Association of Tryptophan 2,3 Dioxygenase Gene Polymorphism With Autism

Rafiqun Nabi,1 Fatema J. Serajee,1 Diane C. Chugani,1,2 Hailang Zhong,1 and A.H.M. Mahbubul Huq1,3*

1Department of Pediatrics, Wayne State University, Detroit, Michigan
2Department of Radiology, Wayne State University, Detroit, Michigan
3Department of Neurology, Wayne State University, Detroit, Michigan

Although elevation of blood and platelet serotonin has been documented in autism, genetic analyses of serotonin transporter gene have given conflicting results. Tryptophan 2,3 dioxygenase (TDO2) is the rate-limiting enzyme in the catabolism of tryptophan, the precursor of serotonin. A mutation that results in decreased activity of the TDO2 can decrease catabolism of tryptophan and increase the level of whole body serotonin. As such it is a potential candidate gene for autism. We have investigated five single nucleotide polymorphisms in the TDO2 gene for association with autistic disorder. One hundred and ninety six multiplex autistic disorder families were tested using transmission disequilibrium test. There was a significant difference in the transmission of a promoter variant to autistic subjects (P = 0.0006). Haplotype analysis also demonstrated significant difference in the transmission of TDO2 haplotypes to autistic subjects (P = 0.0027). Our results suggest the presence of a susceptibility mutation in the TDO2 or a nearby gene, but may also represent a chance finding.

KEY WORDS: autism; TDO2; serotonin

INTRODUCTION

Autistic spectrum disorders [MIM 209850], which include autism, Asperger’s syndrome, and pervasive developmental disorder (PDD) not otherwise specified, are characterized by impairments in communication and social interactions and presence of stereotyped behaviors. Family and twin studies have demonstrated a high monozygotic to dizygotic twin risk ratio and a sibling relative risk between 50 and 100, suggesting that the predisposition to develop autism is largely genetically determined [Folstein and Rutter, 1977; Bailey et al., 1995]. Although there are a number of biological clues that might inform the genetic study of autism, a consistent finding is the elevation of platelet serotonin (5-hydroxy tryptamine, 5-HT) levels in over 30% of the autistic subjects [Cook et al., 1988]. There is also a report of focal and whole brain abnormalities in serotonin synthesis in children with autism [Chugani et al., 1997, 1999]. Further evidence comes from a study in which decreased serotonergic neurotransmission by tryptophan depletion resulted in an exacerbation of symptoms in autistic subjects [McDougle et al., 1996; Tordjman et al., 2001]. However, although elevation of blood and platelet serotonin have been consistently documented in autism, genetic analyses of serotonin transporter gene have given conflicting results [Herault et al., 1993; Cook et al., 1997; Klauck et al., 1997; Lassig et al., 1999; Zhong et al., 1999; Kim et al., 2000; Philippe et al., 2002; Veenstra-VanderWeele et al., 2002].

Tryptophan 2,3 dioxygenase (TDO2) is the rate-limiting enzyme in the catabolism of tryptophan, the precursor of serotonin [Fernstrom, 1983]. A mutation that alters activity of the TDO2 can alter catabolism of tryptophan and level of whole body serotonin. The TDO2 gene is localized to chromosome 4q31.3 [Comings et al., 1991]. We investigated five single nucleotide polymorphisms (SNPs) of the TDO2 gene for association with autism using 196 autistic disorder families from Autism Genetic Resource Exchange (AGRE).

SUBJECTS AND METHODS

Subjects

DNA samples from 196 families were obtained from the AGRE. AGRE, developed and maintained by the Cure Autism Now Foundation, is a central repository of family DNA samples for genetic studies of autism [Geschwind et al., 2001]. All AGRE families included...
at least two affected members with a diagnosis of autism, Asperger syndrome, or pervasive developmental disorder (PDD) not otherwise specified. Diagnoses of AGRE families were confirmed using the Autism Diagnostic Interview-Revised protocol (ADI-R).

Genotyping

Four promoter polymorphisms were examined. Two of the promoter polymorphisms were genotyped using restriction enzyme digestion based assays. Two other promoter polymorphisms were genotyped through single base primer extension assays. A C/A polymorphism in the promoter (dbSNP: rs3755910) was amplified with primer sequences 5'-TGGATGACTGCTAGCTACC-TG-3' and 5'-TTACCTGTCACTAGCAATC-3' and a PCR program of 95 °C for 5 min, 40 cycles of 95 °C (30 sec), 60 °C (45 sec), and 72 °C (45 sec), followed by 72 °C (10 min). Digestion of the 572 bp amplified product with MboI (New England Biolabs Inc., Beverly, MA) resulted in invariant bands of 207 and 75 bp and a bi-allelic polymorphism with 135 and 155 bp bands if the C allele was present and a band of 290 bp if the A allele was present. Similarly a more 5' A/C polymorphism in the promoter region (dbSNP: rs3775085) was amplified with primer sequences 5'-CTAAGCCAGGACACCAGGAA-3' and 5'-TGCAAATGCCATGTATTGA-3' and a PCR program of 95 °C for 5 min, 40 cycles of 95 °C (30 sec), 60 °C (45 sec), and 72 °C (45 sec), followed by 72 °C (10 min). The product (172 bp) was digested by Eco0109I (New England Biolabs Inc.), which selectively digest the A allele, giving two fragments of 131 and 41 bp.

Two additional promoter SNPs (dbSNP: rs3755907, A/G; dbSNP: rs3755908, C/T) and the exon 11 splice region SNP (dbSNP: rs2292537, A/G) were genotyped by a high throughput single-base primer extension assay with oligonucleotide microarrays and fluorescence detection through Orchid Biosciences' SNPstream UHT services. For these SNPs, we genotyped 196 trios, randomly selecting one affected sib from each multiplex family. Single-base primer extension involves the annealing of an oligonucleotide primer to a single-stranded PCR amplicon at a location which lies immediately adjacent to, but not including the polymorphic SNP site, followed by the addition of a DNA polymerase and subsequent enzymatic extension of the primer in the presence only of chain-terminating dideoxynucleotides, which are labeled to facilitate subsequent detection of the identity of the single incorporated nucleotide.

TDO2 Sequencing

The exons and 500 bp of the upstream promoter region were sequenced in 20 autistic subjects. Primers were designed using sequences from the human contig NT_006171 from the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov).

Statistical and Genetic Analysis

The programs PedCheck and Merlin were used to find Mendelian errors [O’Connell and Weeks, 1998; Abecasis et al., 2002]. Mistyping analyses were also performed using SimWalk2 [Sobel and Lange, 1996]. Marker allele frequencies were obtained by counting parental genotypes. Linkage disequilibrium between the markers was analyzed by the Sim Walk2 and GOLD software packages using parental gametic haplotypes [Sobel and Lange, 1996; Abecasis and Cookson, 2000].

Family based association analyses were performed using the transmission disequilibrium test (TDT), where preferential allelic transmission from heterozygous parents to affected offspring is tested by applying the (b – c)²/(b + c) statistics and the χ² test [Spielman and Ewens, 1996]. The transmission ratio (transmitted/not transmitted) and the 95% confidence interval around the transmission ratio were calculated for each allele of the SNPs. Under a multiplicative model the transmission ratio is an estimator of the genotype relative risk [Schaid, 1999]. Haplotypes of the TDO2 gene were constructed on the basis of transmission patterns in families in which both the parents were genotyped. For each haplotype, a test with 1 df for excess transmission of that haplotype was calculated. Finally, a global test was also performed with H = 1 df, by summing the chi-squared values for each haplotype and multiplying the sum by (H – 1)/H, where H is the number of haplotypes for which transmission data are available. This statistic is approximately chi-squared with H – 1 degrees of freedom [Spielman and Ewens, 1996; Martin et al., 1997].

RESULTS

SNPs

We investigated four SNPs in the promoter region and a SNP in the splice region 3’ to exon 11 (Fig. 1). The TDO2 promoter A/C polymorphism (rs3755910) is a rare polymorphism with a frequency of 2.36% for the rare allele in our sample. The rare allele of the other three TDO2 promoter polymorphisms (rs3775085, A/C; rs3755907, A/G; rs3755908, C/T) has approximately 13% frequency, whereas the rare allele of the 3’ splice polymorphism near exon 11 has 7.49% frequency. The allele and genotype frequency are shown in Table I. There was no deviation from Hardy–Weinberg disequilibrium.

The four promoter SNPs were in strong disequilibrium. The standardized pairwise disequilibrium value D’ and coefficient of disequilibrium, D, were calculated using GOLD software package. The coefficient of disequilibrium, D, is the difference between the observed haplotype frequency and the frequency expected under statistical independence. The D’ measure is a proportion of the maximum value of D, whose range

![Fig. 1. Genomic organization of the TDO2 gene showing the location of the polymorphisms (arrows).](image-url)
extends from $-1$ to $+1$, with $-1$ and $+1$ representing complete LD and 0 representing free association. The pairwise SNPs in the promoter region showed that the first three SNPs were in strong LD with $D' > 0.8$ (range 0.85–0.96). The promoter SNPs were in weak linkage disequilibrium with the 3' SNP. The 4th promoter SNP (rs3755910) was in moderate LD with the other promoter SNPs and the 3' SNP. The output from GOLD analyses pairwise LD between the SNPs are shown in Table II.

### Association Analyses

TDT in 196 AGRE families demonstrated significant difference in the transmission of A/C SNP (rs3755910) to autistic subjects ($P = 0.0006$) (Table III). TDT analyses of the first three promoter SNPs and 3' SNP did not reveal any association (Table III). Multiple affected sibs in each family were genotyped for two promoter SNPs (rs3775085 and rs3755910) in each family, TDT in this case may not be a valid test for association [Spielman and Ewens, 1996]. Pedigree disequilibrium test (PDT) provides a general and valid test of linkage disequilibrium in such situation [Spielman and Ewens, 1996]. We analyzed the TDO2 genotype data with PDT, which revealed a nominally positive association of autism with the rs3755910: A/C polymorphism (SUM PDT: $\chi^2 = 4.052$, df = 1, $P = 0.0442$; AVE PDT: $\chi^2 = 6.063$, df = 1, $P = 0.0138$). The rs3755905 A/C promoter polymorphism demonstrated no association with autism by PDT analysis (SUM PDT: $\chi^2 = 0.393$, df = 1, $P = 0.53$; AVE PDT: $\chi^2 = 0.023$, df = 1, $P = 0.88$).

Since neighboring SNPs if sufficiently close, will be inherited with a disease causing SNP, the inheritance of a haplotype can be used to uniquely tag a region of the genome for closer study. Multiple haplotype analysis was performed using TRANSMIT v2.5.2 and results are shown in Table IV. The program estimates the association from probabilities of a haplotype transmission to affected offspring even when there are uncertain marker haplotype assignments. A total of 13 haplotypes were observed. Of these, only three were present with probabilities greater than 2% and these accounted for the majority of the haplotype diversity (95%). Among these three haplotypes, haplotype GTCCA was significantly over transmitted to the autistic probands ($\chi^2 = 5.24$, df = 1, $P = 0.015$). The global $P$ value for the haplotypes is 0.0027 ($\chi^2 = 15.4$, df = 3). When haplotypes

---

**Table I. Allele and Genotype Frequencies in 196 AGRE Families**

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>NT_016606 contig position</th>
<th>Location</th>
<th>Allele</th>
<th>Parents</th>
<th>Autistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3755907</td>
<td>18337737</td>
<td>Promoter</td>
<td>A</td>
<td>0.8747</td>
<td>0.8644</td>
</tr>
<tr>
<td>rs3755907</td>
<td>18339168</td>
<td>Promoter</td>
<td>C</td>
<td>0.8747</td>
<td>0.8553</td>
</tr>
<tr>
<td>rs3775085</td>
<td>18337241</td>
<td>Promoter</td>
<td>A</td>
<td>0.8704</td>
<td>0.8509</td>
</tr>
<tr>
<td>rs3755908</td>
<td>18340462</td>
<td>Promoter</td>
<td>C</td>
<td>0.8796</td>
<td>0.8741</td>
</tr>
<tr>
<td>rs3755910</td>
<td>18355324</td>
<td>Exon 11</td>
<td>A</td>
<td>0.8725</td>
<td>0.8356</td>
</tr>
</tbody>
</table>

**Table II. Linkage Disequilibrium Among the SNPs in TDO2 Gene**

<table>
<thead>
<tr>
<th>SNP1</th>
<th>SNP2</th>
<th>N</th>
<th>df</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>LOP</th>
<th>Cramer</th>
<th>U</th>
<th>D</th>
<th>$D'$</th>
<th>Delta2</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3755907</td>
<td>rs3755908</td>
<td>742</td>
<td>1</td>
<td>679.827</td>
<td>0</td>
<td>100</td>
<td>0.9572</td>
<td>0.8697</td>
<td>0.105</td>
<td>0.963</td>
<td>0.916</td>
</tr>
<tr>
<td>rs3755907</td>
<td>rs3775085</td>
<td>734</td>
<td>1</td>
<td>492.575</td>
<td>0</td>
<td>100</td>
<td>0.8192</td>
<td>0.6039</td>
<td>0.092</td>
<td>0.849</td>
<td>0.671</td>
</tr>
<tr>
<td>rs3755907</td>
<td>rs3755910</td>
<td>741</td>
<td>1</td>
<td>73.465</td>
<td>0</td>
<td>16.99</td>
<td>0.3149</td>
<td>0.118</td>
<td>0.016</td>
<td>0.747</td>
<td>0.099</td>
</tr>
<tr>
<td>rs3755907</td>
<td>rs2292537</td>
<td>738</td>
<td>1</td>
<td>8.648</td>
<td>0.0033</td>
<td>2.48</td>
<td>0.1082</td>
<td>0.0149</td>
<td>0.009</td>
<td>0.146</td>
<td>0.012</td>
</tr>
<tr>
<td>rs3755908</td>
<td>rs3775085</td>
<td>738</td>
<td>1</td>
<td>499.27</td>
<td>0</td>
<td>100</td>
<td>0.8225</td>
<td>0.6086</td>
<td>0.094</td>
<td>0.852</td>
<td>0.677</td>
</tr>
<tr>
<td>rs3755908</td>
<td>rs3755910</td>
<td>746</td>
<td>1</td>
<td>91.521</td>
<td>0</td>
<td>20.96</td>
<td>0.3503</td>
<td>0.1461</td>
<td>0.017</td>
<td>0.866</td>
<td>0.123</td>
</tr>
<tr>
<td>rs3755908</td>
<td>rs2292537</td>
<td>745</td>
<td>1</td>
<td>7.916</td>
<td>0.0049</td>
<td>2.31</td>
<td>0.1031</td>
<td>0.0136</td>
<td>0.009</td>
<td>0.139</td>
<td>0.011</td>
</tr>
<tr>
<td>rs3775085</td>
<td>rs3755910</td>
<td>742</td>
<td>1</td>
<td>66.244</td>
<td>0</td>
<td>15.4</td>
<td>0.2988</td>
<td>0.1067</td>
<td>0.016</td>
<td>0.744</td>
<td>0.089</td>
</tr>
<tr>
<td>rs3775085</td>
<td>rs2292537</td>
<td>732</td>
<td>1</td>
<td>6.61</td>
<td>0.0101</td>
<td>1.99</td>
<td>0.095</td>
<td>0.0115</td>
<td>0.009</td>
<td>0.131</td>
<td>0.009</td>
</tr>
<tr>
<td>rs3755910</td>
<td>rs2292537</td>
<td>740</td>
<td>1</td>
<td>51.212</td>
<td>0</td>
<td>12.08</td>
<td>0.2631</td>
<td>0.0911</td>
<td>0.01</td>
<td>0.491</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Measures of linkage disequilibrium include chi-squared, Cramer’s V, uncertainty coefficient U, Lewontin’s disequilibrium coefficient D and $D'$, and delta-squared measure of disequilibrium. The usual contingency table chi-squared can be calculated, and its significance is estimated from an asymptotic distribution with $(r-1)(c-1)$ degrees of freedom. Cramer’s V is a transformation of the chi-squared statistic into the zero to one interval and is useful for comparing the relative intensity of association between marker pairs. The coefficient of disequilibrium, D, is the difference between the observed haplotype frequency and the frequency expected under statistical independence. The $D'$ measure is a proportion of the maximum value of D, whose range extends from $-1$ to $+1$, with $-1$ and $+1$ representing complete LD and 0 representing free association. U refers to how much information one marker provides on the other’s genotype. It varies between 0 (independent) and 1 (completely dependent). Delta-squared measure of disequilibrium is only defined for bi-allelic markers.
TABLE III. Transmission Disequilibrium Test in 196 AGRE Families

<table>
<thead>
<tr>
<th>Location</th>
<th>dbSNP ID</th>
<th>Allele</th>
<th>T</th>
<th>NT</th>
<th>(\chi^2)</th>
<th>P-value</th>
<th>Ratio (T/NT)</th>
<th>95% CI for ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter</td>
<td>rs3755907</td>
<td>A</td>
<td>32</td>
<td>37</td>
<td>0.36</td>
<td>0.55</td>
<td>0.86</td>
<td>0.53–1.39</td>
</tr>
<tr>
<td>Promoter</td>
<td>rs3755908</td>
<td>G</td>
<td>37</td>
<td>32</td>
<td>0.36</td>
<td>0.55</td>
<td>1.16</td>
<td>0.72–1.89</td>
</tr>
<tr>
<td>Promoter</td>
<td>rs3775085</td>
<td>T</td>
<td>39</td>
<td>32</td>
<td>0.69</td>
<td>0.41</td>
<td>0.82</td>
<td>0.5–1.3</td>
</tr>
<tr>
<td>Promoter</td>
<td>rs3775910</td>
<td>C</td>
<td>86</td>
<td>87</td>
<td>0.01</td>
<td>0.94</td>
<td>0.89</td>
<td>0.73–1.33</td>
</tr>
<tr>
<td>Intron</td>
<td>rs3755910</td>
<td>C</td>
<td>32</td>
<td>39</td>
<td>0.01</td>
<td>0.94</td>
<td>1.01</td>
<td>0.75–1.37</td>
</tr>
<tr>
<td>11 splice</td>
<td>rs2292537</td>
<td>A</td>
<td>30</td>
<td>20</td>
<td>11.76</td>
<td>0.0006</td>
<td>0.26</td>
<td>0.08–0.52</td>
</tr>
</tbody>
</table>

T indicates transmitted and NT indicates not transmitted. Ratio = transmission ratio (T/NT). Under a multiplicative model the transmission ratio is an estimator of the genotype relative risk. 95% CI = 95% confidence interval around the transmission ratio.

were constructed with the four promoter variants only, global P value was less significant \(\chi^2 = 5.5, df = 2, P = 0.09\). Transmission disequilibrium, calculated for pairwise haplotypes for adjacent promoter (rs3755910) and 3’ (rs2292537) markers using the TDT2 implementation in GENEHUNTER 2.0, displayed significant difference in the transmission of the haplotypes to autistic subjects \(\chi^2 = 10.59, df = 3, P = 0.01\). These data are consistent with there being a risk haplotype for the TDO2 gene.

As the more frequent C allele of the dbSNP rs3755910 polymorphism was preferentially transmitted to the autistic subjects, the A/C SNP is likely not the etiologic variant. In order to identify the true susceptibility allele and obtain additional SNP for genotyping, we sequenced 500 bp of the promoter region and exons of TDO2 gene in 20 autistic subjects, including autistic subjects with both AC and CC genotypes at A/C promoter polymorphism (rs3755910). Sequencing did not reveal any additional variants in the promoter and exons, suggesting that an etiologic variant in the TDO2 gene, if any, is not located in an exon.

**Power Analyses**

The program TDT Power Calculator (TDT-PC v 1.2) based on Knapp’s first approximation, was used to estimate power for the TDT analyses [Knapp, 1999; Chen and Deng, 2001]. We calculated the POWER of our studies with the different SNPs to detect a gene effect with genotypic relative risk, g = 6.0 for the homozygote and g = 3.0 for the heterozygote, assuming a significance level, alpha = \(5 \times 10^{-8}\), \(1 \times 10^{-6}\) and 0.0001. The results are shown in Table V. We had sufficient power to detect association at a significance level of alpha = \(1 \times 10^{-6}\) (dbSNP rs3755907, rs3755908, and rs3775085 and rs3755910), and 0.0001 (dbSNP rs2292537) (Table V).

**DISCUSSION**

Although several consensus regions of linkage for autism on many different chromosomes have been identified [Philippe et al., 1999; Buxbaum et al., 2001; IMGSAC, 2001; Shao et al., 2002], the task of identifying the responsible genes remains a formidable problem. Direct analysis of the likely candidate genes and SNPs-based linkage disequilibrium mapping are thus valid strategies for identification of autism susceptibility genes. Published linkage studies do not suggest that the TDO2 gene is located within a potential susceptibility region. However, the finding of increased blood and platelet 5-HT level in autism [Cook et al., 1988] and the critical role of 5-HT during embryogenesis and in the development of the central nervous system [Blue et al., 1991; Bennett-Clarke et al., 1994; Cases et al., 1996; Osterheld-Haas and Hornung, 1996; Yan et al., 1997] suggest that TDO2 deserves investigation as a potential candidate gene that may confer an increased susceptibility to autism. In this report, TDT studies revealed linkage disequilibrium of a promoter variant with autism in 196 multiplex families. Our data are also consistent with the presence of a risk haplotype of TDO2 gene.

**TABLE IV. Estimated Haplotype Probabilities and Chi-Squared Test of Multimarker Haplotypes Using TRANSMIT**

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>3’</th>
<th>Estimated probabilities</th>
<th>O</th>
<th>E</th>
<th>Var (O–E)</th>
<th>(\chi^2)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>0.802</td>
<td>638.39</td>
<td>637.68</td>
<td>48.88</td>
<td>0.010</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>0.091</td>
<td>86.46</td>
<td>73.27</td>
<td>33.172</td>
<td>5.24</td>
<td>0.015</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>G</td>
<td>0.056</td>
<td>42.66</td>
<td>44.48</td>
<td>12.2</td>
<td>0.27</td>
<td>0.558</td>
</tr>
</tbody>
</table>

Markers are shown in order. P1, P2, P3, P4 are four promoter SNPs rs3755907 (A/G), rs3755908 (C/T), rs3775085 (A/C), and rs3755910 (A/C). 3’ indicates splice region SNP (rs2292537, A/C) 3’ to exon 11. TRANSMIT 2.5.4 is used to analyze the data. O = observed transmission of haplotype to affected offspring, E = expected transmission under Mendelian inheritance, Var (O–E) = variance of (O–E), P value calculated for 1 df. Haplotypes were omitted from analysis if the estimated haplotype probabilities were less than 2%. Thirteen haplotypes were observed, but only three haplotypes had frequencies >2%.
Previous association studies of TDO2 gene reveal a significant association of a G→T and a G→A polymorphism in intron 6 and Tourette syndrome (TS), attention deficit hyperactivity disorder, and drug dependence [Comings et al., 1996; Comings, 2001]. Only the association with TS was significant with a Bonferroni correction (P = 0.005). These results suggest the TDO2 gene may be one of the genes involved in TS. The intron 6 G→T variant was significantly associated with platelet 5-HT levels [Comings et al., 1996]. These data are of interest in view of increased blood and platelet 5-HT levels in autism [Cook et al., 1988] and clinical and genetic relationship between TS and Autism [Comings and Comings, 1991].

One caveat of our study is that the dbSNP rs3755910 A/C promoter SNP, which demonstrated significant difference in the transmission to autistic subjects (P = 0.0006) had only 34 informative transmission for TDT analyses, with 27 transmitted to the autistic subjects versus 7 non-transmitted. The more frequent C allele of the rs3755910 polymorphism was preferentially transmitted to the autistic subjects. As rs3755910 is a rare polymorphism with minor allele frequency of 0.0236, this may represent a chance non-transmission of the rare allele. The numbers of informative families for TDT for the additional promoter and intron 11 SNPs (rs3755907, A/G; rs3755908, C/T; rs3775085, A/C, and rs2292537, A/G), which did not reveal any significant association were 69, 71, 173, and 50, respectively. Since the most significant finding was based on the smallest number of families, this could be a chance finding.

In this study, a single promoter SNP was more informative than the haplotypes for TDT analyses. Haplotypes usually are more informative than single SNPs, as mutations occur on particular haplotype backgrounds and are associated with nearby SNPs until recombination or recurrent mutations breaks these associations [Fullerton et al., 2000]. Haplotypes are however not likely to be more informative if compared with a causative SNP or if they are longer than the local length scale for decay of linkage disequilibrium [Judson and Stephens, 2001]. In this study, the rs3755910 A/C promoter SNP demonstrated highly significant (P = 0.0006) association, while the P value for the transmission of the most significant of the three common haplotypes (G→T→C→A) was 0.015. Possible explanations include the rs3755910 A/C promoter SNP being a causative SNP, incomplete knowledge of the length scale for decay of linkage disequilibrium within TDO2 gene, and lack of non-additivity between the contributing SNPs in the haplotypes [Judson and Stephens, 2001].

POWER analyses suggested that we had sufficient power to detect association of dbSNP rs3755910 polymorphism with autism at a significance level of 1 × 10⁻⁵ but not at a genome-wide significance level (alpha = 5 × 10⁻⁸) (Table V). The P values described here are nominal P values and have not been corrected for multiple testing. The P values also do not reach the genome-wide threshold of significance (5 × 10⁻⁵). However, it has been pointed out that with a practical sample size even a fully validated disease-predisposing variant such as APOE-E4 allele for Alzheimer’s disease would yield only a modestly significant P-value [Emahazion et al., 2001]. Thus the nominally positive association of a haplotype and a promoter variant in TDO2 gene may still be of interest in view of the consistent finding of elevated serotonin level in autism and role of serotonin in the embryogenesis of central nervous system.

In conclusion, our data provide suggestive evidence of association of the TDO2 or a nearby gene with autism. Further studies in independent samples will clarify whether the reported findings represent true association or a chance finding.

ACKNOWLEDGMENTS

We gratefully acknowledge the resources provided by the AGRE consortium and the participating AGRE families.

REFERENCES


