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A Genomewide Screen for Autism-Spectrum Disorders: Evidence for a Major Susceptibility Locus on Chromosome 3q25-27

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To identify genetic loci for autism-spectrum disorders, we have performed a two-stage genomewide scan in 38 Finnish families. The detailed clinical examination of all family members revealed infantile autism, but also Asperger syndrome (AS) and developmental dysphasia, in the same set of families. The most significant evidence for linkage was found on chromosome 3q25-27, with a maximum two-point LOD score of 4.31 ($Z_{\max dom}$) for D3S3037, using infantile autism and AS as an affection status. Six markers flanking over a 5-cM region on 3q gave $Z_{\max dom}$ >3, and a maximum parametric multipoint LOD score (MLS) of 4.81 was obtained in the vicinity of D3S3715 and D3S3037. Association, linkage disequilibrium, and haplotype analyses provided some evidence for shared ancestor alleles on this chromosomal region among affected individuals, especially in the regional subisolate. Additional potential susceptibility loci with two-point LOD scores >2 were observed on chromosomes 1q21-22 and 7q. The region on 1q21-22 overlaps with the previously reported candidate region for infantile autism and schizophrenia, whereas the region on chromosome 7q provided evidence for linkage 58 cM distally from the previously described autism susceptibility locus (AUTS1).

Introduction

Infantile autism (MIM 209850) is characterized by abnormalities in reciprocal social interaction and communication, restricted and stereotyped patterns of interests and activities, and the presence of developmental abnormalities by 3 years of age. Together with Asperger syndrome (AS), it belongs to the disease spectrum of pervasive developmental disorders. Although family studies have reported increased rates of language disorders in relatives of autistic probands, the reasons for the observed association between autism and developmental language disorders are currently unknown (Bailey et al. 1995, 1998; Fombonne et al. 1997; Pickles et al. 2000). Comparative studies of autism and developmental language disorders have indicated that the distinction between the phenotype traits is more pronounced in childhood and tends to ameliorate later on (Howlin et al. 2000; Mawhood et al. 2000). Consequently, recommendations have been made for more broadly defined diagnostic criteria for autism (Piven et al. 1997*a;* Folstein et al. 1998).

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Genetic susceptibility to autism is implicated by the findings of increased concordance rates of 69%–98% among MZ twins, compared with rates of 0%–30% among DZ twins (Folstein and Rutter 1977; Steffenburg et al. 1989; Bailey et al. 1995), and a 3%–5% recurrence risk for siblings (Smalley 1997). Eight genome scans performed so far for the autism loci have provided suggestive evidence for linkage to several chromosomal regions (International Molecular Genetic Study of Autism Consortium [IMGSAC] 1998; Collaborative Linkage Study of Autism [CLSA] 1999; Philippe et al. 1999; Risch et al. 1999; Buxbaum et al. 2001; IMGSAC 2001*b;* Liu et al. 2001; Shao et al. 2002*b*), reflecting both genetic and clinical heterogeneity of this complex trait. The most convincing linkage finding has been observed on chromosome 7q. Being originally detected in British sibpair families, the 7q linkage was later replicated in multiple genome scans and in several populations (CLSA 1999; Philippe et al. 1999; Risch et al. 1999; IMGSAC 2001*b;* Buxbaum et al. 2001; Liu et al. 2001). Recently, this locus, designated "AUTS1," was studied in 153 IMGSAC families, resulting in a multipoint LOD score (MLS) of 3.37 at D7S477 (IMGSAC 2001*a*). Another interesting region is 2q, a region for which overlapping linkage results have been generated in several genome scans (Philippe et al. 1999; Buxbaum et al. 2001; IMGSAC 2001*b;* Shao et al. 2002*b*). By analysis of a subset of autistic patients with delayed phrase speech, increased support for linkage on chro-

Table 1

Family Material in Stage I and Stage II

Diagnostic Criterion	Phenotypes	No. in Stage I		No. in Stage II	
		Families	Affected Individuals	Families	Affected Individuals
1	Autism	12	33	19	54
2	Autism, AS	18	42	28	72
3	Autism, AS, dysphasia	19	47	38	87

mosome 2 was obtained (Buxbaum et al. 2001; Shao et al. 2002*a*).

So far, genetic studies of autism-spectrum disorders have mainly focused on infantile autism, with a minority of families or family members representing other trait phenotypes (Philippe et al. 1999; Risch et al. 1999; Liu et al. 2001). Since, in our nationwide collection of multiplex families, approximately one-third of the probands with infantile autism were found to have a first-degree relative with AS or developmental dysphasia, we decided to include these phenotype traits as affection status in our linkage studies. The spectrum of clinical phenotype in our family sample is consistent with the previous findings implying that relatives of autistic probands have an increased risk for language-related cognitive disorders or deficits in social functioning (Bolton et al. 1994; Fombonne et al. 1997; Pickles et al. 2000). The genome scan in multiplex Finnish families affected by the broad phenotype provided evidence for a new autism locus on 3q25-27.

Material and Methods

Family Material

The families with two or more members with the broad phenotype were recruited through a nationwide search of university and central hospitals of Finland and through the Finnish Association for Autism and Asperger Syndrome. Thorough clinical and medical examinations were performed by an experienced child neurologist or pediatrician, by use, as screening instruments, of the Childhood Autism Rating Scale (CARS), the Asperger Syndrome Screening Questionnaire (ASSQ), and the Asperger's Syndrome Diagnostic Interview (ASDI) (Ehlers and Gillberg 1993). Diagnoses were assessed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 4th edition) or ICD10 criteria (World Health Organization 1993). Further, any available information from speech therapists and neuropsychologists was considered in the diagnostic evaluation. We paid particular attention to the classification of the patients with developmental dysphasia. Reassessment of dysphasia was performed by one specialist (R.V.) for all the studied family members. Most of the patients with developmental dysphasia (11/15) had receptive language disorder (F80.2 according to ICD10), which is similar to the DSM-IV diagnosis for mixed receptive-expressive language disorder. Of 15 patients, 2 had the expressive type of dysphasia (F80.1, according to ICD10) similar to the DSM-IV diagnosis for expressive language disorder; however, one of them was diagnosed as having F80.2 in early childhood, and the other was diagnosed later in life, at the age of 10 years. Further, one young male patient with an autistic sibling was first diagnosed to have the expressive type of dysphasia (F80.1) and was handled as such in the analyses. Later, he fulfilled the criteria for autism. In one subject, who was first considered to be dysphasic and was treated as such in the statistical analyses, not all the necessary diagnostic criteria for dysphasia or autism-spectrum disorder were fulfilled. Problems in social interaction and language skills were observed; however, the diagnosis remained unspecified. Families with associative medical conditions, such as fragile-X syndrome, chromosomal aberrations, neurocutaneous syndromes, and profound mental retardation, were excluded. A blood sample was taken from all the available first-degree relatives of the probands. All families are Finnish, except for one, in which the father was of Turkish origin. To determine whether the families had any common ancestors, we performed genealogical analyses using population registers (Varilo et al. 1996). These study protocols were approved by the ethical committees of the Hospital for Children and Adolescents of Helsinki University Hospital and National Public Health Institute, Helsinki. Informed consent for genetic studies was obtained from the subjects or their parents.

Genotyping

The initial genomewide scan (stage I) was performed in 19 multiplex families with a total of 47 affected individuals (33 with infantile autism, 9 with AS, and 5 with developmental dysphasia), by use of 369 microsatellite markers from Weber screening set 6.0 (Sheffield et al. 1995) (table 1 and fig. 1). In stage II, 19 additional families with a total of 40 affected individuals (21 with infantile autism, 9 with AS, and 10 with developmental dysphasia) were included in the studies and were genotyped using markers on nine promising chromosomal regions. Of these 38 families, 17 had participated in the

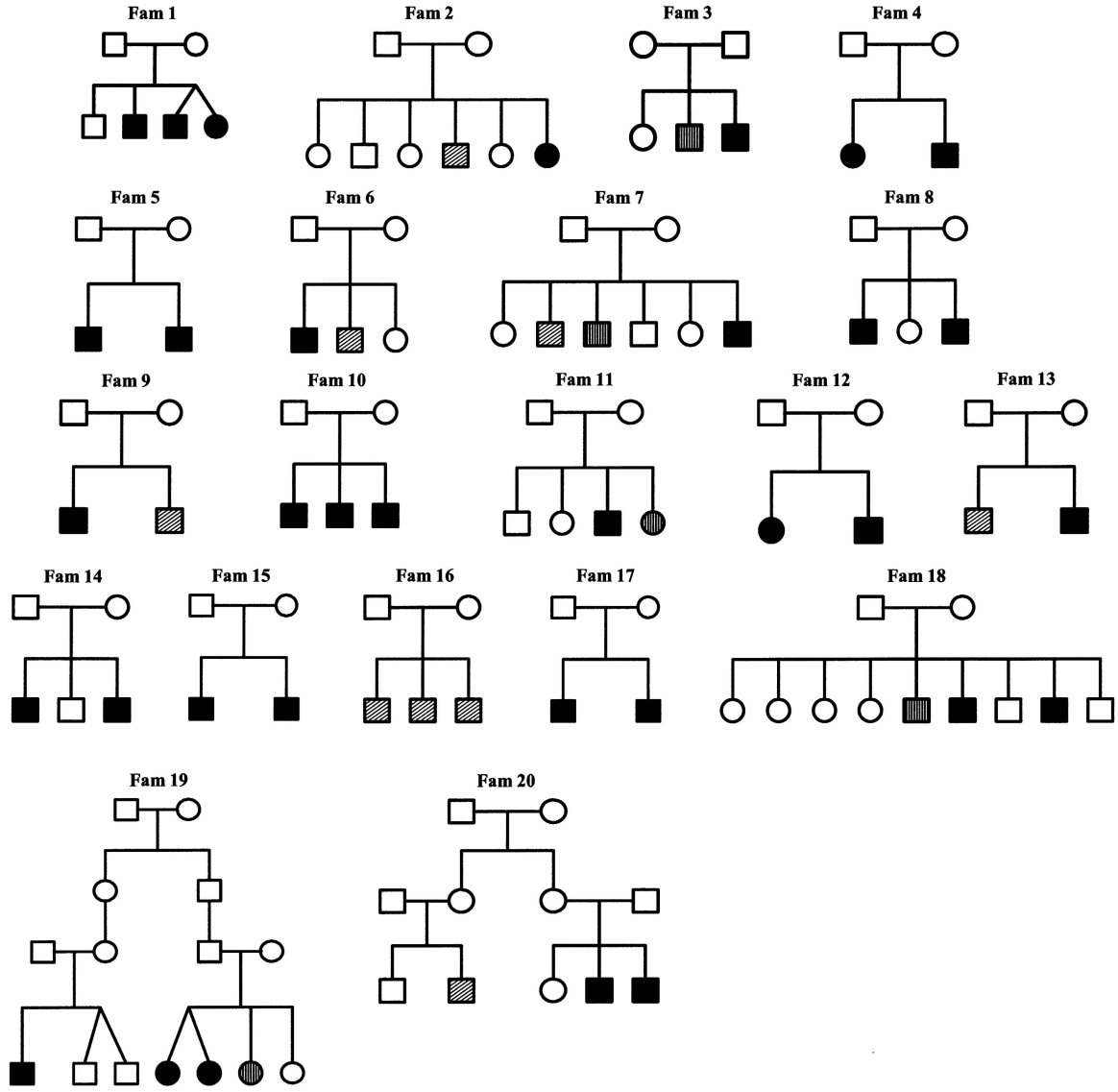

Figure 1 Pedigree structure for 19 families in the initial genomewide screen (stage I). Families 1 and 3 are linked genealogically and were treated as one family. Blackened symbols denote infantile autism, symbols with diagonal lines denote AS, and symbols with vertical lines denote developmental dysphasia.

analysis of the previously published susceptibility loci for autism (Auranen et al. 2000). All the parents were genotyped, except in three families for which one parent was not available and one family for which both parents were unavailable.

DNA was extracted from EDTA-treated blood according to standard procedures (Blin and Stafford 1976). PCR reactions were performed in 15-ml reaction volumes containing 20 ng of genomic DNA, 6 pmol of both primers, 0.2mM of dNTP, 1.5 mM $MgCl_2$, 10 mM TrisHCl, 50 mM KCl, 0.1% Triton X100, and 0.23 U Dynazyme (Finnzymes Oy). The reactions were performed using an MJ Research thermocycler and a hotstart procedure with Dynazyme that was added only after the first denaturation step of 5 min at 95°C. The DNA amplification was carried out in 35 cycles as follows: 30 s at temperature specific for each primer (49–62°C), 30 s at 72°C and 30 s at 95°C. An elongation step of 5 min at 72°C terminated the reaction after the last annealing. Electrophoretic allele separation was performed using an ABI automated DNA sequencer (Applera), and genotypes were assigned using the Genotype 2.0 software (Applera). Markers that failed to amplify reliably were replaced by markers from the Généthon marker map. The fine-mapping markers were selected from the Marshfield and the Genetic Location Database maps.

The genotypes were assigned by two independent individuals. For each marker, the allele frequencies were calculated using the pedigree data itself. For genotype-error elimination, the PEDCHECK, MENDEL, and SIMWALK2 programs were utilized (Lange et al. 1988; Sobel and Lange 1996; O' Connell and Weeks 1998). Haplotypes were constructed using the GENEHUNTER program (Kruglyak et al. 1996).

Statistical Analysis

In all analyses, individuals were designated as either affected or unknown. All available samples of first-degree relatives of the probands were genotyped. For statistical analyses, the families were classified into three nested diagnostic categories defined by diagnostic criterion 1, families with infantile autism only (19 families); diagnostic criterion 2, families with infantile autism and AS (28 families); and diagnostic criterion 3, families with all three phenotypes (38 families). The maximum two-point LOD scores (Z_{max}) under homogeneity were calculated using the MLINK program of the LINKAGE package (Lathrop and Lalouel 1984; Lathrop et al. 1986), and tests of heterogeneity and calculations of the proportion of families showing linkage (α) were performed using the HOMOG program (Ott 1986). Affecteds-only analysis was performed under both autosomal dominant and autosomal recessive modes of inheritance allowing no phenocopies, and the disease-allele frequency was set at 10^{-4}. Sib-pair analysis was performed using the SIBPAIR program (Kuokkanen et al. 1996). Nonparametric maximum multipoint LOD scores (MLS) were calculated using the MAPMAKER/SIBS and GENEHUNTER programs on autosomal and X-chromosomal markers respectively (Kruglyak and Lander 1995; Kruglyak et al. 1996). This analysis was restricted to the information of the sib-pair families, and the extended families were excluded from the analyses. Consequently, 17 of 19 families fulfilling diagnostic criterion 1, 25 of 28 families fulfilling diagnostic criterion 2, and 35 of 38 families fulfilling diagnostic criterion 3 were analyzed. In stage II, we used the information of sib-pair families in parametric multipoint calculations and in the construction of shared haplotypes. Parametric MLSs were calculated utilizing the SIMWALK 2.81 program (Sobel and Lange 1996). Association analysis was performed by calculating the *P* values for the transmission/disequilibrium test (TDT) using Mendel 4.0 (Lange et al. 2001), and likelihood-based haplotype relative risk using the ANALYZE package (Terwilliger 1998). Gamete-competition analysis (Sinsheimer et al. 2000), as implemented in MENDEL 4.0 (Lange et al. 2001), was used to estimate the degree of apparent bias in transmission of alleles of the markers on chromosomes 1, 7, and 3q to the affected offspring. Linkage disequilibrium (LD) between the markers was calculated using the GenePop 3.3 program. The extent of nonrandom allelic association between pairs of loci was monitored in the transmitted and nontransmitted alleles from the parents. Differences in the allele and genotype frequency distributions in the transmitted and nontransmitted alleles from the parents were evaluated by an exact test for population differentiation. These tests calculate the tail probability (*P* value) for Fisher's exact test. Additionally, two-point LD analyses were performed using the DISLAMB program (Terwilliger 1995).

Results

Genealogical Studies

We studied the church parish records (birth registers) and the registers in the National Archives of Finland in order to identify putative common ancestors for all 39 study families (Varilo et al. 1996). We were able to construct two extended pedigrees merging five core families with common ancestors in the 18th century. Two families with five affected individuals could be linked (Fam 1 and 3 in figs. 1 and 4), as well as three families with one affected individual (pedigree not shown), all originating from central Finland. The genealogical information was used in the two-point linkage analyses as well as in TDT and HRR analyses. In 20 (51%) of 39 families studied, at least one grandparent originated from central Finland, suggesting some clustering of the clinical phenotype in this regional subisolate.

Primary Genome Scan (Stage I)

Diagnostic criterion 1.—Among 19 families included in the initial genome scan, a total of 12 had two or more sibs diagnosed as having infantile autism (table 1). In linkage analyses of these families, nine chromosomal regions showed a $Z_{max\,rec} > 1.0$ (table 2 and fig. 2*a*). These regions on chromosomes 1, 3p, 3q, 9, 12, 14, 17, 18, and 21, as well as the AUTS1 locus on chromosome 7q, were selected for fine mapping (stage II) with 19 additional families with autism, AS, and dysphasia. The most encouraging findings in stage I were obtained on chromosome 3q, on which several markers flanking a 15-cM interval showed $Z_{max\,dom} > 1.0$ (table 2).

Diagnostic criteria 2 and 3.—When the affection status of the siblings was broadened to include not only infantile autism but also AS (diagnostic criterion 2) and dysphasia (diagnostic criterion 3), the number of families with two or more affected individuals increased from 12 to 18 and 19, respectively. The markers showing some evidence for linkage ($Z_{max} > 1$) in both diagnostic criterion 2 and 3 families were on chromosomes 1, 3p, 3q, 6, 19, and X (figs. 2*b* and 2*c*). On chromosome 1, $Z_{max\,dom} = 1.76$ ($\theta = 0.00$; $\alpha = 1.0$) and $Z_{max\,dom} =$

Table 2

Markers Showing $Z_{\max rec}$ >1.0 for Patients with Autism in Stage I

Marker	Distance (cM)	$Z_{\max rec}$	θ	α
D1S1675	149.20	1.0	0	1.0
D3S3038	44.81	1.60	.14	.64
D3S4009	137.0	1.60	0	.59
D3S3554	152.62	1.64	.10	1.0
D3S3053	181.87	1.10	.04	.65
D3S2427	188.29	1.45	.16	1.0
D3S2418	215.84	1.40	.12	1.0
D9S158	161.71	1.62	0	.64
D12S2078	149.60	1.02	.14	1.0
D14S297	31.75	1.53	.10	1.0
D17S784	116.86	1.43	.04	.51
D18S59	.00	1.22	0	.40
D21S1440	36.1	1.29	0	.44

NOTE.—LOD score values. The θ values and the proportion of families showing linkage (α) are allowed to vary between 0 and 0.5 and between 0 and 1.0, respectively.

1.42 ($\theta = 0.1$; $\alpha = 1.0$) were observed at D1S1675 in individuals affected under criterion 2 and criterion 3, respectively. On chromosome 3p, the highest LOD scores were found at D3S3608 ($Z_{\max rec} = 2.84$; $\theta = 0.06$; $\alpha = 0.74$) and $Z_{\max rec} = 3.13$ ($\theta = 0.08$; $\alpha = 0.80$), on chromosome 3q at D3S3554 with $Z_{\max rec} = 1.79$ ($\theta = 0.04$; $\alpha = 0.64$) and $Z_{\max rec} = 2.40$ ($\theta = 0.06$; $\alpha = 0.73$), on chromosome 6 at D6S1021 with $Z_{\max rec} = 1.58$ ($\theta = 0.02$; $\alpha = 0.48$) and $Z_{\max rec} = 1.72$ ($\theta = 0.04$; $\alpha = 0.53$), and on chromosome 19 at D19S433 with $Z_{\max rec} = 1.92$ ($\theta = 0.0$; $\alpha = 0.47$) and $Z_{\max rec} = 1.25$ ($\theta = 0.0$; $\alpha = 0.33$) under criterion 2 and 3, respectively. On chromosome X, markers DXS7132 and DXS6789, located ~10 cM apart, resulted in $Z_{\max dom} = 1.48$ ($\theta = 0.14$; $\alpha = 1.0$) and $Z_{\max dom} = 1.20$ ($\theta = 0.0$; $\alpha = 0.64$) under criterion 2 and $Z_{\max dom} = 1.89$ ($\theta = 0.12$; $\alpha = 1.0$) and $Z_{\max dom} = 1.82$ (at $\theta = 0.0$; $\alpha = 0.68$) under criterion 3, respectively. Promising pairwise LOD scores on chromosome X prompted us to perform nonparametric multipoint analysis under criterion 3, using the GENEHUNTER program. The maximum MLS of 2.75 ($P = .002733$) was obtained close to marker DXS7132 in this set of families (fig. 3*b*).

On chromosome 7, five of six markers covering a 30-cM region from D7S1824 to D7S550 showed a $Z_{\max dom}$ >1.0 under criterion 3. $Z_{\max dom} = 3.04$ ($\theta = 0.0$; $\alpha = 1.0$) was obtained under criterion 3 at D7S2462, with $Z_{\max dom} = 3.04$ ($\theta = 0.0$; $\alpha = 1.0$), located ~58 cM telomerically from the recently published autism locus (IMGSAC 2001*a*). Parametric multipoint analysis was performed for each of the diagnostic classes with SIMWALK 2.81. Under criterion 3, a maximum MLS of 3.66 under the dominant model of inheritance was observed at D7S2462 with α value of 1.0 (fig. 3*a*). The corresponding LOD scores under criterion 2 were systematically lower (table 2).

Fine-Mapping Results (Stage II)

Nine chromosomal regions showing two-point LOD scores >1.0 in the initial scan were fine mapped with an additional 60 markers. At this stage, 19 additional families with 40 individuals affected with autism, AS, or dysphasia were included in the analyses (table 1).

Chromosomes 7, 9, 12, 14, 17, 18, and 21.—On chromosome 7, analysis of the AUTS1 locus with five markers (D7S480, D7S1804, D7S2437, D7S684, and D7S1824) did not give evidence for linkage using diagnostic criterion 1 and 2 (pairwise LOD scores <1). Under criterion 3, a $Z_{\max dom} = 1.05$ ($\theta = 0$; $\alpha = 0.44$) was obtained at D7S1824.

Fine mapping on chromosomes 9, 12, 18, and 21 (table 3) did not increase the evidence for linkage when compared to the initial genome-scan results (stage I). On chromosome 14, a $Z_{\max dom}$ of 1.60 ($\theta = 0$; $\alpha = 1.0$) was detected at D14S297 using diagnostic criterion 1 (table 3). The evidence for linkage was less significant under criterion 2 and 3 in stage II when compared with stage I results. On chromosome 17, two additional flanking markers, D17S1806 and D17S1830, located ~2 cM apart were analyzed (table 3). Under criterion 1, all three markers provided suggestive evidence for linkage with $Z_{\max rec}$ of 1.50 ($\theta = 0.1$; $\alpha = 0.66$) at D17S1806, $Z_{\max rec}$ of 1.82 ($\theta = 0.1$; $\alpha = 0.7$) at D17S784 and $Z_{\max rec}$ of 1.78 ($\theta = 0$; $\alpha = 0.37$). The best $Z_{\max dom}$ of 2.16 ($\theta = 0$; $\alpha = 0.45$) were obtained for D17S784 under criterion 3.

Chromosome 3.—On chromosome 3p, the highest LOD scores were obtained under criterion 3 (38 families; 87 affected individuals) at D3S3038 ($Z_{\max rec} = 1.53$; $\theta = 0.06$; $\alpha = 0.36$) and D3S3659 ($Z_{\max rec} = 1.72$; $\theta = 0.0$; $\alpha = 0.38$) which are located ~2.6 cM apart. Inclusion of additional families and adoption of a dominant model of inheritance resulted in lower LOD scores in stage II (table 3) when compared to the results in stage I (table 2).

For chromosome 3q, a total of 29 markers in an ~85 cM interval were genotyped (average intermarker distance 2.7 cM). The highest two-point LOD score of $Z_{\max dom} = 4.31$ ($\theta = 0.0$; $\alpha = 1.0$) and ASP LOD score of 4.21 (identical-by-descent [IBD] sharing 82%) were observed at D3S3037 under criterion 2 (table 3). In the 5-cM interval between D3S2421 and D3S3730, six markers gave significant evidence for linkage under locus homogeneity [$Z_{\max dom}$ ($\theta = 0$) > 3.0)] (table 3). Under criterion 1, the highest ASP LOD score of 3.16 (IBD sharing 84%) was generated at D3S3037 (table 3). Un-

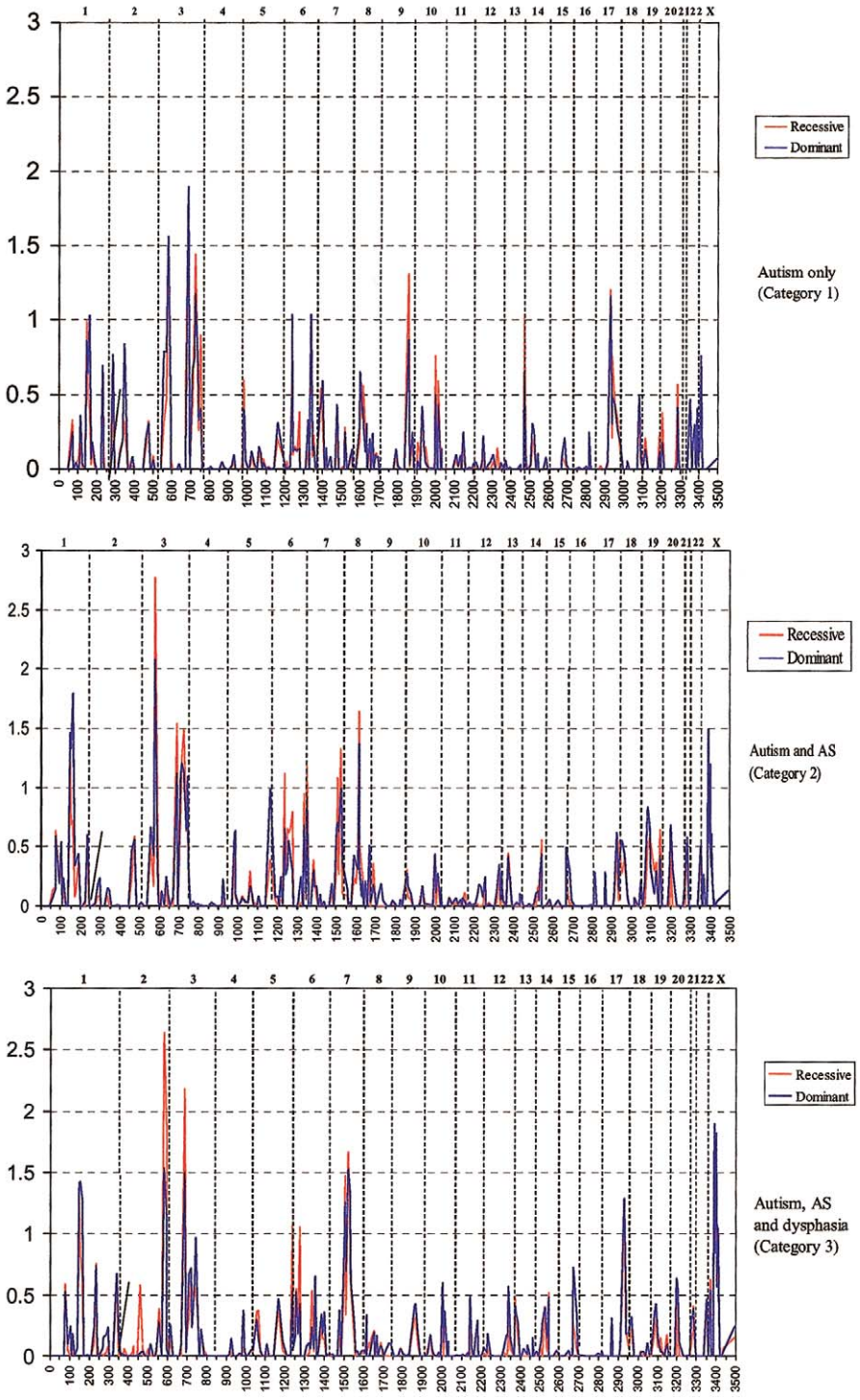

Figure 2 The two-point LOD scores under homogeneity obtained from the whole genomewide screen under different diagnostic categories: diagnostic criterion 1, families with infantile autism (*A*);. diagnostic criterion 2, families with infantile autism and AS (*B*);. and diagnostic criterion 3, families with infantile autism, AS, and developmental dysphasia combined (*C*).

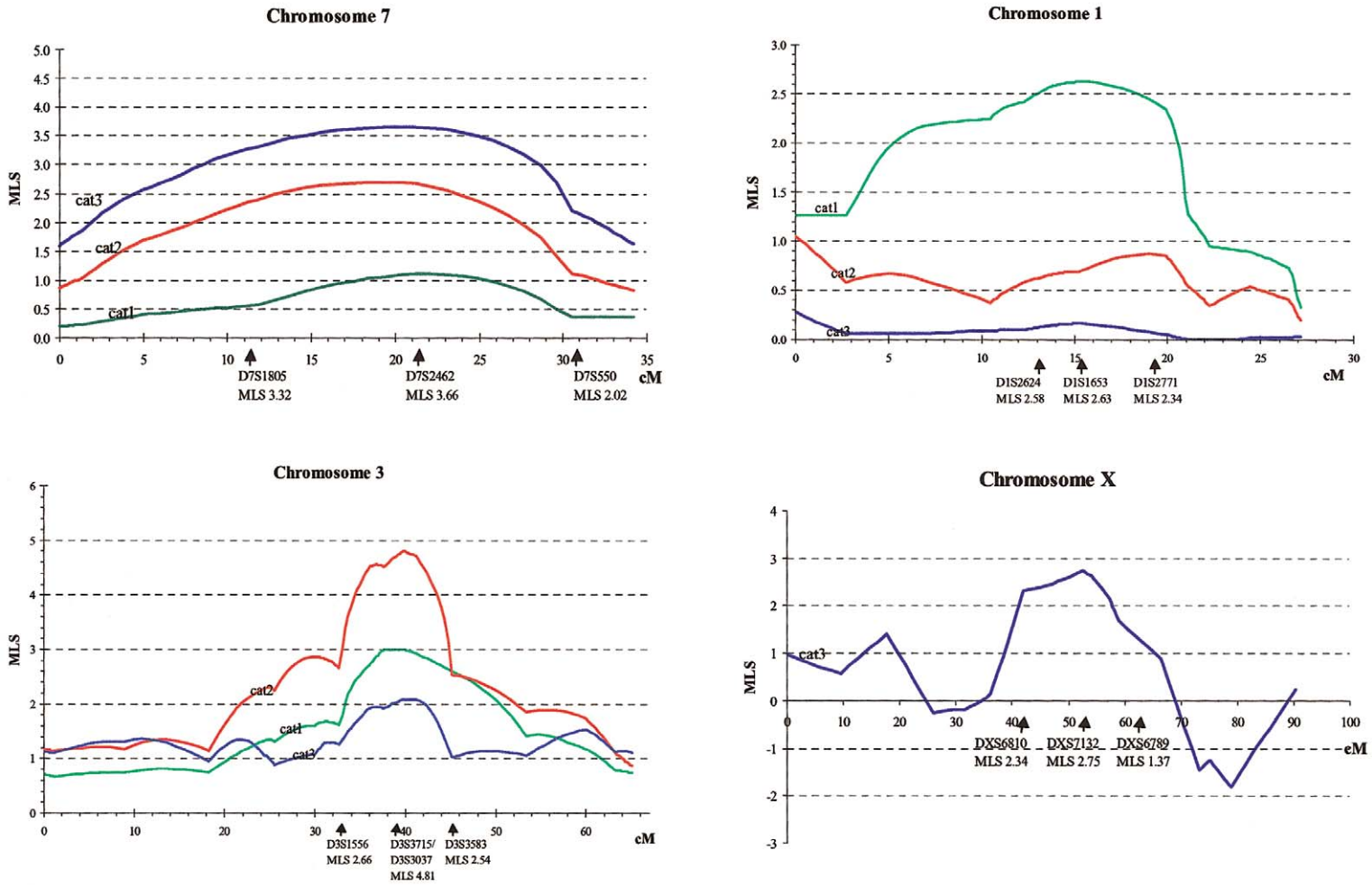

Figure 3 Parametric multipoint LOD score (MLS) analysis, which allows for heterogeneity, was performed using the SIMWALK 2.81 program in different diagnostic categories. On chromosome X, the MLS analysis was performed using the GENEHUNTER program with diagnostic criterion 3. Cat1 = diagnostic criterion 1, Cat2 = diagnostic criterion 2, and Cat3 = diagnostic criterion 3.

Table 3

Maximum Pairwise LOD Scores ($Z_{max\ dom}$) and Affected Sib-Pair Scores (ASP) in Different Diagnostic Criteria in Stage II

		Score for Criterion					
		1		2		3	
Marker	Distance (cM)	$Z_{max\ dom}$	ASP	$Z_{max\ dom}$	ASP	$Z_{max\ dom}$	ASP
D1S1675	149.20	1.98	1.69	2.63	1.67	1.07	.95
D1S498	155.89	2.32	1.40	.59	.51	.10	.09
D1S2715	159.32	1.76	.84	.27	.10	.15	.17
D1S2125	163.34	1.58	1.32	.88	.25	.20	.00
D1S1653	164.09	1.52	.93	1.03	.47	.19	.00
D1S2771	168.52	1.20	.82	1.45	.99	.10	.10
D3S3038	44.81	.46	.11	.38	.40	.76	.96
D3S3659	47.44	.35	.18	.86	.38	1.26	.42
D3S3053	181.87	1.69	2.01	2.68	2.73	2.06	1.88
D3S1556	183.47	1.55	1.18	2.56	1.66	1.76	.89
D3S2421	186.68	2.30	1.27	***3.51***	2.80	1.20	1.21
D3S2427	188.29	2.40	2.10	***3.05***	2.48	.81	.80
D3S3676	188.29	1.75	1.56	2.18	1.85	.79	.70
D3S3041	188.29	2.74	2.15	***3.55***	2.88	1.55	1.41
D3S3715	190.43	2.22	.68	2.37	1.65	.55	.49
D3S3037	190.43	2.88	***3.16***	***4.31***	***4.21***	2.65	2.28
D3S3699	191.79	2.27	2.08	***3.04***	2.49	.47	.46
D3S3730	191.79	1.96	1.64	***3.21***	2.72	1.49	1.24
D3S3583	195.60	1.51	.84	.97	.87	.57	.57
D9S158	161.71	.63	.65	.48	.16	.19	.01
D12S2078	149.60	1.02	.71	1.19	.67	.33	.22
D14S297	31.75	1.60	1.38	.22	.22	.03	.04
D17S1806	114.41	1.25	1.16	.86	.61	1.24	.94
D17S784	116.86	1.67	1.23	1.92	.22	2.16	.63
D17S1830	116.86	1.44	.98	1.11	.20	1.12	.43
D18S59	.00	.55	.72	.41	.44	.04	.00
D21S1440	36.10	.41	.53	.27	.23	.16	.21

Note.—Markers showing LOD scores >3.0 are set in boldface italics.

der criterion 2, a $Z_{max\ rec}$ of 4.21 was observed at D3S3037 ($\theta = 0.1$; $\alpha = 1.0$). Parametric MLS analysis on the chromosome 3q region that allows for locus heterogeneity resulted in a maximum MLS of 4.81 with an α value of 1.0 under criterion 2 and using the dominant mode of inheritance (fig. 3*c*). In multipoint analyses, the maximum MLS values of 3.01 ($\alpha = 1.0$) and 2.08 ($\alpha = 0.65$) were observed at D3S3037 under criteria 1 and 3, respectively (fig. 3*c*).

Chromosome 1.—A total of 15 additional markers covering ~27 cM were genotyped, the average intermarker distances being 1.4 cM. Under the dominant model of inheritance the highest $Z_{max\ dom}$ of 2.63 was generated under criterion 2 for D1S1675 (table 3). Under the recessive model, the best pairwise LOD scores were obtained under criterion 1: at D1S1675 ($Z_{max\ rec} = 1.98$; $\theta = 0.12$; $\alpha = 1.0$) 2.7 cM D1S534 ($Z_{max\ rec} = 1.11$; $\theta = 0.22$; $\alpha = 1.0$) 4 cM D1S498 ($Z_{max\ rec} = 1.64$; $\theta = 0.16$; $\alpha = 1.0$) – 7.5 cM – D1S2125 ($Z_{max\ rec} = 1.73$; $\theta = 0.06$; $\alpha = 0.98$) – 0.8 cM D1S1653 ($Z_{max\ rec} = 1.14$; $\theta = 0.18$; $\alpha = 1.0$) – 6.8 cM – APOA2 ($Z_{max\ rec} = 1.01$; $\theta = 0.2$; $\alpha = 1.0$). ASP analysis resulted in a significant IBD sharing ratios for alleles of the markers D1S1675 (80%) and D1S2125 (85%) under criterion 1. Parametric multipoint analysis was performed under the dominant mode of inheritance in all diagnostic criteria, resulting in a maximum MLS of 2.63 at D1S1653 (fig. 3*d*) under criterion 1.

Haplotype analysis for the linked chromosome 3 region.—Given that common ancestors from Central Finland were identified in our genealogical search for the families 1 and 3, included in the genome scan, we monitored for shared chromosomal segments on 3q. Interestingly, all three patients with autism in family 1 shared identical haplotypes over the 20-cM chromosomal region between D3S3053 and D3S2436, comprising a total of 12 markers. In this family, the same maternal and paternal alleles were transmitted to all affected sibs (fig. 4). In family 3, the sharing of identical haplotype was observed in the autistic and healthy siblings, but not in the male patient with dysphasia being separated by 15 generations from the family 1 affected siblings (fig. 4).

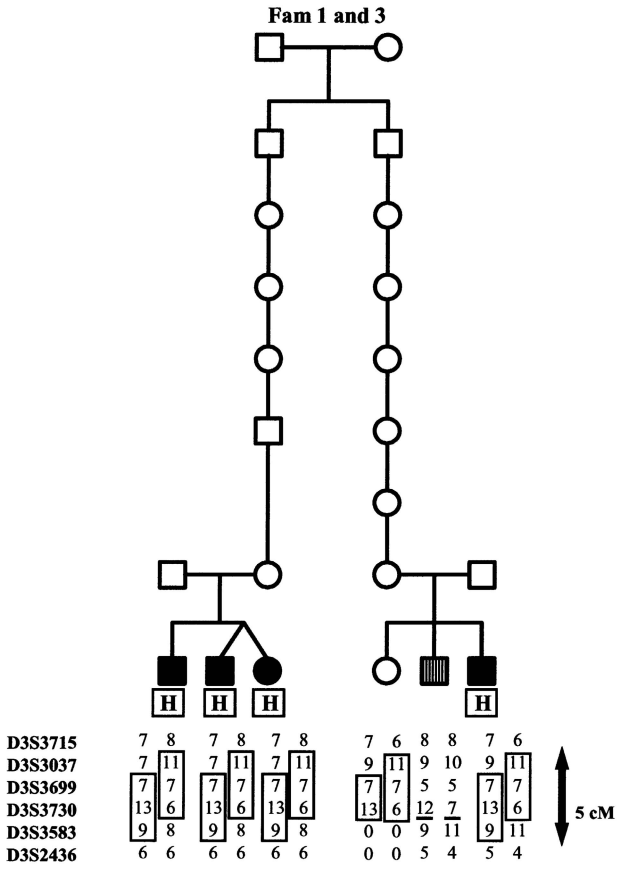

Figure 4 A common ancestor originating from central Finland was detected for core families 1 and 3. The shared chromosomal segment on 3q25-27 was observed in four patients with autism. Blackened symbols denote infantile autism, and symbols with vertical lines denote developmental dysphasia.

We calculated the probability for two relatives, as distantly related as an offspring in nuclear family 1 and an offspring in nuclear family 3, to share one allele IBD at any randomly selected location. The probability of sharing one allele IBD is only 0.000122, making by-chance effect a highly unlikely possibility.

To further address the issue of a restricted pool of ancestral alleles for autism-associated DNA region among affected individuals in this study, we monitored for marker haplotypes across the 4 cM chromosomal region on 3q25-27 covered by six markers. The draft sequence available at the National Council for Biotechnology Information (NCBI) Web site was used to order the markers (fig. 5). Haplotypes were constructed using the GENEHUNTER program and were monitored in 17 families fulfilling diagnostic criterion 1 (36 affected individuals), 25 families fulfilling criterion 2 (53 affected individuals), and in 35 families fulfilling criterion 3 (75 affected individuals), as well as in 18 sib-pair families originating from Central Finland (41 affected individuals). No single shared haplotype could be identified among all families (fig. 5). However, chromosome 3 haplotypes shared by at least two affected sibs were detected in 34 of 35 core families. Allele 11 of D3S3037 was observed in 65% of affected siblings in families originating from the Central Finland, compared to 24% of affected siblings in families originating outside Central Finland. In the control chromosomes of the whole study material (nontransmitted alleles of the parents) the frequency of this allele was 19%.

To test whether there are fewer founder alleles among affected individuals than among control individuals, we used the Genepop 3.3 program to evaluate the LD across the key markers in 3q. In alleles transmitted to the individuals affected under criterion 2, some evidence for LD was observed between D3S3715 and D3S3037, located at the same contig ~30 kb apart (NT_005503.6) ($P = .0108$). In individuals affected under diagnostic

Family	Markers and contig accession number					
	D3S2427 NT_022674.3	D3S3676 NT_022458.4	D3S3041 NT_022458.4	D3S3715 NT_005503.4	D3S3037 NT_005503.4	D3S3699 NT_005950.5
Central Finland						
1	18	7	9	**8**	**11**	**7**
2	**20**	**6**	**10**	**8**	**11**	**7**
4	18	5	8	**8**	**11**	4
9	**20**	**6**	**10**	**8**	10	6
13	15	10	6	7	**11**	6
19	15	**6**	**10**	**8**	10	0
11	22	7	7	7	**11**	**7**
32	22	4	8	6	**11**	6
20	15	7	9	6	**11**	**7**
14	22	9	9	6	**11**	6
48	**20**	**6**	11	6	10	5
15	**20**	7	8	**8**	10	6
16	24	**6**	9	**8**	**11**	4
51	**20**	9	**10**	**8**	9	6
18	24	**6**	8	9	10	**7**
60	19	11	9	9	**11**	**7**
150	17	**6**	9	9	**11**	6
Outside Central Finland						
5	15	9	8	**8**	10	**7**
6	4	0	**10**	7	8	4
7	17	**6**	**10**	**8**	10	4
8	21	11	**10**	**8**	**11**	**7**
14	19	11	9	**8**	10	6
10	21	**6**	11	**8**	8	6
19	18	**6**	8	7	9	0
24	**20**	**6**	4	7	6	5
27	18	**6**	9	7	12	0
28	**20**	9	**10**	6	12	**7**
12	**20**	**6**	9	**8**	**11**	4
13	4	12	6	**8**	12	**7**
42	23	**6**	**10**	**8**	10	6
17	4	9	**10**	6	6	0
62	**20**	9	**10**	**8**	**11**	**7**
128	19	7	8	**8**	9	**7**
133	23	9	6	6	**11**	**7**

Figure 5 The observed chromosomal segment of ~4 cM on 3q in 34 core families. Five markers were localized on the contig map of NCBI. The families are divided into two groups, according to the birthplaces of the grandparents.

criterion 3, evidence for LD emerged from markers D3S3715 and D3S3699, separated by 1.4 cM (P = .0960). In alleles of affected individuals in families from Central Finland, some evidence for LD could be detected across a 2.14-cM region between loci D3S2427 and D3S3715 (P = .0562). No significant LD could be detected across the markers in alleles not transmitted to affected individuals (P = .2484). These data would imply for a restricted pool of ancestral alleles for autism-associated DNA region among patients.

Association Analyses

Association analyses were performed in all diagnostic categories, for the whole study sample and separately, using 34 patients affected under diagnostic criterion 2 who originated from Central Finland. Because of the relatively small sample size, the asymptotic P values listed for the gamete competition should be considered approximations of the actual significance. The markers for association studies were selected on the basis of positive linkage findings.

On chromosome 3q25-27, a statistically significant TDT result was observed under criterion 1 (P = .0127) (table 4*a*). In this patient group, the test for haplotype relative risk by likelihood ratio test (HRR-LRT) showed evidence for association with D3S3041 (P values of .0387) (table 4). For marker D3S3730, evidence for association was detected in TDT analysis under criterion 2 (P = .0488). The most significant evidence for association was generated under criterion 2 in families originating from Central Finland with P = .0077 in TDT and P = .0063 in gamete-competition analysis. When monitoring the allele composition between the transmitted and nontransmitted alleles using Genepop 3.3 analysis, differences in were pronounced in the different groups of families analyzed. At D3S3699, P values of .00002 (diagnostic criterion 1), .00147 (diagnostic criterion 2), and .00585 (diagnostic criterion 3) were generated. In families from Central Finland that met criterion 2, P values of .00013 and .02 were observed at D3S3037 and D3S3699.

In families from Central Finland, an allelic association was observed for two markers. For marker D3S3037, allele 11 was observed in 52% of the disease chromosomes and in 14% of the control chromosomes in families (P = .0203; λ = 0.421). For marker D3S3699, 58% of autism chromosomes shared allele 7; this allele was present in 16% of the control chromosomes in individuals affected under criterion 1 (P = .0120; λ = 0.479).

Also, for markers on chromosome 1, association analyses showed evidence for allelic association in all diagnostic classes for marker D1S2771, which was located ~4 cM telomerically from D1S1653 (table 4). In TDT analysis, the best P value, .0004, was detected for marker D1S2771 under criterion 1. Evidence of association in TDT was also observed for D1S1653 in individuals affected under criteria 1 and 2 (table 4).

Discussion

The most promising locus to emerge from our genome-wide scan was on chromosome 3q, representing a new locus for the autism-spectrum phenotype. The markers in this region showed linkage and some evidence for association, as well as increased intermarker LD, implying shared ancestral alleles among affected individuals in the vicinity of markers D3S3037 and D3S3699. Markers on 1q also provided some evidence for linkage in our study sample of 38 families.

Table 4

Results of Association and Gamete-Competition Analyses in Stage II

		Results for											
		Criterion 1			Criterion 2			Criterion 3			Central Finland (Criterion 2)		
Marker	Distance (cM)	HRR-LRT	TDT	GC	HRR-LRT	TDT	GC	HRR-LRT	TDT	GC	HRR-LRT	TDT	GC
Chromosome 3:													
D3S2427	188.29	.5000	.0567	.5649	.5000	.0717	.3539	.5000	.1760	.5179	.5000	.1582	.3562
D3S3676	188.29	.5000	.3130	.0853	.5000	.2550	.0321	.5000	.1330	.0660	.5000	.3606	.1410
D3S3041	188.29	.0387	.8050	.5676	.0705	.5580	.3202	.5000	.2910	.1259	.5000	.3532	.3079
D3S3715	190.43	.5000	.4080	.1956	.0828	.3140	.2733	.5000	.7920	.6542	.4971	.2325	.3648
D3S3037	190.43	.5000	.1050	.1330	.5000	.2070	.2308	.5000	.4310	.4176	.5000	.0077	.0063
D3S3699	191.79	.1252	.0127	.0524	.5000	.5370	.4484	.2347	.6530	.6709	.5000	.2180	.0801
D3S3730	191.79	.5000	.1380	.2834	.3585	.0488	.1082	.0952	.0807	.0996	.5000	.1900	.2064
D3S3583	195.60	.5000	.6690	.6733	.5000	.4850	.4803	.5000	.4680	.3546	.5000	.2397	.5144
D3S2436	203.28	.5000	.8480	.8816	.5000	.2380	.4710	.5000	.1350	.2204	.1917	.3716	.7458
Chromosome 1:													
D1S1653	164.09	.4957	.0069	.0231	.5000	.0327	.13637	.5000	.1850	.38575	.5000	.3710	.2764
D1S2771	168.52	.0020	.0004	.0028	.0075	.0006	.13080	.1125	.0232	.14521	.0418	.0051	.0091

Note.—GC = Gamete competition.

While we established the specific clinical diagnoses for the affected individuals in our study sample, we found many relatives of autistic individuals to present the broad phenotypic spectrum of autism. The family members with AS and developmental dysphasia were diagnostically classified, and this information was used in the linkage analyses to define three diagnostic categories. To be able to fully compare our results with the previously published genomewide screens, infantile autism was selected as a target diagnostic group. Consequently, the loci for fine mapping were selected on the basis of the data from a primary genome scan performed in 12 families and using infantile autism (diagnostic criterion 1) as an affection status.

Our results on chromosome 1p, although only suggestive, can be interpreted as a replication of the suggestive linkage reported by Risch et al. (1999) in 139 sibships with mixed American origin. They reported a maximum MLS of 2.15 at D1S1675 in the largest genomewide screen for autism published so far (Risch et al. 1999). We obtained the $Z_{\text{max dom}}$ of 1.98 with the same marker, D1S1675, in 19 families meeting criterion 1 and the $Z_{\text{max dom}}$ of 2.63 in 28 families meeting criterion 2. Some evidence for linkage also emerged for the long arm of chromosome 1. For families meeting criterion 1, we found a maximum MLS of 2.63 near D1S1653, located ~13 cM distally from marker D1S1675. No linkage reports in autism have been reported in this chromosomal region, but, interestingly, an MLS of 6.50 close to marker D1S1653 was recently reported in a study of schizophrenia (Brzustowicz et al. 2000). It is of interest that earlier family studies have shown increased rates of schizoid personality traits in families with autism. These neuropsychiatric disorders could share a susceptibility gene or genes in this particular chromosomal region (Piven et al. 1994, 1997*a,* 1997*b;* Murphy et al. 2000).

Chromosomes 7q and X provided some linkage evidence in families meeting criterion 3. The putative autism-predisposing region on chromosome 7q colocalizes with the locus for specific language development (SPCH1), for which the gene was recently identified (Fisher et al. 1998; Ashley-Koch et al. 1999; Lai et al. 2000; Lai et al. 2001; IMGSAC 2001*a*). In our families, fine mapping of the previously reported AUTS1 locus did not show any evidence for linkage under diagnostic criterion 1 or 2 on this region. The best result under criterion 3, a $Z_{\text{max dom}}$ of 1.05 ($\theta = 0$, $\alpha = 0.44$), was detected at D7S1824. Interestingly, a $Z_{\text{max dom}}$ of 3.04 was obtained at D7S2462 locating ~58 cM telomerically from the AUTS1 locus (IMGSAC 2001*a*). This particular marker also showed evidence for association in our study sample in TDT and gamete competition test. Liu et al. (2001) reported a maximum MLS of 2.13 with 110 sib-pair families with a broad phenotype (autism, AS, or PDD) at D7S483 that resides ~4.6 cM proximal from our best marker D7S2462. Taken together, our results provide further support for a locus predisposing to autism-spectrum disorders, locating markedly telomerically than the previously published AUTS1 locus (IMGSAC 2001*a*).

For the X chromosome, a $Z_{\text{max dom}}$ of 1.89 was obtained at DXS7132 in families meeting criterion 3, and the nonparametric MLS analysis resulted in a maximum MLS of 2.75 close to this same marker. One earlier genome scan has produced positive signals on chromosome X (Liu et al. 2001). The MLS of 2.56 was obtained at DXS1047, located ~30 cM distally from our peak of linkage, using 110 multiplex families and the broad phenotype classification. The increased male-to-female ratio in PDD is well established (Skuse 2000). Further studies based on putative linkage findings on chromosome X could provide a molecular basis for this difference.

Loci on chromosome 14q and 17q provided only suggestive evidence for linkage. These findings could still be interesting, since some positive LOD scores have previously been reported for chromosome 14 in British families (IMGSAC 1998) and for chromosome 17 in American families (Risch et al. 1999). For chromosome 17, the locus was reported in the mixed American sample set (Risch et al. 1999), with markers locating at a significant distance, 104 cM, proximal to our region. A Z_{max} of 2.19 at D17S784 was previously reported for familial schizophrenia (Brzustowicz et al. 2000). Thus, as in the case of chromosome 1q, these findings raise the possibility of shared susceptibility gene(s) underlying infantile autism and schizophrenia.

Recently, association strategies for mapping complex human disorders have been strongly advocated, especially in isolated populations (Shifman and Darvasi 2001). The families in our study originate from the isolated population of Finland, with a limited number of founders and multiple population bottlenecks, especially in regional isolates with little immigration and rapid expansion (Peltonen et al. 1999). In the Finnish population, the mapping for predisposing loci has been accomplished for many common diseases, including colon cancer (Peltomaki et al. 1993), diabetes (Mahtani et al. 1996), multiple sclerosis (Kuokkanen et al. 1997), familial combined hyperlipidemia (Pajukanta et al. 1998), schizophrenia (Ekelund et al. 2000), and asthma (Laitinen et al. 2001). On the basis of the initial linkage finding on chromosome 3q, this particular region was analyzed for association in families originating from a subisolate of central Finland. The observed linkage was supported by the association observed at D3S3037 in families from central Finland. Further, when monitoring for the intermarker LD in disease alleles of individuals affected under criterion 2, we found increased LD in-

tervals with markers on the same region, suggesting a restricted number of ancestor chromosomes in this regional subisolate.

Careful definition of the phenotype is one of the major challenges in mapping complex disease traits—particularly behavioral neuropsychiatric phenotypes. Splitting the phenotype into endophenotypes or using a broad clinical phenotype has resulted in significant evidence for predisposing loci in linkage analyses of some phenotypes (Brzustowicz et al. 2000; Stober et al. 2000). Here, the best results were obtained with the broad autism phenotype (infantile autism and AS), partly because the broad classification increased the number of affected family members from 54 to 87, increasing the information content in the affected-only analyses. The results indicate a novel gene locus (AUTS2) for autism-spectrum disorders on chromosome 3q25-27. The significance of this finding is supported by the suggestive association and skewing of the marker alleles in disease chromosomes from the genetically isolated Finnish subpopulation from Central Finland, which may facilitate the positional cloning of a susceptibility gene. Replication studies are warranted to confirm whether this region contains a predisposing gene shared by other populations or whether it is a rare locus unique to the Finns.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Généthon, http://www.genethon.fr/php/index.php
Genepop, http://wbiomed.curtin.edu.au/genepop/
Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/ldb.html
Marshfield, http://research.marshfieldclinic.org/genetics/
NCBI, http://www.ncbi.nlm.nih.gov/ (for draft sequence used to order markers)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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