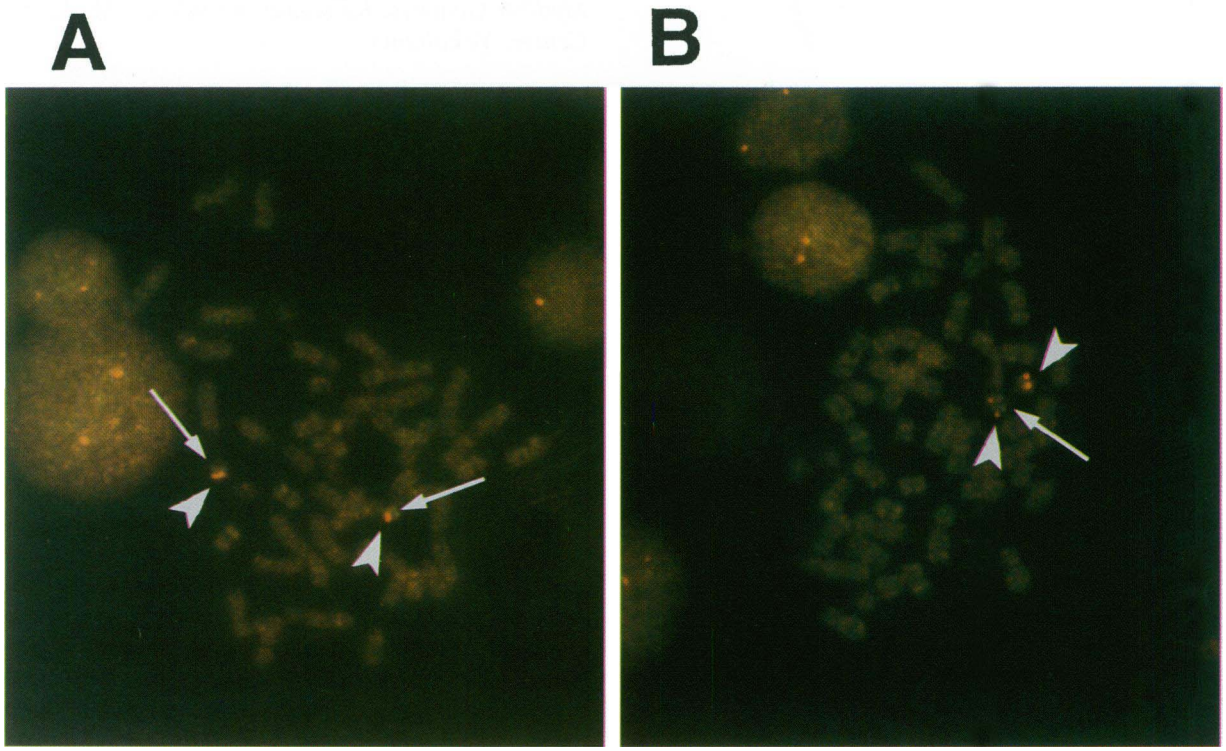

Figure 2 FISH analysis for 22q11 deletion. Biotin-labeled test probes located in the common large deletion (*arrows*) and digoxigenin-labeled control probes at 22q13 (*arrowheads*) were simultaneously hybridized on metaphase chromosomes of a patient with conotruncal anomaly face syndrome and then were identified by avidin-FITC (green) and antidigoxigenin-rhodamine (red). During examination of ADUBP, both green and red signals were detected on both chromosomes 22 of the patient, indicating that the locus had not been deleted (*A*). However, one of the chromosomes 22 of the patient did not show a green signal during examination of cHKAD26, indicating that the cHKAD26 locus had been deleted (*B*).

of the following features: congenital cardiac conotruncal anomaly, evidence of thymic abnormalities, hypocalcemia, or dysmorphic facial appearances corresponding to the characteristics of the syndromes that have been reported to be implicated in 22q11 deletion. Two loci outside the common deletion were also examined: proximal marker p22/34 (*D22S9*) and distal marker pClambda3 (*IGLC*). As a result, a total of 37 patients showed hemizygosity with at least one of the six probes locating in the common deletion; the patients were dizygous at *D22S9* and *IGLC* loci, which indicates that all of them had interstitial deletions. Thirty-three of these patients were found to have large interstitial deletion encompassing all of the six loci (type 1 deletion). Three patients were found to be hemizygous at ADUBP, TUPLE1, N25, cos77, and D0832 loci and dizygous at cHKAD26 (type 2 deletion). Therefore, these three patients were identified as having smaller deletions spanning the centromeric five loci and including the SRO. Since type 1 and type 2 deletions comprise the ADU breakpoint, the pathogenesis of these patients can be reasonably explained by haploinsufficiency of the gene(s) at the ADU breakpoint.

It is interesting to note that the remaining one patient who was not found to be hemizygous at the five centromeric loci, including the ADUBP, TUPLE1, and N25 within the SRO, proved to be hemizygous only at the most-telomeric locus, cHKAD26 (type 3 deletion). In FISH analysis, the signals of cHKAD26 could be detected on only one chromosome 22 of the patient in all 50 metaphase cells examined (fig. 2). The dosage analysis probed by *Eco*RI subfragment of cHKAD26 also showed that the patient was hemizygous at this locus. Since Southern analysis, with several restriction enzymes, of this patient probed with ADUBP revealed no rearranged bands, this patient apparently had no genetic alterations at the ADU breakpoint (data not shown). This patient has pulmonary atresia and tetralogy of Fallot with typical conotruncal anomaly face (Burn et al. 1993). Thus, this patient constitutes the first reported case of CATCH22 phenotype with a unique 22q11 deletion not containing the ADU breakpoint.

The results are summarized as a deletion map in figure 3. This map indicates that there are at least two regions critical for the development of CATCH22 syndrome. One is the SRO containing the ADU breakpoint, and

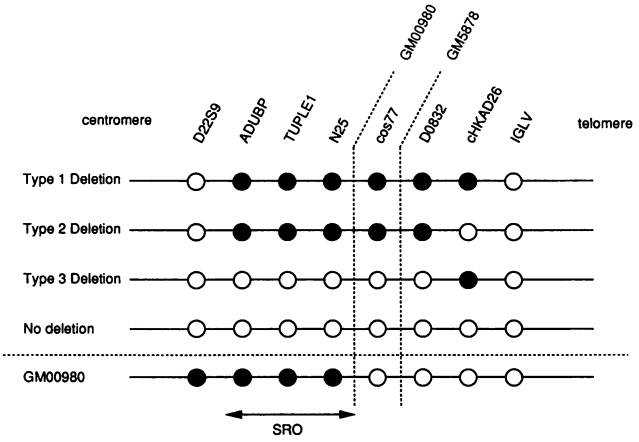

Figure 3 Deletion map of patients with CATCH22 syndrome. The locus names and the breakpoints of the two translocations are listed at the top. Open circles indicate dizygosity, and blackened circles, hemizygosity. Deletions have been subgrouped into three types listed at the *left*. Note that the type 3 deletion does not contain ADUBP or SRO.

the other, defined by the type 3 deletion, is the more telomeric region in the common large deletion. The latter region was deleted in all but three patients with 22q11 deletion. Haploinsufficiency of genes located in this region may also play crucial roles in the development of the phenotype of the syndrome. Since the deletion of each region seems to result in a similar phenotype, the genes in each region may function in the same cascade of fetal development. As an alternative, a *cis*-acting element may be located in either of the regions and have a position effect on the gene(s) in another region. On the other hand, the type 2 and type 3 deletions apparently do not overlap, as far as could be determined with the six markers. However, when the chromosomal region between D0832 and cHKAD26 is analyzed in detail, overlapping deletion may be found. Analysis of the YACs in this region is therefore in progress.

To conclude, there are at least two critical regions in the common large deletion of patients with CATCH22 syndrome. Our present findings favor the possibility that CATCH22 syndrome is not a single-gene disorder but a contiguous-gene syndrome.

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References

Augusseau S, Jouk S, Jalbert P, Prieur M (1986) DiGeorge syndrome and 22q11 rearrangements. Hum Genet 74:206

Budarf ML, Collins J, Gong W, Roe B, Wang Z, Bailey LC, Sellinger B, et al (1995) Cloning a balanced translocation associated with DiGeorge syndrome and identification of a disrupted candidate gene. Nat Genet 10:269–278

Burn J, Takao A, Wilson D, Cross I, Momma K, Wadey R, Scambler P, et al (1993) Conotruncal anomaly face syndrome is associated with a deletion within chromosome 22q11. J Med Genet 30:822–824

Demczuk S, Aledo R, Zucman J, Delattre O, Desmaze C, Dauphinot L, Jalbert P, et al (1995) Cloning of a balanced translocation breakpoint in the DiGeorge syndrome critical region and isolation of a novel potential adhesion receptor gene in its vicinty. Hum Mol Genet 4:551–558

Fu W, Borgaonkar DS, Ladewig PP, Weaver J, Pomerance HH (1976) Structural aberrations of the long arm of chromosome no. 22. Clin Genet 10:329–336

Halford S, Wadey R, Roberts C, Daw SCM, Whiting JA, O'Donnell H, Dunham I, et al (1993*a*) Isolation of a putative transcriptional regulator from the region of 22q11 deleted in DiGeorge syndrome, Shprintzen syndrome, and familial congenital heart disease. Hum Mol Genet 2:2099–2107

Halford S, Wison DI, Daw SCM, Roberts C, Wadey R, Kamath S, Wickremasinghe A, et al (1993*b*) Isolation of a gene expressed during early embryogenesis from the region of 22q11 commonly deleted in Digeorge syndrome. Hum Mol Genet 2:1577–1582

Inazawa J, Ariyama T, Tokino T, Tanigami A, Nakamura Y, Abe T (1994) High-resolution ordering of DNA markers by multi-color fluorescent in situ hybridization of prophase chromosomes. Cytogenet Cell Genet 65:130–135

Kelley RI, Zackai EH, Emanuel BS, Kistenmacher M, Greenberg F, Punnett H (1982) The association of the DiGeorge anomalad with partial monosomy of chromosome 22. J Pediatr 101:197–200

Kurahashi H, Akagi K, Inazawa J, Ohta T, Niikawa N, Kayatani F, Sano T, et al (1995) Isolation and characterization of a novel gene deleted in DiGeorge syndrome. Hum Mol Genet 4:541–549

Kurahashi H, Akagi K, Karakawa K, Nakamura T, Dumanski JP, Sano T, Okada S, et al (1994) Isolation and mapping of

cosmid markers on human chromosome 22, including one within the submicroscopically deleted region of DiGeorge syndrome. Hum Genet 93:248–254

Lamour V, Lécluse Y, Desmaze C, Spector M, Bodescot M, Aurias A, Osley MA, et al (1995) A human homolog of the *S. cerevisiae* HIR1 and HIR2 transcriptional repressors cloned from the DiGeorge syndrome critical region. Hum Mol Genet 4:791–799

Lindsay EA, Greenberg F, Shaffer LG, Shapira SK, Scambler PJ, Baldini A (1995) Submicroscopic deletions at 22q11.2: variability of the clinical picture and delineation of a commonly deleted region. Am J Med Genet 56:191–197

Wadey R, Daw S, Taylor C, Atif U, Kamath S, Halford S, O'Donnell H, et al (1995) Isolation of a gene encoding an integral membrane protein from the vicinty of a balanced translocation breakpoint associated with DiGeorge syndrome. Hum Mol Genet 4:1027–1033

Wilson DI, Burn J, Scambler P, Goodship J (1993) DiGeorge syndrome: part of CATCH 22. J Med Genet 30:852–856

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Detection of Linkage to Affective Disorders in the Catalogued Amish Pedigrees: A Reply to Pauls et al.

To the Editor:

We have reported evidence for linkage of a region of chromosome 18 markers to affective illness in 22 bipolar (BP) pedigrees (Berrettini et al. 1994). The pedigree series included 21 U.S. pedigrees collected by us (Berrettini et al. 1991, 1994) and part of Amish pedigree 884 (NIGMS Human Genetic Mutant Cell Repository 1995) referred to as panel 3 in the catalog and also known as "the right extension." The rest of 884 was never genotyped by us, because it did not fit the criteria for inclusion, as described elsewhere (Berrettini et al. 1994).

Pauls et al. (1995) have recently studied whether this linkage can be detected in the entire catalogued Amish pedigrees (884 and 1075) (NIGMS Human Genetic Mutant Cell Repository 1995) in four of the marker loci reported by Berrettini et al. (1994). The authors conclude that the Amish data contain no significant susceptibility locus for BP illness in this region of chromosome 18. We find that the data published by Pauls et al. are not conclusive with regard to the presence or absence of any susceptibility locus under the nonparametric analyses presented, and, although the sample size is extremely small, it could also be interpreted as consistent with our findings.

In Berrettini et al. (1994), evidence for linkage was found with affected-sib-pair (ASP) and multilocus affected-pedigree-member (APM) analyses (Weeks and Lange 1988; Bailey-Wilson and Elston 1994). Affection status models were affection status model 1 (ASM1), which includes BPI and BP2 and schizoaffective (SA), and ASM2, which included ASM1 and recurrent unipolar disorder (UP); the quoted statistics that follow are from ASM2. Multilocus APM analysis showed significant sharing of marker alleles among affected persons, for five contiguous markers (D18S40, D18S45, D18S44, D18S66, and D18S56), with P values from $<1 \times 10^{-4}$ to 7×10^{-4} under weighting functions $f(p) = 1$ and $f(p) = 1/\sqrt{p}$. Since publication, we have performed multilocus ASP analysis (Goldgar 1990). The P values for the multilocus analyses ranged between .003 and .00008, depending on the set of markers analyzed.

LOD Score Analyses with Specified Genetic Model

Under a dominant model, Pauls et al. (1995) found a maximum LOD score of 1.31 for D18S53 in the right extension under an affection status model that includes BP disorder and major depression, which is about the same as that found for this pedigree by Berrettini et al. (1994) (LOD score = 1.25). When the rest of the pedigrees were included, the LOD score was >-2. Pauls et al. (1995) imply that the observed LOD scores in the right extension are a "false positive," finding them reminiscent of previous reports of linkage of this pedigree on chromosome 11p15 (which did not replicate). They assert that the "right extension" of Amish pedigree is more likely to have false-positive results than the other Amish pedigrees. In a simulation study they perform, where the marker was unlinked to the disease gene, 2.6% of the replicates gave a LOD score >1.0 for the right-extension pedigree but only 0.6% of replicates of the rest of the pedigrees had LOD scores >1.0. The argument that the false-positive rate in the first pedigree is too high is flawed, because, asymptotically, one would expect a LOD score of 1.0 to occur 3.2% of the time (assuming a two-tailed χ^2 test). Thus the finding that 2.6% of replicates have this value is consistent with theory and does not suggest that this pedigree is prone to false-positive results. Their further assertion that "presumably, these observations also hold true for nonparametric (ASP) linkage analyses" is a speculation based on their incorrect interpretation (Pauls et al. 1995, p. 641).

LOD scores of ≥ 1 were reported by us in the 1994 paper only as illustrative results in single pedigrees, and not as a positive linkage result in any one pedigree or in the entire series. It does not appear appropriate to use such a result, or the results of Pauls et al., in a