

ORIGINAL RESEARCH ARTICLE

Serotonin transporter gene and autism: a haplotype analysis in an Irish autistic population

J Conroy¹, E Meally², G Kearney², M Fitzgerald², M Gill^{1,2} and L Gallagher^{1,2}

¹Department of Genetics, Smurfit Institute, Trinity College, Dublin, Ireland; ²Department of Psychiatry, Trinity College, Dublin, Ireland

The role of the serotonin transporter (5-HTT) in the development of neuropsychiatric disorders has been widely investigated. Two polymorphisms, an insertion/deletion in the promoter region and a 12 repeat allele in a variable nucleotide tandem repeat (VNTR) in intron 2, drive higher expression of the 5-HTT gene. Four studies have shown nominally significant excess transmission of alleles of the 5-HTT gene in autism, while three studies have reported no excess transmission. This present study investigates the role of 5-HTT in the genetically homogenous Irish population. In all, 84 families were genotyped for five polymorphisms (three SNPs, a VNTR and an in/del). The analysis of allele transmissions using the transmission disequilibrium test (TDT) was undertaken and indicated preferential transmission of the short promoter allele (TDT P -value = 0.0334). Linkage disequilibrium between markers was calculated and haplotypes were assessed for excess transmission and odds ratios (ORs) to affected children. A number of haplotypes, especially those involving and surrounding SNP10, showed evidence of association. The ORs ranged from 1.2 to 2.4. The most significant haplotype associated with transmission to affected probands was the SNP10–VNTR–SNP18 haplotype ($\chi^2 = 7.3023$, $P = 0.0069$, odds ratio = 1.8). This haplotype included the 12 repeat allele of the VNTR, which is associated with increased expression and may play a subtle role in the early development of the brain in affected probands.

Molecular Psychiatry (2004) 9, 587–593. doi:10.1038/sj.mp.4001459

Published online 6 January 2004

Keywords: neuropsychiatric disorders; autism; serotonin transporter; transmission disequilibrium testing; odds ratio; haplotypes; brain development

Introduction

Autism (OMIM 209850) is a neurodevelopmental disorder of childhood onset characterised by impairments in the three core areas of social interaction, communication and behaviour. The prevalence of core autism is estimated at between 5.5 and 20 in 10 000.^{1,2} The role of genetics in the aetiology of autism is undisputed. There is increased monozygotic vs dizygotic concordance in twins (60–91%: 0%).^{3,4} The sibling recurrence rate of 4.5% is approximately 40 times the base population prevalence.⁵ The estimated heritability using a multithreshold liability model has been calculated at 91–93%.⁴ These observations are incompatible with a simple Mendelian mode of inheritance. Statistical modelling suggests that between 3 and 7, and possibly 15 or more, loci may play a role in autism.^{6–8}

Candidate genes are difficult to identify due to a limited understanding of the underlying aetiology and pathophysiology of the disorder. Few of those

investigated have shown strong evidence for association. The most replicated findings have been found for the GABRB3 (gamma-aminobutyric acid A receptor beta 3) and the serotonin transporter (5-HTT).^{9–15} Genome screens using affected relative pair families have been undertaken in order to detect and highlight genomic regions of interest. The results published to date indicate evidence for linkages on a number of chromosomal regions. The IMGSAC group found some evidence for linkage around the locus for the 5-HTT on Ch 17q. Using the VNTR in intron 2 as a marker, a single point maximum LOD score (MLS) of 3.6 and a multipoint MLS of 2.34 were found.¹⁶ Another genomewide screen has also detected linkage around the 5-HTT gene, this time producing an MLS of 2.83.¹⁷

Broadly speaking, serotonin (5-hydroxytryptamine, 5-HT) has been implicated in a variety of behavioural and psychological processes, including mood, obsessive-compulsive symptoms, cognition, pain perception, sleep and social interaction and affiliation.^{18–20} Elevated platelet serotonin in individuals with autism and their first-degree relatives has been consistently described, and this has led to considerable investigation of the role of serotonin in the aetiology of the disorder.^{21–23} Moreover, some patients with autistic

Correspondence: J Conroy, Department of Genetics, Smurfit Institute of Genetics, Trinity College, Dublin 2, Ireland.
E-mail: conroyju@tcd.ie
Received 02 October 2003; revised 02 October 2003; accepted 14 October 2003

disorder respond positively to 5-HTT inhibitors, for example, fluvoxamine, with regard to obsessional and repetitive behavioural phenotypes.²⁴ Finally, serotonin also functions to regulate cell proliferation, migration and differentiation in neuronal tissue, and variations of expression could play a role in neurodevelopment.^{25–27}

A major modulator of serotonergic neurotransmission is the 5-HTT, which directly controls the uptake of 5-HT in presynaptic nerves. The 5-HTT gene (SLC6A4) is located on chromosome 17q11.2 and contains 14 exons spread over ~35 kb.²⁶ There are three extensively studied polymorphisms within the 5-HTT gene, a 44 bp insertion/deletion in the promoter region, a variable nucleotide tandem repeat (VNTR) (12, 10 or 9 repeats) in an intronic region and a G to T transversion in a putative polyadenylation site in the 3' untranslated region (UTR) of the gene. The basal activity of the long promoter variant has been shown to be three-fold higher than that of the short variant.²⁸ The 12 repeat allele is the most common variant of the VNTR and has been shown to drive higher expression in embryonic mouse rostral hind-brain.²⁷

Klauck *et al*¹² and Yirmiya *et al*²⁹ reported preferential transmission of the long promoter variant in autism. One study by Tordjman *et al*³⁰ reported the transmission of the short promoter allele in severely affected individuals, and also found the transmission of the long promoter allele in the sample group as a whole. Cook *et al*¹¹ found evidence of increased transmission of the short allele in 86 autistic trios. In addition, there are several studies that have found no association with either long or short alleles^{13–15}. Most recently, Kim *et al*³¹ described the characterisation of further polymorphisms in the 5-HTT gene. These authors also described association with several SNPs surrounding the VNTR.

The present study investigates the role of 5-HTT in the genetically homogeneous Irish population and uses a haplotype-based approach using some of the polymorphisms described by Kim *et al*.³¹

Materials and methods

Sample description

Affected trios (probands and both parents) were recruited through schools and parent support groups. Subjects were assessed by the ADI-R,³² ADOS-G,³³ neurological examination, skin examination with Wood's lamp and karyotyping/Fragile X testing. All subjects met the ADI-R criteria for autism and the ADOS-G criteria for autism/autism spectrum disorder. Individuals were excluded if they had a known medical cause of autism (eg tuberous sclerosis, extreme prematurity, congenital rubella), IQ < 35 (or mental age < 18 months) or chromosomal abnormalities/fragile X. Ethical approval was obtained from the Eastern Regional Health Authority, Child and Adolescent Psychiatry Ethics Committee, Ireland.

Genotyping

DNA was extracted from blood (or buccal swabs) using the phenol/chloroform extraction method. The polymerase chain reaction was used to amplify the genomic regions of interest.

PCR amplification of a 741 bp fragment in the 3'UTR region of the 5-HTT gene containing a putative polyadenylation signal sequence was performed in a 25 μ l volume, containing 60 ng of genomic DNA, 20 pmol of each primer, 200 μ mol of each dNTP, 50 mM of KCl, 10 mM of Tris HCl (pH 9), 1.5 mM of MgCl₂, 0.01% gelatine and 1 U of *Taq* polymerase. This product was digested with 2 U of *Mse*I for 3–4 h and then visualised as G alleles (741 bp) or T alleles (689 + 52 bp) on a 2% agarose gel stained with ethidium bromide.

PCR amplification of a region of the 5-HTT VNTR region was carried out as described by Cook *et al*.¹¹ In all, nine (345 bp), 10 (360 bp) and 12 (390 bp) repeat alleles were resolved on a 4% agarose gel stained with ethidium bromide.

PCR amplification of the promoter region containing short alleles (484 bp) and/or long alleles (528 bp) of the 5-HTT gene was also carried out as described by Cook *et al*.¹¹ However, given the GC-rich content of this region, dGTP was replaced with a dGTP: 7-deaza dGTP mix (in a ratio of 1:1). Primers were designed spanning the promoter region. The PCR products were visualised on a 2% agarose gel stained with ethidium bromide.

Genotyping of both SNP10 and SNP18 were carried out using the SNaPshot MethodTM of single base extension (Applied Biosystems) that employs the principle of extending an unlabelled oligonucleotide primer in the presence of fluorescent dye.³⁴ Each of the four ddNTPS is tagged by a different fluorescent dye. This allows the specific allele products to be differentiated from each other when run on an ABI Genetic Analyser (ABI PRISM 377 DNA Sequencer). The initial PCRs were carried out in conditions similar to those described above. The PCR cycle conditions were as follows: denaturation at 95°C for 3 min, 46 cycles (SNP18) or 42 cycles (SNP10) of 30 s at 95°C, 30 s at 56°C (SNP10) or 58°C (SNP18), 30 s at 72°C, a final denaturation step of 72°C for 10 min completed the amplification. The PCR product (5 μ l) was treated with 1 U of shrimp alkaline phosphatase (SAP) and 1 U of *Exonuclease I* (ExoI). This causes the degradation of the initial primers and nucleotides, and allows the extension PCR to occur cleanly. The treated PCR product (2 μ l) was used as a template in the extension PCR, which also required 1 μ l of the SNaPshot Reagent mix, 1.5 μ l of buffer (160 mM Tris-HCl, pH 9, 4 mM magnesium chloride) and 0.4 μ l of extension primer (5 pmol/ml) and water (to make up to a final volume of 10 μ l). The resulting product was again treated with 1 U of SAP and run on an ABI 377. Genotyping was performed using Genotyper (version 2.5.1).

Data analysis

The statistics used in this study to look at the transmission of individual markers were the transmission disequilibrium test (TDT) and the haplotype-based haplotype relative risk (HHRR).^{35,36} Both these tests minimise any pitfalls associated with population stratification. In these tests, the 'controls' constitute the nontransmitted alleles and the χ^2 test was used to assess the significance of the resulting tables. The transmission of haplotypes was tested using the program TRANSMIT (www.hgmp.mrc.ac.uk/Menu/transmit.html). In a method similar to the TDT and HHRR tests, the transmission of genetic markers from parents to offspring was examined. D' values between markers were evaluated using the program GOLD (www.well.ox.ac.uk/asthma/GOLD). It is important to note that corrections for multiple testing were not carried out. Odds ratios (ORs) were calculated using a reference allele or haplotype, which consisted of all other alleles or haplotypes as in the formula $a/a+c$ divided by $b/b+d$, where a is the number of transmissions of the risk allele, b is the number of nontransmissions of the risk allele, c is the number of transmissions of all other alleles and d is the number of nontransmissions of all other alleles. A power study was conducted using the 'TDT for discrete traits' application of the 'Genetic Power Calculator'.³⁷

Given that the scale of the gene effect is unknown, and assuming that it is a susceptibility gene for the development of autism, power calculations were performed under the following conditions: number of trios = 100, high-risk allele frequency (A) = 0.25, disease prevalence = 0.002, genotypic relative risk of heterozygote (Aa) = 2 and genotypic relative risk of homozygote (AA) = 3. Given these parameters, it was shown that this study had a power of 0.762, although any changes in these parameters led to a rapidly decreasing power.

Results

In all, 84 families were genotyped for the 5-HTT promoter, VNTR polymorphism SNP10 and SNP18, while 78 families were genotyped for the 5-HTT 3'UTR polymorphism. It should be noted that SNP11 was not included in the analysis due to difficulties with genotyping; however, it appears that this SNP is in complete LD with the VNTR and therefore would not provide additional information (EH Cook, personal communication). The results of individual marker TDT analyses are shown in Table 1. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium. No significant increase was observed in the transmission of the 12 repeat VNTR or for allele 1 (G) of the 3' UTR variant. Preferential transmission of the short promoter allele was observed ($\chi^2 = 4.5252$, P -value = 0.0334).

The analysis of haplotypes is presented in Table 2. The Examination of two marker haplotypes revealed an increased transmission of several haplo-

Table 1 TDT results

	Transmitted	Not transmitted
5-HTT promoter variant		
Long (0.64)	34	55
Short (0.36)	55	34
TDT $\chi^2 = 4.5252$, P -value = 0.0334, relative risk = 1.6176		
5-HTT SNP10		
C (0.62)	54	42
T (0.38)	42	54
TDT $\chi^2 = 1.2608$, P -value = 0.2615, relative risk = 1.2857		
5-HTT VNTR variant		
12 repeat (0.60)	45	37
9 + 10 repeat (0.02, 0.38)	37	45
TDT $\chi^2 = 1.00$, P -value = 0.3347, relative risk = 1.159		
5-HTT SNP18		
G (0.58)	46	37
A (0.42)	37	46
TDT $\chi^2 = 0.7707$, P -value = 0.3800, relative risk = 1.2432		
5-HTT 3'UTR variant		
G (0.47)	42	31
T (0.53)	31	42
TDT $\chi^2 = 1.3712$, P -value = 0.2416, relative risk = 1.3548		

Note: allele frequencies were calculated from parental genotypes.

Allele frequencies for each polymorphism are shown in parentheses.

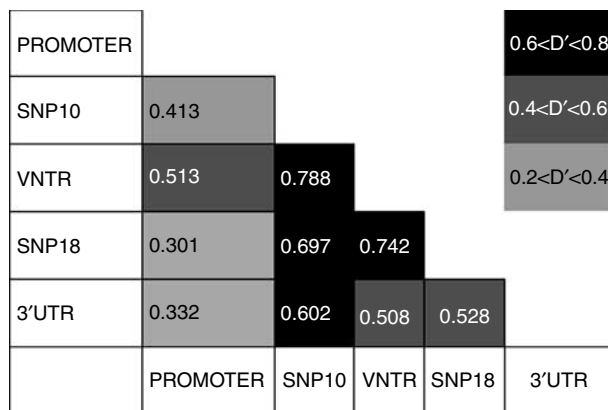
types. These include the haplotype constructed from the short promoter allele and allele 1 (the C allele) of SNP10 ($\chi^2 = 4.2277$, $P = 0.0398$), and also the haplotype constructed from allele 1 of the SNP10 and the 12 repeat allele of the VNTR ($\chi^2 = 5.8916$, $P = 0.0152$). In addition, when the markers promoter SNP10 and the VNTR were analysed together, a significant preferential transmission of the short promoter-C allele-12 repeat VNTR haplotype ($\chi^2 = 6.341$, $P = 0.0118$) to autism cases was observed. Transmission of an SNP10-VNTR-SNP18-3'UTR haplotype (C-12rpt-G-G) was the most significant producing a χ^2 value of 10.888 with a corresponding P -value of 0.0052. Interestingly, almost all associated haplotypes, with the exception of haplotype VNTR-SNP18-3'UTR (12rpt-G-G) ($\chi^2 = 4.4189$, $P = 0.0355$), contained allele 1 of SNP10. Linkage disequilibrium (LD) measurement (D') analysis demonstrates the presence of LD between markers across the locus with D' values between 0.513 and 0.788 across the 5-HTT gene (Figure 1).

ORs were calculated for single polymorphisms and for each two-, three-, four- and five-marker haplotypes. The results are presented in Figure 2. ORs varied from 1.269 (SNP18 G allele) to 2.4034 (four-marker haplotype involving the short promoter, C allele of SNP 10, 12 repeat VNTR and the G allele of SNP18). It was found that the OR increased, as

Table 2 Haplotype transmission and OR data

Markers	Alleles	Observed transmission	Expected transmission	χ^2	Haplotype <i>P</i> -value	OR	Global <i>P</i> -value
Promoter–SNP10	S-C	40.054	32.919	4.2277	0.0398	1.7414	0.0784
SNP10–VNTR	C-12rp	56.688	47.932	5.8916	0.0152	1.6923	0.0776
VNTR–SNP18	12rp-G	94.08	86.601	3.0407	0.0812	1.4203	0.1964
SNP18–3'UTR	G-G	68.804	61.566	3.0933	0.0786	1.4512	0.3398
Promoter–SNP10–VNTR	S-C-12rp	30.951	23.819	6.341	0.0118	2.0436	0.0821
SNP10–VNTR–SNP18	C-12rp-G	50.531	41.129	7.6501	0.0057	1.8406	0.2323
VNTR–SNP18–3'UTR	12rp-G-G	68.143	59.578	4.4189	0.0355	1.6088	0.1901
Promoter–SNP10–VNTR–SNP18	S-C-12rp-G	29.163	21.869	7.6866	0.0085	2.4034	0.1121
SNP10–VNTR–SNP18–3'UTR	C-12rp-G-G	42.177	32.946	10.888	0.0052	2.062	0.5650
Promoter–SNP10–VNTR–SNP18–3'UTR	S-C-12rp-G-G	24.981	18.717	5.878	0.0153	2.206	0.1766

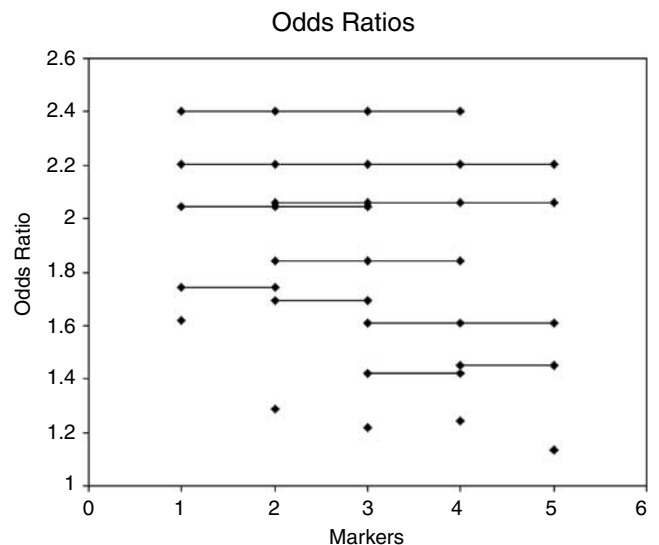
Haplotype *P*-values represent the *P*-value for the transmission of indicated variants of the haplotype. The global *P*-value represents the overall significance for the haplotype using all possible variants. S = short promoter variant; 12rp = 12 repeat variant of intron 2 VNTR.

**Figure 1** Measures of LD (*D'*) across the 5-HTT gene.

neighbouring polymorphisms were included in the haplotype analyses. A good example of this involves the 12 repeat VNTR, in which the OR of developing autism having inherited this allele increases from ~1.35 to 1.70 when the C allele of SNP10 is added, and continues to increase until all polymorphisms, except the G allele of the 3'UTR, are included, which gives the highest OR of 2.4. Interestingly, ORs decrease as one moves closer to the 3'-end of the gene (see Table 2 and Figure 2).

Discussion

Five 5-HTT polymorphisms were analysed for association with autistic disorder using a family-based study design. The short promoter allele was the only individual allele that reached significance (*P*-value = 0.0334, see Table 1). Haplotypes constructed from alleles that were individually overtransmitted showed significant excess transmission for all two-marker combinations, with the exception of the VNTR–SNP18 haplotype (Table 2). Moving to three- and four-marker haplotypes, it is apparent that each haplotype that contains SNP10 (allele 1, or cytosine)

**Figure 2** OR of each haplotype. Marker 1: The short promoter allele; marker 2: C allele of SNP10; marker 3: 12 repeat VNTR; marker 4: G allele of SNP18; marker 5: G allele of 3'UTR polymorphism.

is statistically significant. The most significant haplotype is that surrounding the VNTR (SNP10–VNTR–SNP18, haplotype 12rp-G-G, $\chi^2 = 7.6501$, *P*-value = 0.0057, OR = 2.4, 95% CI, 1.2–4.8). It is interesting to note that as one moves towards the 5'-end of the gene, the ORs calculated for the haplotypes increase, which could suggest that the polymorphism(s) linked to autism are at this end of the gene. In addition, the observed pattern of associated haplotypes may suggest that risk is associated with a combination of polymorphisms working together to alter the function or levels of the 5-HTT produced. It is also possible that more than one haplotype may confer risk at this locus.

Our findings contradict those of Klauck *et al*¹² and Yirmiya *et al*,²⁹ who have reported an association with the long promoter allele. Cook *et al*¹¹ reported

the transmission of the short promoter allele, although this finding was not replicated in further 81 new trios.³¹ However, the long promoter–12 repeat VNTR haplotype did reach significance ($P = 0.021$).³¹ More recently, Tordjman *et al*³⁰ found preferential transmission of the long promoter variant in their total sample. However, when the patient sample was divided into those mildly and severely affected based on the social and communication domains of the ADI, significant transmission of the short allele was found in those patients who were severely affected. Finally, Persico *et al*,¹⁴ Maestrini *et al*¹³ and Betancur *et al*¹⁵ reported no associations between autism and the promoter variants. It is possible that these conflicting results may be resolved with further investigation using increased sample size, haplotype analysis and the extra SNPs reported by Kim *et al*.³¹ The results presented here tend to agree with Cook *et al*'s initial report and that of Tordjman. It is also possible that the contrasting long and short allele findings may occur because these variants are in LD with a causative variant but differ between populations. Alternatively, one must consider that there may be molecular *cis* effects between the long promoter and 12 repeat VNTR when they both occur on the same haplotype. Another likely reason for the confounding results is that the underlying mechanisms of hyperserotonemia are heterogeneous.³⁸

Phenotypic heterogeneity must also be considered in the discussion of the conflicting findings outlined above. Phenotypic variation is a problem in any psychiatric genetics study and autism is no exception. The sample described here underwent rigorous assessment using the ADI-R and ADOS-G assessments to define the phenotype. However, heterogeneity is seen even within a sample meeting criteria for the narrow diagnosis of autism using these diagnostic tools. Subgrouping the sample in order to compare particular aspects of the phenotype, for example, social abnormalities or restrictive and repetitive behaviours with the genotypic information might be useful. However, this is not straightforward as the phenotypic data are qualitative in nature. Cluster analysis has been proposed as an alternative to determine genetically relevant aspects of the phenotype and the comparison of genotypic and phenotypic data in larger samples could prove useful.³⁹ With regard to the autistic samples described both here and above, the range of severity and IQ levels are broad and this also contributes to heterogeneity and is probably responsible for the conflicting findings.

There are a number of polymorphisms within the 5-HTT gene, which can directly influence the levels of protein produced. The basal activity of the long promoter variant has been shown to be three-fold higher than that of the short variant.²⁸ Associated haplotypes also contained the 12 repeat VNTR (allele 1) and this allele has been shown to be a stronger positive transcriptional regulatory element in comparison to the 10 repeat allele (allele 2) in mouse rostral hindbrain, where the 5-HTT gene is known to

Table 3 Primer sequence and PCR conditions

SNP name	Primers (F + R)	Extension primer	Annealing temp. (°C)	Restriction digest enzyme	Polymorphism
Promoter	F: 5'-GGC GTT GCC GCT CTG AAT GC-3' R: 5'-GAG GAC TGA GCT GGA CAA CCA-3'	NA	61	NA	Insertion/deletion of 44 bp in promoter
HTTSNP10	F: 5'-TTA TTT TAA AAG CCT ATC AAG C-3' R: 5'-CCC CAG GCT CAA GAA TGC	5'-TG AGC AGG GTG AGG TTA TGG AGA-3'	56	NA	T/C transition in promoter IB
VNTR	F: 5'-TGG ATT TCC TTC TCT CAG TGA TTG G-3' R: 5'-TCA TGT TCC TAG TCT TAC GCC AGT G-3'	NA	57	NA	9, 10 or 12 repeats in intron 2
HTTSNP18	F: 5'-GAC ACT CAT TCC CCA GCG TA-3' R: 5'-TGT GCA AAT CAG AAA GGT CCA T-3'	5'-GAA CAC ATG GTT TTA TTC TGG AGC C-3'	58	NA	G/A transition in intron 2
3'UTR	F: 5'-CCG CTT GAA TGC TGT GTA ACA CAC-3' R: 5'-GTA CCC TTC CAA TAA TAA CCT CC-3'	NA	56	MseI	G/T polymorphism

NA = not applicable.

be expressed during brain development.²⁷ The Development of the brain is a very complex process, and it is not inconceivable that altering levels of serotonin transporter expression, due to the short promoter allele or a 12 repeat VNTR allele or both, may produce very subtle differences, and that these altered levels, in conjunction with other factors, may contribute to the development of autism. Chugani *et al.*⁴⁰ have shown differing serotonin synthesis capacities between typically developing children and children with autism (lower in children with autism).

The serotonergic system is complex and many proteins are involved in the release and reuptake of serotonin into the presynaptic neuron. It is possible that 5-HTT variants do not contribute to the hyperserotonemia observed in the autistic population. Polymorphisms in serotonin receptors may contribute genetic risk, and indirectly be responsible for increased 5-HT blood levels. However, preliminary studies have not shown associations with 5-HT_{2A} receptor⁴¹ or with variants within the 5-HT₇ receptor gene.⁴² Studies with markers in other genes involved in the metabolism of serotonin have also proved negative, for example, tryptophan hydroxylase,⁴³ DOPA decarboxylase.⁴⁴ Conflicting results have been described for monoamine oxidase A (MAO-A) responsible for the degradation of serotonin. Yirmiya *et al.*⁴⁵ observed a trend for an association between an MAO-A genotype and IQ in a study involving 33 families with one autistic proband and 15 families with two affected probands. However, Phillippe *et al.*⁴⁶ reported no association with MAO-A genotypes in 38 families with two affected sibs and one family with two half affected sibs. Further study is required to evaluate the possible role of MAOA, and other genes involved in controlling serotonin levels in the aetiology of autism (Table 3).

Acknowledgements

We thank all the families who participated in this study. This study was supported by funding from the Health Research Board, The Wellcome Trust and the National Alliance for Autism Research.

References

- Fombonne E. The epidemiology of autism: a review. *Psychol Med* 1999; **29**: 769–786.
- Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children. *JAMA* 2001; **285**: 3093–3099.
- Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G *et al.* A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. *J Child Psychol Psychiatry* 1989; **30**: 405–416.
- Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E *et al.* Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995; **25**: 63–77.
- Jorde LB, Hassstedt S, Ritvo ER, Mason-Brothers A, Freeman BJ, Pingree C *et al.* Complex segregation analysis of autism. *Am J Hum Genet* 1991; **49**: 932–938.
- Pickles A, Bolton P, Macdonald H, Bailey A, Le Couteur A, Sim CH *et al.* Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. *Am J Hum Genet* 1995; **57**: 717–726.
- Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J *et al.* A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet* 1999; **65**: 493–507.
- Pritchard J. Are rare variants responsible for susceptibility to complex diseases? *Am J Hum Genet* 2001; **69**: 124–137.
- Buxbaum JD, Silverman J, Smith CJ, Greenberg DA, Kilifarski M, Reichert J *et al.* Association between a GABRB3 polymorphism and autism. *Mol Psychiatry* 2002; **7**: 311–316.
- Shao Y, Cuccaro M, Hauser ER, Raiford KL, Menold MM, Wolpert CM *et al.* Fine mapping of autistic disorder to chromosome 15q11–q13 by use of phenotypic subtypes. *Am J Hum Genet* 2003; **72**: 539–548.
- Cook Jr EH, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A *et al.* Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry* 1997; **2**: 247–250.
- Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A. Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet* 1997; **6**: 2233–2238.
- Maestrini E, Lai C, Marlow A, Matthews N, Wallace S, Bailey A *et al.* Serotonin transporter (5-HTT) and gamma-aminobutyric acid receptor subunit beta3 (GABRB3) gene polymorphisms are not associated with autism in the IMGSA families. *Am J Med Genet* 1999; **88**: 492–496.
- Persico AM, Militeri R, Bravaccio C, Schneider C, Melmed R, Conciatori M *et al.* Lack of association between serotonin transporter gene promoter variants and autistic disorder in two ethnically distinct samples. *Am J Med Genet* 2000; **96**: 123–127.
- Betancur C, Corbex M, Spieleswoy C, Philippe A, Laplanche JL, Launay JM *et al.* Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. *Mol Psychiatry* 2002; **7**: 67–71.
- International Molecular Genetic Study of Autism Consortium (IMGSA). A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet* 2001; **69**: 570–581.
- Yonan A, Alarcon M, Cheng R, Magnusson PK, Spence SJ, Palmer AA *et al.* A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet* 2003; **73**: 886–897.
- Chen H, Clark M, Goldman D. Quantitative autoradiography of 3H-paroxetine binding sites in rat brain. *J Pharmacol Toxicol Methods* 1992; **27**: 209–216.
- Hensler JG, Ferry R, Labow DM, Kovachich GB, Frazer A. Quantitative autoradiography of the serotonin transporter to assess the distribution of serotonergic projections from the dorsal raphe nucleus. *Synapse* 1994; **17**: 1–15.
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1531.
- Schain R, Freedman D. Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. *J Pediatr* 1961; **58**: 315–320.
- Abramson RK, Wright H, Carpenter R, Brennan W, Lumpuy O, Cole E *et al.* Elevated blood serotonin in autistic probands and their first-degree relatives. *J Autism Dev Disord* 1989; **19**: 397–407.
- Leboyer M, Philippe A, Bouvard M, Guillaud-Bataille M, Bondoux D, Tabuteau F, Feingold J *et al.* Whole blood serotonin and plasma beta-endorphin in autistic probands and their first-degree relatives. *Biol Psychiatry* 1999; **45**: 158–163.
- McDougle CJ, Naylor S, Cohen DJ, Volkmar FR, Heninger GR, Price LH. A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Arch Gen Psychiatry* 1996; **53**: 1001–1008.
- Lauder J. Ontogeny of the serotonergic system in the rat: serotonin as a developmental signal. *Ann NY Acad Sci* 1990; **600**: 297–313.
- Ramamoorthy S, Bauman A, Moore KR, Han H, Yang-Feng T, Chang AS *et al.* Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci USA* 1993; **90**: 2542–2546.

- 27 MacKenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc Natl Acad Sci USA* 1999; **96**: 15251–15255.
- 28 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D *et al*. Allelic variation of human serotonin transporter gene expression. *J Neurochem* 1996; **66**: 2621–2624.
- 29 Yirmiya N, Pilowsky T, Nemanov L, Arbelle S, Feinsilver T, Fried I *et al*. Evidence for an association with the serotonin transporter promoter region polymorphism and autism. *Am J Med Genet* 2001; **105**: 381–386.
- 30 Tordjman S, Gutknecht L, Carlier M, Spitz E, Antoine C, Slama F *et al*. Role of the serotonin transporter gene in the behavioral expression of autism. *Mol Psychiatry* 2001; **6**: 434–439.
- 31 Kim SJ, Cox N, Courchesne R, Lord C, Corsello C, Akshoomoff N *et al*. Transmission disequilibrium mapping at the serotonin transporter gene (SLC6A4) region in autistic disorder. *Mol Psychiatry* 2002; **7**: 278–288.
- 32 Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L *et al*. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. *J Autism Dev Disord* 1989; **19**: 185–212.
- 33 Lord C, Risi S, Lambrecht L, Cook Jr EH, Leventhal BL, DiLavore PC *et al*. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* 2000; **30**: 205–223.
- 34 Norton N, Williams N, Williams HJ, Spurlock G, Kirov G, Morris DW *et al*. Universal, robust, highly quantitative SNP allele frequency measurement in DNA pools. *Hum Genet* 2002; **110**: 471–478.
- 35 Spielman RS, McGinnis R, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993; **52**: 506–516.
- 36 Terwilliger JD, Ott J. A haplotype-based ‘haplotype relative risk’ approach to detecting allelic associations. *Hum Hered* 1992; **42**: 337–346.
- 37 Purcell S, Sham P. Genetic Power Calculator (2001–2003) <http://statgen.iop.kcl.ac.uk/gpc/>.
- 38 Cook Jr EH, Leventhal BL, Heller W, Metz J, Wainwright M *et al*. Platelet serotonin studies in hyperserotonemic relatives of children with autistic disorder. *Life Sci* 1993; **52**: 2005–2015.
- 39 Tadevosyan-Leyfer O, Dowd M, Mankoski R, Winklosky B, Putnam S, McGrath L *et al*. A principal components analysis of the autism diagnostic interview—revised. *J Am Acad Child Adolesc Psychiatry* 2003; **42**: 864–872.
- 40 Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J *et al*. Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. *Ann Neurol* 1999; **45**: 287–295.
- 41 Hérault J, Petit E, Martineau J, Cherpi C, Perrot A, Barthelemy C *et al*. Serotonin and autism: biochemical and molecular biology features. *Psychiatry Res* 1996; **65**: 33–43.
- 42 Lassig JP, Vachirasomtoon K, Hartzell K, Leventhal M, Courchesne E, Courchesne R *et al*. Physical mapping of the serotonin 5-HT(7) receptor gene (HTR7) to chromosome 10 and pseudogene (HTR7P) to chromosome 12, and testing of linkage disequilibrium between HTR7 and autistic disorder. *Am J Med Genet* 1999; **88**: 472–475.
- 43 Hérault J, Perrot A, Barthelemy C, Buchler M, Cherpi C, Leboyer M *et al*. Possible association of c-Harvey-Ras-1 (HRAS-1) marker with autism. *Psychiatry Res* 1993; **46**: 261–267.
- 44 Lauritsen MB, Borglum A, Betancur C, Philippe A, Kruse TA, Leboyer M *et al*. Investigation of two variants in the DOPA decarboxylase gene in patients with autism. *Am J Med Genet* 2002; **114**: 466–470.
- 45 Yirmiya N, Pilowsky T, Tidhar S, Nemanov L, Altmark L, Ebstein RP. Family-based and population study of a functional promoter-region monoamine oxidase A polymorphism in autism: possible association with IQ. *Am J Med Genet* 2002; **114**: 284–287.
- 46 Philippe A, Guilloud-Bataille M, Martinez M, Gillberg C, Rastam M, Sponheim E *et al*. Paris Autism Research International Sibpair Study. Analysis of ten candidate genes in autism by association and linkage. *Am J Med Genet* 2002; **114**: 125–128.