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Genome–wide scan for autism susceptibility genes

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Family and twin studies have suggested a genetic component in autism. We performed a genome-wide screen with 264 microsatellites markers in 51 multiplex families, using non-parametric linkage methods. Families were recruited by a collaborative group including clinicians from Sweden, France, Norway, the USA, Italy, Austria and Belgium. Using two-point and multipoint affected sib-pair analyses, 11 regions gave nominal *P*-values of 0.05 or lower. Four of these regions overlapped with regions on chromosomes 2q, 7q, 16p and 19p identified by the first genome-wide scan of autism performed by the International Molecular Genetic Study of Autism Consortium. Another of our potential susceptibility regions overlapped with the 15q11–q13 region identified in previous candidate gene studies. Our study revealed six additional regions on chromosomes 4q, 5p, 6q, 10q, 18q and Xp. We found that the most significant multipoint linkage was close to marker *D6S283* (maximum lod score = 2.23, *P* = 0.0013).

INTRODUCTION

Autism (MIM 209850) is an aetiologically heterogeneous syndrome. Approximately 10–25% of autism cases are due to known medical conditions, involving environmental factors or genetic disorders (1,2). The cause remains unknown in the other cases. The risk of developing autism is ~50–100 times greater for siblings of autistic individuals than for the general population (3). Twin studies have shown a much higher concordance for monozygotic than for dizygotic twins, suggesting a strong genetic component to autism (4). Candidate gene studies with family-based case controls have demonstrated linkage disequilibrium between autism and a marker in the γ-aminobutyric $acid_A$ receptor subunit gene (*GABRB3*), and between autism and polymorphisms of the serotonin transporter gene (*5-HTT*) (5–7). The first genome-wide screen for autism, conducted by the International Molecular Genetic Study of Autism Consortium (IMGSAC), has suggested the involvement of six different chromosomal regions (4, 7, 10, 16, 19 and 22) (8). We undertook a full genome-wide screen for autism susceptibility loci, using the non-parametric sib-pair method in 51 multiplex families.

RESULTS

Fifty-one families (described in Table 1) including at least two siblings or half-siblings affected by autism were recruited. Full genotype information was available for all parents except four fathers. All the families were Caucasian, 18 were from Sweden (35%), 15 from France (29%), six from Norway (12%), five from the USA (10%), three from Italy (6%), two from Austria (4%) and two from Belgium (4%). The mean age of the probands was 13.5 years (4–44) and the sex ratio was 2.85 (77 boys and 27 girls). The non-verbal IQ distribution was: IQ >70 for 12.5% of the patients, between 50 and 70 for 30.5%, and <50 for 57%.

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Table 1. Description of families

	Number of families	
	Autosomal screening	X chromosome screening
Two affected siblings	47	47
Two affected siblings: two half-siblings	0	2
Three affected siblings	1	1
Three affected siblings: two full and one half-sibling	1	1
Total number of affected sib-pairs	51	55
Sex of affected sib-pairs		
Male/male	25	29
Male/female	22	22
Female/female	4	4

Table 2. Genome scan non-parametric linkage results: maximum lod score (MLS) values for two- or multipoint analyses (significance level ≤5%).

Locus	Position (cM)[a]	Two-point analysis		Multipoint analysis	
		MLS	*P*-value	MLS[b] (% IBD sharing)	*P*-value
D2S382	210.4	0.98	0.0160		
PEAK 2	223.1			0.64 (62.7)	0.0659
D2S364	231.5	0.65	0.0400		
PEAK 4	226.1			0.88 (61.0)	0.0352
D4S1535	226.1	0.92	0.0199		
D5S417	0.0	0.75	0.0315		
PEAK 5	0.0			0.84 (61.3)	0.0391
D6S283	128.0	1.02	0.0149		
PEAK 6	132.8			2.23 (68.6)	0.0013
PEAK 7	135.3			0.83 (59.0)	0.0401
D7S486	135.3	0.52	0.0609		
D10S217	196.9	0.61	0.0460		
PEAK 10	212.0			0.84 (66.0)	0.0391
D15S128	0.0	0.66	0.0402		
PEAK 15	41.1			1.10 (63.0)	0.0201
D15S118	41.1	0.76	0.0305		
D16S3075	10.6	0.62	0.0456		
PEAK 16	17.1			0.74 (60.5)	0.0506
D18S68	60.0	1.47	0.0046		
PEAK 18	60.0			0.62 (58.5)	0.0695
D19S226	24.1	1.17	0.0102		
PEAK 19	24.1			1.37 (61.9)	0.0103
DXS996	0.0	0.89	0.0210		
PEAK X	0.0			0.40 (60.0)	0.0900

[a]Position, in cM (Haldane function), of loci from pter to qter. Marker frequencies were estimated from the data using the computer program ILINK from the Linkage software package (28). Inter-locus distances were estimated from the data using the VITESSE program (29).
[b]Multipoint MLS values were maximized over the 'possible triangle' (MAPMAKER/SIB version 2.1) except for X-marker data (ASPEX/sib-phase, version 1.14).

The results of two-point affected sib-pair analysis (SIBPAIR) are summarized in Table 2. A maximum lod score (MLS) >0.6 (nominal $P < 0.05$) was obtained for 12 of the 264 (4.5%) markers tested on chromosomes 2, 4, 5, 6, 10, 15, 16, 18, 19 and X. The highest two-point MLS values were for chromosomes 18 (*D18S68*, MLS = 1.47, $P = 0.0046$), 19 (*D19S226*, MLS = 1.17, $P = 0.0102$) and 6 (*D6S283*, MLS = 1.02, $P = 0.0149$).

These potential regions were also significant at the 5% level in multipoint analysis except for those on chromosomes 2 ($P = 0.0659$), 18 ($P = 0.0695$) and X ($P = 0.0900$) (Fig. 1 and

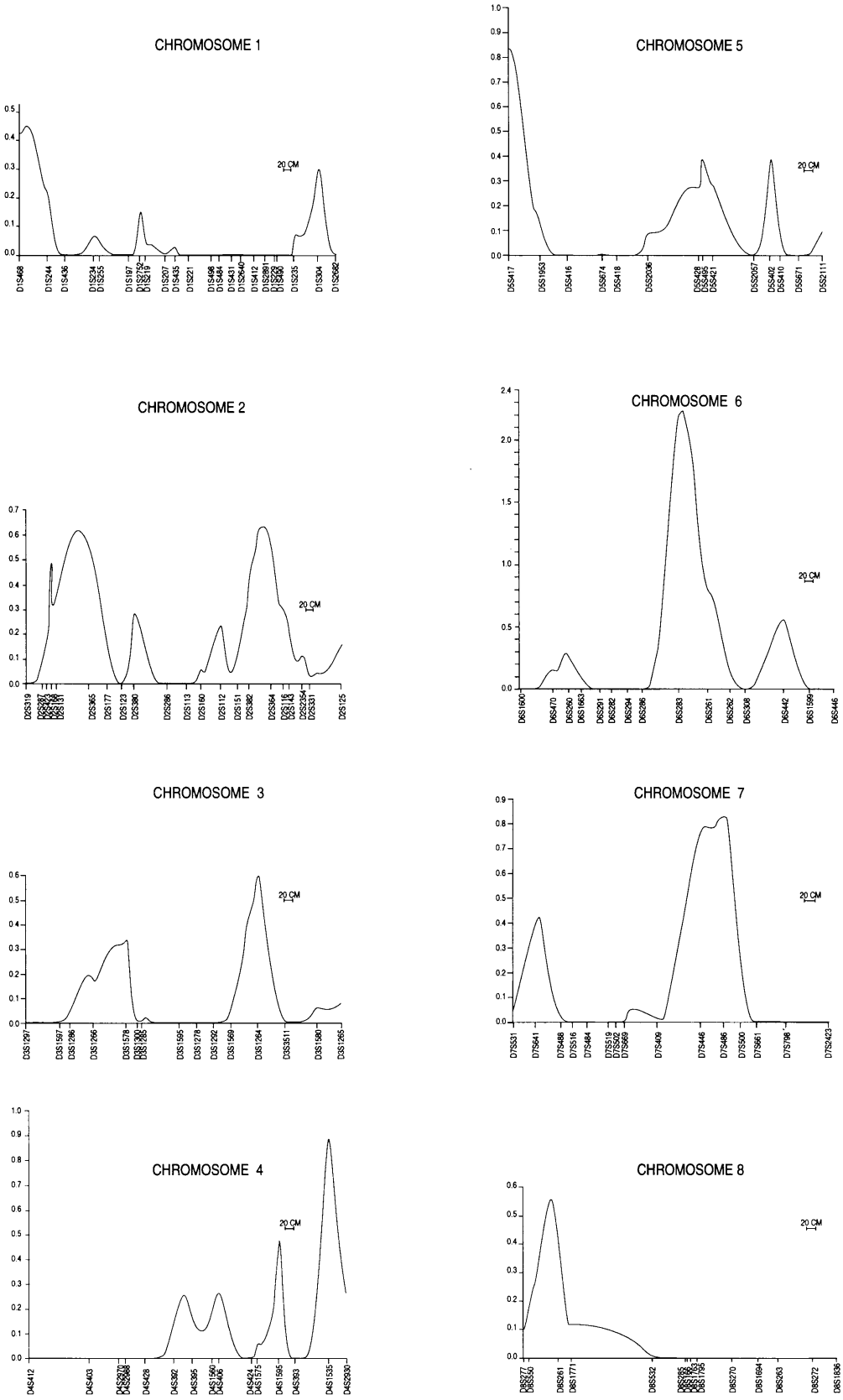

Figure 1. Multipoint MLS values at each point location for each chromosome.

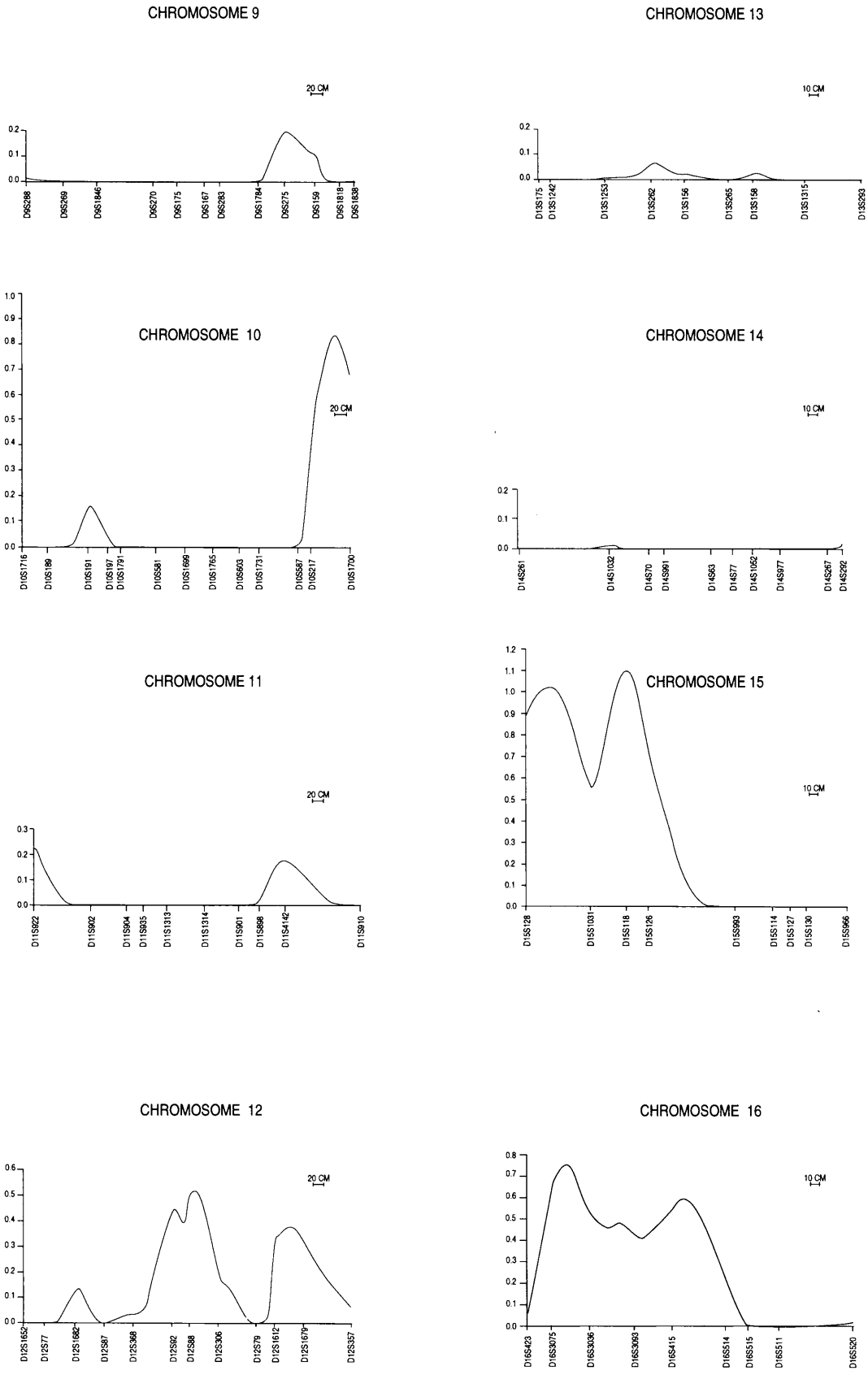

Figure 1. *Continued*

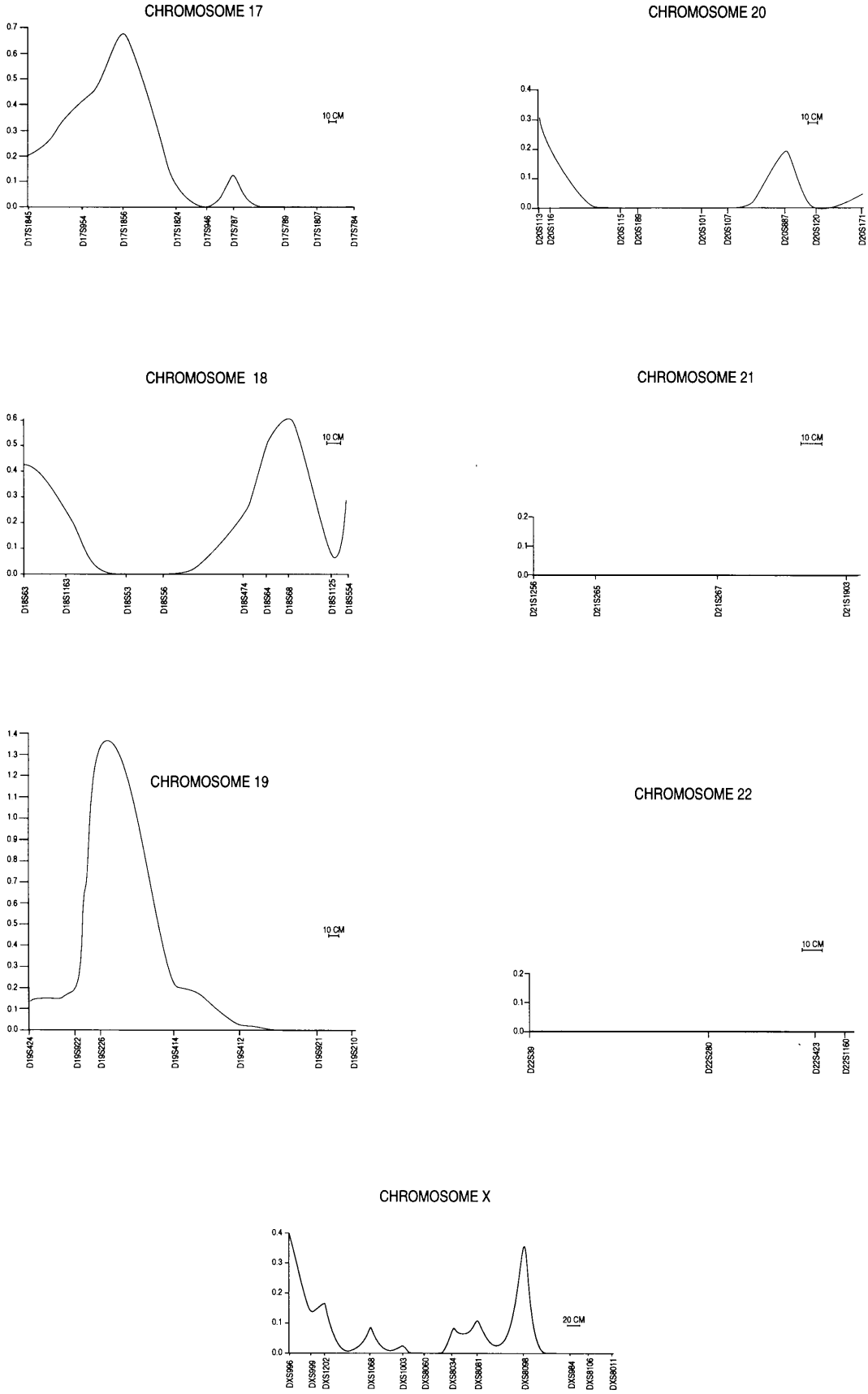

Figure 1. *Continued*

Table 2). Another potential susceptibility region was identified on chromosome 7 (MLS = 0.83, $P = 0.0401$). The most significant result was obtained for a region close to marker *D6S283* (MLS = 2.23).

DISCUSSION

Our genome-wide scan revealed 11 chromosomal regions positively linked to autism with nominal *P*-values of 5% or lower. The multitests problem of these linkage analyses raises the challenging issue of what constitutes a statistically significant finding (9–11). It is also argued that to localize a gene and to replicate its localization require different criteria for significance (9,12). Nonetheless, there is agreement that replication studies are essential to distinguish true from false-positive linkage findings. Our study is, to our knowledge, the first replication study of the IMGSAC findings, the first genome-wide scan of autism (8). Using the genome-wide thresholds derived by Lander and Krugylak (9), none of our positive findings constitutes a 'significant' linkage (i.e. $P < 2.2 \times 10^{-5}$). In our independent sample of autism families, the number of markers significant at the 5% level is close to that expected by chance (13 out of 264). Although the statistical inference is weaker, some of our positive findings show, however, evidence consistent with excess sharing in regions reported by other candidate gene studies or by the IMGSAC group.

Four regions, on chromosomes 2q, 7q, 16p and 19p were identified that overlapped with those reported by the IMGSAC study. Two of these four regions gave the most significant scores in the IMGSAC study: chromosome regions 7q and 16p had peak MLS values of 3.55 and 1.97, respectively, in the English subgroup of 56 affected sib-pair families. However, for the total sample of 87 families, the evidence for linkage of autism to these regions was weaker (MLS = 2.53 and 1.51, respectively) (8). Interestingly, the proband of one affected sib-pair in our initial recruitment was excluded due to microduplication of 16p13. Another patient with autism and partial duplication of 16p has been reported (13). These data reinforce the hypothesis that a susceptibility locus for autism maps on chromosome 16p.

On the other hand, the present study did not replicate three positive results reported by IMGSAC. We found no evidence for excess of alleles shared identical by descent (IBD) on chromosomes 4p, 10p or 22p. There may be several reasons for these conflicting results: false-negative results due to our smaller sample size, false-positive results, genetic heterogeneity between data sets within or between studies, or differences in the diagnostic criteria used in the two studies. We evaluated the power of our family data set (given the observed family structures: sibship sizes, number of affected and unaffected sibs, and DNA availability) to detect an excess of marker alleles IBD sharing (y) of 64 and 59%, corresponding to a locus-specific sibling recurrence risk (λs) of ~2 or 1.6, respectively. These are the values estimated in the total IMGSAC family data from the most significant findings, i.e. chromosomes 7q and 16p markers. Power estimates of our data set (rate of significant replicates out of 2000) were evaluated for different maximum MLS values (data not shown). Our family sample has good power to detect linkage when λs is ≥2. Weaker genetic effects, as expected, are unlikely to be found. For instance, to replicate a finding at the 5% level, the power of our data set is 81 or 44% when $y = 64$ or 59%, respectively.

We also identified a potential susceptibility region, with positive results for three adjacent markers, on chromosome 15 (q11–q15). This region included the critical imprinted region for Prader–Willi and Angelman syndromes. Pericak-Vance *et al.* (14) reported weak linkage with autism for an overlapping region, in 15q11.2–q13, for a sample of 37 multiplex families. Cook *et al.* (5) demonstrated linkage disequilibrium between autism and the γ-aminobutyric acid receptor subunit gene *GABRB3*, which maps to 15q11.2–q12. Several case reports have also described chromosomal abnormalities of the 15q11–q13 region associated with autistic features (15–17).

Finally, our study revealed six other regions potentially involved in autism on chromosomes 4q, 5p, 6q, 10q, 18q and Xp. Excess of alleles shared IBD was most significant on chromosome 6q (MLS = 2.23, IBD = 68.6%). Based on estimated sharing probabilities ($Z_0 = 0.157$, $Z_1 = 0.313$, $Z_2 = 0.107$), the 6q-specific λs was 1.59. Interestingly, Cao *et al.* (18) provided evidence for a schizophrenia susceptibility locus on 6q13–q26 in independent data sets. Possible candidate genes in this region include those for myristoylated alanine-rich protein kinase C substrate (*MACS*), which may be involved in the development of the central nervous system, glutamate receptor 6 (*GRIK6*) and G protein-coupled receptor 6 (*GPR6*), which is most strongly expressed in the putamen.

Fine mapping of the regions identified is under way. Nevertheless, because of the large number of tests in genome-wide approaches, these findings require confirmation on other independent panels of families.

MATERIALS AND METHODS

Families

Families with at least two siblings or half-siblings fulfilling the DSM IV criteria for autistic disorder (19) and the Autism Diagnostic Interview (ADI) algorithm for ICD-10 childhood autism (20) were recruited for an international collaborative autism sib-pair project, involving seven countries (Austria, Belgium, France, Italy, Norway, Sweden and the USA). Subjects were included only after thorough clinical and medical examinations comprising a full exploration of medical and family history, physical (including meticulous skin examination involving Wood's light assessment), neuropsychological [appropriate IQ test or Vineland interview (21)] and neurological examination, standard karyotyping and fragile-X testing (either karyotype in folic acid-depleted medium or molecular genetic testing for the trinucleotide repeat expansion in the *FMR-1* gene), brain imaging and blood and urine analysis. Cases diagnosed in which there were associated organic conditions, such as phenylketonuria, tuberous sclerosis, neurofibromatosis, hypomelanosis of Ito, Rett syndrome, Moebius syndrome, Duchenne muscular dystrophy, Down's syndrome, fragile X syndrome or other established chromosomal disorders, were excluded. However, severe mental retardation, epilepsy, mild motor co-ordination disorders, mild hearing deficits, attention deficits and tics were not exclusion criteria. Blood samples were taken from affected siblings and both parents. The study was approved by the ethical committees

of the collaborating organizations. Informed consent forms were completed by the parents of each child included in the study.

Genotyping

Blood samples were collected from both parents and affected sib-pairs. DNA was extracted and lymphoblastoid cell lines generated. If one parent was unavailable, blood samples were also taken from unaffected siblings to increase the probability of inferring the genotype of the missing parent.

A complete genomic screen was performed with 252 autosomal and 12 X-linked microsatellite markers in the Gnthon Laboratory (Evry, France) (22). We used fluorescent primers. Polymerase chain reaction (PCR) was performed in a total volume of 50 µl, containing 80 ng of genomic DNA, 50 pmol of each primer, 0.125 mM dNTPs and 1 U *Taq* polymerase. The amplification buffer (1×) contained 10 mM Tris base pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100 and 0.01% gelatin. Reactions were performed using a hot-start procedure, with *Taq* polymerase added only after a first denaturation step of 5 min at 96°C. DNA was amplified in 35 cycles of denaturation (94°C for 40 s) and annealing (55°C for 30 s). An elongation step (72°C for 2 min) terminated the reaction after the last annealing. PCR products were combined into pools and typed using the semi-automated Genescan/Genotyper system on ABI 373A sequencing machines.

Linkage analysis

Non-parametric likelihood methods were used for linkage analysis. Pairwise linkage was conducted using the SIBPAIR program (23), which provides a likelihood-based test statistic for linkage. The likelihoods over all families are maximized as a function of the rate of marker alleles IBD (y) among affected sibs, and the likelihood ratio test statistic T is calculated against the null hypothesis of $y = 0.5$ ($T = 2\mathrm{Ln}[L(y)/L(y = 0.5)]$). The statistic follows a χ^2 distribution with 1 degree of freedom and can thus be expressed as a lod score, MLS = T/2ln(10). Multipoint affected sib-pair linkage analysis, which uses information from all markers simultaneously, was performed using the program MAPMAKER/SIBS (24) which tests linkage by the maximum likelihood ratio approach (25). At any chromosomal location, the likelihood of the observed marker information among affected sib-pairs is maximized as a function of the proportion of pairs sharing two, one and zero alleles IBD, and is compared, through a likelihood ratio test, with the likelihood of the marker data under the null hypothesis of no linkage (i.e. $T = 2\mathrm{Ln}[L(Z_2, Z_1, Z_0)/L(Z_2 = 0.25, Z_1 = 0.5, Z_0 = 0.25)]$). An evaluation of the λs attributed to a locus can be calculated as $0.25/Z_0$ (24). Holmans has shown that the power of the test is increased when imposing constraints among Z parameters such as the possible triangle test ($2Z_0 \leq Z_1$ and $Z_1 \leq 1/2$) and that the resulting distribution of T is a mixture of χ^2s with 1 and 2 degrees of freedom (26). The statistic can also be reported as a lod score: MLS = T/2ln(10). For X-chromosome marker data, the likelihood ratio test is a function of one parameter only (i.e. $T = 2\mathrm{Ln}[L(Z_1, 1 - Z_1)/L(Z_1 = 0.5, Z_0 = 0.5)]$). The resulting distribution of T is a χ^2 distribution with 1 degree of freedom. Because the X chromosome version of MAPMAKER/SIBS program is not functional, multipoint MLS values for X marker data were computed using the sib-phase program of ASPEX package (25,27).

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REFERENCES

1. Ritvo, E.R., Mason-Brothers, A., Freeman, B.J., Pingree, C., Jenson, W.R., McMahon, W.M., Petersen, P.B., Jorde, L.B., Mo, A. and Ritvo, A. (1990) The UCLA–University of Utah epidemiologic survey of autism: the etiologic role of rare diseases. *Am. J. Psychiatry*, **147**, 1614–1621.
2. Gillberg, C. and Coleman, M. (1996) Autism and medical disorders: a review of the literature. *Dev. Med. Child Neurol*., **38**, 191–202.
3. Bolton, P., Macdonald, H., Pickles, A., Rios, P., Goode, S., Crowson, M., Bailey, A. and Rutter, M. (1994) A case–control family history study of autism. *J. Child Psychol. Psychiatr*., **35**, 877–900.
4. Smalley, S.L. (1997) Behavioral genetics '97. Genetic influences in childhood-onset psychiatric disorders: autism and attention-deficit/hyperactivity disorder. *Am. J. Hum. Genet*., **60**, 1276–1282.
5. Cook, E.H., Courchesne, R.Y., Cox, N.J., Lord, C., Gonen, D., Guter, S.J., Lincoln, A., Nix, K., Haas, R., Leventhal, B.L. and Courchesne, E. (1998) Linkage-disequilibrium mapping of autistic disorder, with 15q11–q13 markers. *Am. J. Hum. Genet*., **62**, 1077–1083.
6. Cook, E.H., Courchesne, R., Lord, C., Cox, N.J., Yan, S., Lincoln, A., Haas, R., Courchesne, E. and Leventhal, B.L. (1997) Evidence of linkage between the serotonin transporter and autistic disorder. *Mol. Psychiatry*, **2**, 247–250.
7. Klauck, S.M., Poustka, F., Benner, A., Lesch, K.-P. and Poustka, A. (1997) Serotonin transporter (*5-HTT*) gene variants associated with autism? *Hum. Mol. Genet*., **6**, 2233–2238.
8. International Molecular Genetic Study of Autism Consortium (1998) A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum. Mol. Genet*., **7**, 571–578.
9. Lander, E. and Kruglyak, L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genet*., **11**, 241–247.
10. Elston, R.C. (1997) 1996 William Allan Award Address. Algorithms and interferences: the challenge of multifactorial diseases. *Am. J. Hum. Genet*., **60**, 255–262.
11. Terwilliger, J.D., Shannon, W.D., Lathrop, G.M., Nolan, J.P., Goldin, L.R., Chase, G.A. and Weeks, D.E. (1997) True and false positive peaks in genomewide scans: applications of length-biased sampling to linkage mapping. *Am. J. Hum. Genet*., **61**, 430–438.
12. Lermark, A. and Ott, J. (1998) Sometime it's hot, sometimes it's not. *Nature Genet*., **3**, 213–214.
13. Hebebrand, J., Martin, M., Ksrner, J., Roitzheim, B., de Braganca, K., Werner, W. and Remschmidt, H. (1994) Partial trisomy 16p in an adolescent with autistic disorder and Tourette's syndrome. *Am. J. Med. Genet*., **54**, 268–270.
14. Pericak-Vance, M.A., Wolpert, C.M., Menold, M.M., Bass, M.P., Delong, G.R., Beaty, L.M., Zimmerman, A., Potter, N., Gilbert, J.R., Vance, J.M., Wright, H.H., Abramson, R.K. and Cuccaro, M.L. (1997) Linkage evidence supports the involvement of chromosome 15 in autistic disorder (AUT). *Am. J. Hum. Genet*., **61** (Suppl.), A40.
15. Martinsson, T., Johannesson, T., Vujic, M., Sjsstedt, A., Steffenburg, S., Gillberg, C. and Wahlstrsm, J. (1996) Maternal origin of inv dup (15) chromosomes in infantile autism. *Eur. Child Adolescent Psychiatry*, **5**, 185–192.
16. Cook, E.H., Lindgren, V., Leventhal, B.L., Courchesne, R., Lincoln, A., Shulman, C., Lord, C. and Courchesne, E. (1997) Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am. J. Hum. Genet*., **60**, 928–934.

17. Schroer, R.J., Phelan, M.C., Michaelis, R.C., Crawford, E.C., Skinner, S.A., Cuccaro, M., Simensen, R.J., Bishop, J., Skinner, C., Fender, D. and Stevenson, R.E. (1998) Autism and maternally derived aberrations of chromosome 15q. *Am. J. Hum. Genet.*, **76**, 327–336.
18. Cao, Q., Martinez, M., Zhang, J., Sanders, A.R., Badner, J.A., Cravchik, A., Markey, C.J., Beshah, E., Guroff, J.J., Maxwell, M.E., Kazuba, D.M., Whiten, R., Goldin, L.R., Gershon, E.S. and Gejman, P.V. (1997) Suggestive evidence for a schizophrenia susceptibility locus on chromosome 6q and a confirmation in an independent series of pedigrees. *Genomics*, **43**, 1–8.
19. American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. APA, Washington, DC.
20. Lord, C., Rutter, M. and Le Couteur, A. (1994) Autism Diagnostic Interview—Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J. Aut. Dev. Dis.*, **24**, 659–685.
21. Sparrow, S.S., Balla, D. and Cicchetti, D.V. (1984) *Vineland Adaptative Behavior Scales*. American Guidance Service, Circle Pines, MN.
22. Dib, C., Faur, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Milasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J. and Weisenbach, J. (1996) The Gnthon human genetic linkage map. *Nature*, **380**, 152–154.
23. Terwilliger, J. (1996) Program SIBPAIR: sibpair analysis on nuclear families. ftp://linkage.cpmc.columbia.edu .
24. Kruglyak, L. and Lander, E.S. (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am. J. Hum. Genet.*, **37**, 439–454.
25. Risch, N. (1990) Linkage strategies for genetically complex traits. I. Multilocus models. *Am. J. Hum. Genet.*, **46**, 222–228.
26. Holmans, P. (1993) Asymptotic properties of affected-sib-pair linkage analysis. *Am. J. Hum. Genet.*, **52**, 362–374.
27. Hinds, D. and Risch, N. (1996) The ASPEX package: affected sib-pair mapping. ftp://lahmed.stanford.edu/pub/aspex .
28. Lathrop, G.M. and Lalouel, J.M. (1984) Easy calculations of LOD scores and genetic risks on small computers. *Am. J. Hum. Genet.*, **36**, 460–465.
29. O'Connell, J.R. and Weeks, D.E. (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. *Nature Genet.*, **11**, 402–408.