Suggestive evidence for association of D2S2188 marker (2q31.1) with autism in 143 Sicilian (Italian) TRIO families
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We have screened 143 Sicilian (Italian) families with one autistic child to verify, by a linkage disequilibrium approach, the involvement of the 2q31.1 region in the cause of the disease in these families. Our study design includes the use of intrainfamilial association to prevent a population stratification bias and ethnic homogeneity of the sample. The results of our analysis provided suggestive evidence of the occurrence of transmission disequilibrium between autism and the D2S2188 polymorphism in Sicilian TRIO families, a finding which provides further and independent support to the hypothesis of the existence of a susceptibility gene (or genes) for autism on chromosome 2q. \textit{Psychiatr Genet} 15:149–150 © 2005 Lippincott Williams & Wilkins.

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Introduction
D2S2188 (Genebank no. Z52467) is the STR marker, on chromosome 2, to date displaying the highest LOD score value on a genomewide screen in multiplex autistic families [International Molecular Genetic Study of Autism Consortium (IMGSAC), 2001]. The general aim of our study was to verify, by a linkage disequilibrium-based approach, the involvement of the 2q31.1 region in autistic disorder in 143 Sicilian families with one affected child. This analysis uncovered suggestive evidence of transmission disequilibrium of the D2S2188 marker in Sicilian TRIO families, a finding that provides further and independent support to the hypothesis of the existence of a susceptibility gene (or genes) for autism on chromosome 2q (IMGSAC, 2001; Macstrini \textit{et al.}, 2000; Buxbaum \textit{et al.}, 2001; Shao \textit{et al.}, 2002).

Patients and methods
All patients (128 boys and 15 girls accounting for a male/female ratio of 8.53, mean age 12.43 years; SD ± 5.71 years) are mentally retarded and met DSM-IV criteria for autistic disorder. In addition, patients were assessed by the (1) Childhood Autism Rating Scale, (2) Brunet–Lezine test, (3) Weschler Intelligence Scale for Children-Revised (WISC-R), (4) Psychoeducational Profile Revised (PEP-R), (5) Griffith’s Mental Developmental Scales and (6) Leiter International Performance Scale. Patients who were excluded from the study include those displaying at least one of the following: neurological focal signs or seizures, chromosomal abnormalities, \textit{FKR1} gene mutation (fragile X syndrome) and other neurological diseases such as phenylketonuria, neurofibromatosis, tuberous sclerosis and encephalopathies due to congenital infections. ‘Sicilian ancestry’ for more than 95% of all patients was ascertained, for at least two generations, by enquiring about the place of birth of their maternal and paternal grandparents. Informed consent was obtained from patients’ parents. Typing of D2S2188 in all TRIO families included isolation of genomic DNA from peripheral blood, polymerease chain reaction (PCR) and electrophoresis by an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Details on the protocols used (primers, PCR and sequencing reactions etc.) are available, upon request, directly from the authors. The Hardy–Weinberg equilibrium was tested by the $\chi^2$ test for goodness of fit. Analysis of transmission disequilibrium from 193 heterozygote parents was carried out using extended transmission disequilibrium test (ETDT) ( Spielman \textit{et al.}, 1993) (program of David Curtis: ftp://ftp.genie.ucl.ac.uk/pub/packages/decurtis, version 1.8) and multialelic transmission disequilibrium test (MTDT) ( Spielman \textit{et al.}, 1993).
Table 1: Extended transmission disequilibrium test (ETDT) and multiallelic transmission disequilibrium test (MTDT) analyses of D2S2188 in Sicilian autistic TRIO families

<table>
<thead>
<tr>
<th>Allele no. (%)</th>
<th>Not passed</th>
<th>Passed</th>
<th>χ²</th>
<th>P Values**</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 (0.5)</td>
<td>2</td>
<td>1</td>
<td>0.667</td>
<td>0.4142</td>
</tr>
<tr>
<td>131 (1.0)</td>
<td>4</td>
<td>2</td>
<td>6.400</td>
<td>0.0114</td>
</tr>
<tr>
<td>133 (7.9)</td>
<td>28</td>
<td>12</td>
<td>1.166</td>
<td>0.2904</td>
</tr>
<tr>
<td>135 (25.3)</td>
<td>79</td>
<td>66</td>
<td>0.095</td>
<td>0.7570</td>
</tr>
<tr>
<td>137 (8.6)</td>
<td>20</td>
<td>22</td>
<td>0.200</td>
<td>0.6547</td>
</tr>
<tr>
<td>141 (7.0)</td>
<td>39</td>
<td>47</td>
<td>0.953</td>
<td>0.3290</td>
</tr>
<tr>
<td>143 (4.3)</td>
<td>5</td>
<td>18</td>
<td>7.348</td>
<td>0.0097</td>
</tr>
<tr>
<td>145 (2.0)</td>
<td>9</td>
<td>12</td>
<td>0.800</td>
<td>0.3711</td>
</tr>
</tbody>
</table>

MTDT: χ² = 15.85, df = 6, P = 0.045 (for MTDT calculation alleles 125 and 147 were pooled). *N* = 560; Allele-wise ETDT: χ² = 19.62, df = 9, P = 0.021; Genotype-wise ETDT: χ² = 38.76, df = 25, P = 0.039. **No allele yielded significant P values after correction for multiple testing.

1996; Buxbaum et al., 2002) tests. Further details on the application of the MTDT test can be obtained directly from the authors.

Results and discussion

Overall, 10 alleles were identified for the D2S2188 dinucleotide STR and their sizes ranged from 125 to 147 bp (Table 1). Alleles no. 127 and no. 129 were never observed in our families' samples. An analysis of the Hardy-Weinberg equilibrium was carried out separately for the group of patients and their parents and did not uncover significant differences between the observed and expected number of both homozygotes and heterozygotes (P > 0.17). As shown in Table 1, both allele-wise (χ² = 19.62, df = 9, P = 0.021) and genotype-wise (χ² = 38.76, df = 25, P = 0.039) transmission disequilibrium tests are nominally significant. In contrast, individual analysis of the alleles did not yield significant P values, after Bonferroni's correction. Whereas the P value required to achieve significance at an overall level of 0.05 for 10 alleles is 0.005, a value of 0.0067 was obtained for allele no. 143, which displays the highest transmission distortion (see Table 1). Similar to allele-wise analysis, MTDT also yielded a nominally significant P value (χ² = 15.85; df = 8; P = 0.045). In summary, the results of our analyses provide suggestive and preliminary evidence of an association between autistic disorder and D2S2188 in Sicilian TRIO families. These findings, which need to be confirmed by the analysis of additional markers flanking D2S2188, should also be interpreted in the light of the significant differences distinguishing these from previous studies (IMGSAC, 2001; Buxbaum et al., 2001; Shao et al., 2002) also reporting significant linkage of autism to 2q. These differences would include clinical diagnosis of patients and their geography (environment) and ethnicity (genetic structure). For instance, the failure to use the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994) and Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 1989) for diagnosing autism in Sicilian patients makes the present study at variance with the standard that has been established in other genetic studies of autism. On the other hand, it should be pointed out that in the paper of IMGSAC (2001), the D2S2188 marker, while yielding a high maximum LOD score (MLS) of 3.74, did not display allele association with autism spectral disorders, a finding that may reflect a more genetically heterogeneous composition of the IMGSAC sample than that of the Sicilian families. In this context, the strengths of our study design include the use of intrafamilial association to prevent a population stratification bias and the ethnic homogeneity of the sample. Notwithstanding the limitations and peculiarities of our study, it is compelling that the results of our initial analysis confirm previous and independent linkage (IMGSAC 2001; Buxbaum et al., 2001; Shao et al., 2002) of autism to chromosome 2q.

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References


