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ORIGINAL RESEARCH ARTICLE

The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample

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Genetic variation of the dopamine transporter gene (DAT1) is of particular interest in the study of attention-deficit hyperactivity disorder (ADHD), since stimulant drugs interact directly with the transporter protein. Association between ADHD and the 10-repeat allele of a 40-bp VNTR polymorphism that lies within the 3′-UTR of DAT1 was first reported in 1995, a finding that has been replicated in at least six independent samples from Caucasian populations. We analysed the DAT1 polymorphism in a sample of 110 Taiwanese probands with a DSM-IV diagnosis of ADHD and found evidence of increased transmission of the 10-repeat allele using TRANSMIT ($\chi^2 = 10.8$, 1 d.f., $p = 0.001$, OR=2.9, 95% CI 1.4–6.3). These data give rise to a similar odds ratio to that observed in Caucasian poplulations despite a far higher frequency of the risk allele in this Taiwanese population; 82.3% in the un-transmitted parental alleles and 94.5% in the ADHD probands. These data support the role of DAT1 in ADHD susceptibility among Asian populations.
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Introduction

Investigation of variation at the dopamine transporter gene (DAT1) is of particular interest in the study of attention deficit hyperactivity disorder (ADHD), since stimulant drugs interact directly with the transporter protein. It is, therefore, of considerable interest that several studies have reported association between ADHD and the 10-repeat allele of a 40-bp VNTR polymorphism that lies within the 3′-UTR of DAT1. To date, there have been 11 published studies in clinical ADHD samples, six that support an association with the 10-repeat allele and five that do not.[1–10] We have analysed the DAT1 polymorphism in a sample of 110 Taiwanese probands with a DSM-IV diagnosis of ADHD and find evidence of increased transmission of the 10-repeat allele using TRANSMIT ($\chi^2 = 10.8$, df = 1, $P = 0.001$, OR = 2.9, 95% CI 1.4–6.3). These data give rise to a similar odds ratio to that observed in previous studies of Caucasian individuals, despite a far higher frequency of the risk allele.

Over the past 5 years considerable progress has been made in the identification of polymorphic variation within monoamine system genes that are associated with the ADHD phenotype. In particular, replicated associations have been reported with the dopamine D4 and D5 receptors (DRD4 and DRD5),[4,11–13] the dopamine transporter (DAT1) and the serotonin 1B receptor (5HT1B)[14,15] genes. Although the identification of such consistent findings is unusual in the study of human behavioural disorders, uncertainties remain over their validity and further evidence is required before we can be confident that the reported findings represent true genetic associations. For the known markers that are currently reported to be associated with ADHD, odds ratios have been estimated at 1.4, 1.16, 1.4 and 1.48 for DRD4,[11] DAT1,[5] DRD5 (unpublished data) and 5HT1B,[14] respectively, on the basis of meta-analyses of available data. Of these, the lowest odds ratio estimate comes from the meta-analysis of a VNTR polymorphism within the 3′-untranslated region of DAT1, in which both linkage and association has been reported between the 10-repeat allele and ADHD ($\chi^2 = 3.45$, $P = 0.06$, OR = 1.16). Despite the low overall level of evidence, this association remains interesting since the number of replications is high with six out of 11 independent datasets finding significant evidence for the association.[1–10] Furthermore, we reported significant evidence of heterogeneity between the data sets, suggesting that there may be identifiable causes for differences between the studies ($\chi^2 = 22.64$, df = 8, $P = 0.004$).[5]

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Here, we report data from a Taiwanese sample in which the frequency of the 10-repeat allele is far higher than that found in Caucasian samples. Association of the DAT1 VNTR in this sample was investigated using the haplotype-based haplotype relative[16] and the transmission disequilibrium test (TDT).[17] As in our previous study,[5] we restricted analysis to the single hypothesis generated from the original report of Cook *et al*[1] That is, there is excess transmission of the 10-repeat allele from parents who are heterozygote for that allele, to their offspring with ADHD. We genotyped a total of 59 complete parent–offspring trios, 45 duos with only one parent and six singletons with no parental DNA available. The frequency of the 10-repeat allele in this population is higher than that seen in Caucasian samples, with a frequency of 94.5% among the 110 ADHD probands and 82.3% among nontransmitted parental alleles of the complete trios ($\chi^2 = 6.6$, df = 1, $P = 0.01$, OR = 2.72, 95% CI = 1.2–6.4). The observed difference in allele frequencies between transmitted and nontransmitted alleles among the complete trios was slightly more significant due to a higher proband frequency of 96.8% in this subgroup ($\chi^2 = 7.9$, df = 1, $P = 0.005$, OR = 4.5, 95% CI = 1.3–16.4).

TDT analysis of the complete trios identified 21 informative parental meioses, in which 16 10-repeat alleles were transmitted compared to five that were not transmitted ($\chi^{2=}5.8$, $P = 0.02$, OR = 3.2). Inclusion of parent–proband duos provided a similar estimate of the transmission ratio, which is likely to be an unbiased estimate due to the very high frequency of the 10-repeat allele (25 transmitted *vs* 12 nontransmitted, $\chi^2 = 4.6$, $P = 0.03$, OR = 2.1). To gain maximum power from the available data set and minimise any potential bias from missing parental genotypes, we extended the analysis to include the duos and singletons in addition to the complete trios using the TRANSMIT program,[18] although this test is no longer robust to the possible effects of stratification. This estimated that 208 10-repeat alleles were transmitted against 198.06 expected transmissions ($\chi^2 = 10.8$, df = 1, $P = 0.001$, OR = 2.9, 95% CI 1.4–6.3).

Although the confidence intervals are wide due to the small sample size, these data appear to give rise to an odds ratio that is similar to that seen in previous studies that consisted predominantly of Caucasian probands of European descent. Given the considerable difference in the frequency of the 10-repeat allele in this population, that may reflect differences in the underlying haplotype structure, the similarity in the odds ratios is consistent with the view that the VNTR polymorphism itself is the functional variant that confers risk for ADHD. This possibility is raised by two recent reports that suggest that the VNTR polymorphism itself may give rise to altered levels of DAT1 expression.[19,20]

However, the strength of the association is slightly higher than that reported in most studies, raising the possibility that the 10-repeat allele shows a stronger association with a nearby functional significant variant (FSV) in this Taiwanese population compared to Caucasian populations. This would predict that associations with risk/protective haplotypes and/or FSVs will be stronger than with the VNTR marker alone and might provide an explanation for the heterogeneity seen across studies; a hypothesis that gains support from the studies of Cathy Barr[7] and Mike Gill (personal communication), who both show an increase in the strength of association with haplotypes across the DAT1 gene. Power tables support this view by illustrating the dramatic loss of power to detect associations, that derives from disparity in allele frequencies or reduced levels of linkage disequilibrium (LD) between a marker locus and FSV locus (Table 1).

Alternatively, differences in ascertainment and cultural differences in the expression of behavioural phenotypes and/or the identification of cases may explain the strength of the association in this population. The sample consists predominantly of the combined subtype of ADHD and an initial report of a meta-analysis of DAT1 data in clinical ADHD samples, suggests that the strength of this association is far higher with this subtype than with the inattentive subtype (Waldman, personal communication).

Table 1 Power Table for detecting association to an FSV contributing 1% to genetic variance in a sample of 5000 unselected individuals

Size of effect (FSV heritability) %	*FSV allele frequency (p)*	*Marker allele frequency (m)*	*Proportion of observed LD out of maximum possible LD (D')*	*% Power at α=0.01*
1	0.1	0.1	1	100
1	0.5	0.5	1	100
1	0.1	0.1	0.5	70
1	0.5	0.5	0.5	71
1	0.1	0.5	1	31
1	0.5	0.1	1	31
1	0.1	0.5	0.5	6
1	0.5	0.1	0.5	6

This illustrates the dramatic loss of power that occurs where there is an allele frequency disparity between the FSV locus and marker locus as well as the effect of reduced *D*' values

We suggest that further mapping studies of this gene focus on identifying associated risk/protective haplotypes and other potential FSVs that give rise to the association between DAT1 and ADHD. Further work should be aimed at clarifying the role of the VNTR polymorphism in the regulation of dopamine transporter activity and the mechanism for ADHD susceptibility.

Methods

Sample collection

The sample consists of 110 children with ADHD diagnosed between the ages of 5–15 years and available parents.. ADHD cases were ascertained from the Child Psychiatric Clinics in the Chang Gung Memorial Hospital in Taipei area, Taiwan. A total of 92 (83.6%) were males. IQ was 50–69 in 13%, 70–89 in 44%, 90–119 in 40% and >120 in 1%. A diagnosis of ADHD was made according to DSM-IV criteria following completion of a standard maternal interview (kiddie-SADS)[21] and completion of parent and teacher Conner's revised rating scales.[22] In all 78% had the combined subtype and 22% the inattentive subtype of ADHD. With regard to co-morbidity, 4% had Tourettes syndrome and 4% oppositional defiant disorder. Two cases with autism were excluded from the study. No other neurological or behavioural disorders were identified. Comorbid developmental conditions were identified as follows: pervasive developmental disorder (6%), mixed receptive and expressive language disorder (10%), expressive language disorder (11%), receptive language disorder (0%), specific reading disorder (11%), specific spelling disorder (3%), specific arithmetic disorder (0%), mixed specific disorder of scholastic skills (0%), specific developmental disorder of motor function (5%), other specific developmental disorders (2%).

Genotyping

The VNTR polymorphism was amplified on an MJ PTC-225 thermal cycler (MJ Research) in a hot-start protocol involving an initial 5-min denaturing step at 95°C, followed by 38 cycles of 93°C for 1 min and 72°C for 1 min. Primers used were 5′- TGT GGT GTA GGG AAC GGC CTG AG-3′ and 5′- CTT CCT GGA GGT CAC GGC TCA AGG-3′. The reaction mix included 75 ng of genomic DNA, 1.5 mM $MgCl_2$, 20 mM dNTP's, 10 mM 10XPCR Buffer (PE Applied Biosystems) and 1 unit of *Taq* polymerase (added separately 30 s into the denaturing step). PCR products were run out on a 2% agarose gel.

Statistical analysis

Tests of association were performed using the haplotype-based haplotype relative risk (HHRR),[16] the transmission disequilibrium test (TDT)[17] and TRANSMIT.[18] Calculations for the association power table were made using the genetic power calculator (http://statgen.iop.kcl.ac.uk) which is described in detail by Sham *et al*.[23]

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