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Family-Based Association Study of Serotonin Transporter Gene Polymorphisms in Attention Deficit Hyperactivity Disorder: No Evidence for Association in UK and Taiwanese Samples

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Five independent studies have reported associations between serotonin transporter gene (*5-HTT*) polymorphisms and attention deficit hyperactivity disorder (ADHD). Four studies found evidence for association between the long-allele of a 44-base pair insertion/deletion polymorphism (5-HTTLPR), one of the studies found association to a variable number tandem repeat within intron 2, another to the T-allele of a single base pair substitution in the 3′-untranslated regions and another reported preferential transmission of a haplotype of the three markers (long-allele/10-repeat-allele/T-allele). One further study found no evidence for these associations. We investigated the association of these three markers in two samples of ADHD patients from the United Kingdom (n = 197) and Taiwan (n = 212), using within-family tests of association. No association was found between any of the three markers in either of the two populations. Although we found some evidence for the preferential transmission of a rare haplotype (long-allele/9-repeat-allele/T-allele; $\chi^2 = 4.5$, $P = 0.034$), we concluded that this most likely occurred by chance factors alone.

KEY WORDS: serotonin transporter gene; attention deficit hyperactivity disorder; ADHD; association studies; gene mapping

Attention deficit hyperactivity disorder (ADHD) is a common highly heritable neuropsychiatric disorder characterized by developmentally inappropriate levels of hyperactivity, inattention, and impulsivity. Current estimates indicate that 3%–6% of school age children are diagnosed with ADHD [Swanson et al., 2000]. ADHD is a complex, multifactorial disorder and the precise etiology factors are unknown, although polymorphic variants within several genes that regulate dopamine and related neurotransmitter pathways have been associated with ADHD in several studies [Asherson, 2004].

The gene encoding the human serotonin transporter (5-HTT) is located at chromosome 17q11.2. Three common polymorphisms associated with 5-HTT have been described; a 44-base pair repeat insertion/deletion in the promoter region (5-HTTLPR) [Heis et al., 1996], a variable number tandem repeat in intron 2 (HTT-VNTR2) [Lesch et al., 1994], and a single nucleotide polymorphism (SNP) within the 3′-untranslated region (HTT-3′UTR-SNP) [Battersby et al., 1999].

To date, five independent research groups have studied association between these 5-HTT polymorphisms and ADHD. Four studies reported evidence for association and linkage between the long-allele of a putatively functional 5-HTTLPR polymorphism and ADHD. Manor et al. [2001] reported a significant decrease in the short/short genotype using a family-based analysis of 98 trios ($P = 0.008$). Seeger et al. [2001] found a positive association of the long-allele with using case-control analysis of 80 ADHD probands and 163 controls ($P = 0.004$). Kent et al. [2002] investigated 113 ADHD parent proband trios and reported a significant association when case-control data was pooled with the previous published datasets from Manor et al. [2001] and Seeger et al. [2001]. Zoroğlu et al. [2002] found the short/short genotype was significantly lower in 71 cases compared to 120 controls ($P = 0.018$).

Zoroğlu et al. [2002] also reported that the 12/12 genotype of HTT-VNTR2 was significantly less frequent in 70 cases compared to 128 controls ($P = 0.001$). Association has also been reported for preferential transmission of the T-allele of the HTT-3′UTR-SNP and significant preferential transmission of a haplotype containing the T-allele, the 10-repeat allele of HTT-VNTR2 and the long-allele of 5-HTTLPR [Kent et al., 2002]. A more recent study however found no association between either 5-HTTLPR or HTT-VNTR2 using family-based as well as case-control analyses [Langley et al., 2003]. The aim of this study was to further investigate the relationship between these three polymorphisms of 5-HTT in two samples of ADHD probands from the UK and Taiwan.

For the UK sample DNA was available from 197 ADHD probands, both parents in 133 families, and from only the mother in 64 families. One hundred and seventeen of the ADHD probands had at least one sibling who was also genotyped. Cases were referred for assessment if they were thought by experienced clinicians to have a diagnosis of the combined subtype of ADHD under DSM-IV criteria, with no significant Axis I co-morbidity apart from oppositional defiant disorder (ODD) and conduct disorder (CD). Research criteria were established using standardized interview and application of operational criteria for DSM-IV combined type as described previously [Mill et al., 2004].

The Taiwanese sample consisted of 212 children with ADHD diagnosed between the ages of 5 and 15 years. Both parents were available for 114 families, only the mother for 59 families and only the father for 23 families. ADHD cases were ascertained from the Child Psychiatric Clinics in the Chang Gung Memorial Hospital in Taipei area, Taiwan. A diagnosis of ADHD was made according to DSM-IV criteria following completion of a standard maternal interview [KIDDIE-SADS,

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TABLE I. ETDT, HHRR, and TRANSMIT Analysis of 5-HTTLPR

	UK samples		Taiwanese samples		Combined samples	
	L	S	L	S	L	S
ETDT						
Transmitted	56	63	42	36	98	99
Non-transmitted	63	56	36	42	99	98
χ^2 (*P*-value)	0.412 (0.521)		0.462 (0.497)		0.005 (0.943)	
HHRR						
Transmitted	158	119	63	188	221	307
Non-transmitted	165	112	57	194	222	306
χ^2 (*P*-value)	0.364 (0.546)		0.394 (0.530)		0.004 (0.950)	
TRANSMIT						
Transmitted	217	165	124	280	341	445
Expected	220	162	114	290	338	448
χ^2 (*P*-value)	0.161 (0.689)		3.192 (0.074)		0.153 (0.696)	

Kaufman et al., 1997] and completion of parent and teacher Conner's revised rating scales [Conners, 1995]. 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. Autism cases were excluded from the study. No other neurological or behavioral disorders were identified.

Genotyping followed routine procedures. The 5-HTTLPR promoter region polymorphism (44 base pair insertion/deletion) and the 17 base pair "HTT-VNTR" polymorphism were genotyped using the methods described in Zoroğlu et al. [2002]. The HTT-3′UTR-SNP was amplified using primers described in Kent et al. [2002]. Genotype data was analyzed using the extended transmission disequilibrium test (ETDT) [Sham and Curtis, 1995] and the haplotype-based haplotype relative risk test (HHRR) [Terwilliger and Ott, 1992] on complete trios, and TRANSMIT for the analysis of the complete datasets including families with missing parental data [Clayton, 1999].

Evidence for linkage disequilibrium (LD) between the three markers was examined by calculating D′ and r^2 statistics. Polymorphism pair VNTR2/3′UTR showed significant LD in both UK and Taiwanese samples ($D' = 0.73$, $r^2 = 0.49$ and $D' = 0.77$, $r^2 = 0.49$, respectively). 5-HTTLPR/VNTR2 showed highly significant moderate LD ($D' = 0.51$, $r^2 = 0.39$ and $D' = 0.44$, $r^2 = 0.28$, respectively). 5-HTTLPR/3′UTR demonstrated weak LD ($D' = 0.35$, $r^2 = 0.34$ and $D' = 0.27$, $r^2 = 0.24$, respectively).

For 5-HTTLPR we found no significant preferential transmission of the long-allele in either the UK or Taiwanese samples. There was also no evidence of preferential transmission by combining UK and Taiwanese samples together (Table I). We found no evidence for a decreased transmission of the short/short genotype. For HTT-VNTR2, three alleles (9-repeat, 10-repeat, and 12-repeat) were detected in our samples. The common alleles were the 10-repeat and 12-repeat alleles. There was no significant association in either the UK sample, the Taiwanese sample, or the two samples combined together (Table II). For HTT-3′UTR-SNP, TDT we also found no evidence of preferential transmission in the UK sample, the Taiwanese sample, or the two combined samples (Table III).

We also completed a haplotype analysis of the three markers and found some evidence for preferential transmission of the long-allele//9-repeat-allele/T-allele haplotype in the UK sample (ETDT: $\chi^2 = 4.5$, $P = 0.034$; HHRR: $\chi^2 = 3.6$, $P = 0.058$; TRANSMIT: $\chi^2 = 1.9$, $P = 0.167$). However the haplotype is rare with only seven transmissions versus one non-transmission from TDT analysis and frequencies of 6% versus 0.8% from HHRR analysis. There was no evidence for this haplotype association in the Taiwanese sample or for association with other haplotypes.

In summary, this study investigated the relationship between three 5-HTT polymorphisms that had previously been reported to be associated with ADHD, in two clinically ascertained samples from the UK and Taiwan. Both samples had previously shown association with dopamine transporter polymorphisms [Curran et al., 2001; Chen et al., 2003] and the UK sample with SNAP-25 [Mill et al., 2002, 2004] in addition to more minor evidence for association with other genes [Asherson, 2004]. The data showed no evidence for an association between ADHD and any of the three serotonin

TABLE II. ETDT, HHRR, and TRANSMIT Analysis of VNTR

	UK samples			Taiwanese samples			Combined samples		
	Allele			Allele			Allele		
	9	10	12	9	10	12	9	10	12
ETDT									
Transmitted	8	52	62	1	16	19	9	68	81
Non-transmitted	5	62	55	0	19	17	5	81	72
χ^2, 2df (*P*-value)	1.399 (0.497)			1.644 (0.440)			2.071 (0.355)		
HHRR									
Transmitted	8	101	170	1	19	233	9	120	403
Non-transmitted	5	111	163	0	22	231	5	133	394
χ^2, 2df (*P*-value)	1.318 (0.517)			1.615 (0.446)			1.929 (0.381)		
TRANSMIT									
Transmitted	8	157	225	1	44	341	9	201	566
Expected	7	156	227	1	44	341	8	203	565
χ^2, 2df (*P*-value)	0.324 (0.850)			0.692 (0.706)			0.413 (0.813)		

TABLE III. ETDT, HHRR, and TRANSMIT Analysis of 3′ UTR

	UK samples		Taiwanese samples		Combined samples	
	G	T	G	T	G	T
ETDT						
Transmitted	64	57	32	40	96	97
Non-transmitted	57	64	40	32	97	96
χ^2 (*P*-value)	0.405 (0.524)		0.891 (0.345)		0.005 (0.943)	
HHRR						
Transmitted	110	152	213	45	323	197
Non-transmitted	103	159	221	37	324	196
χ^2 (*P*-value)	0.388 (0.534)		0.929 (0.335)		0.004 (0.949)	
TRANSMIT						
Transmitted	158	222	319	85	477	307
Expected	159	221	324	80	477	307
χ^2 (*P*-value)	0.014 (0.906)		0.723 (0.395)		0.001 (0.971)	

transporter gene polymorphisms in either of the UK or Taiwanese samples. The power of our samples was a limiting factors and it remains possible that very small genetic effects were missed in our analysis. To detect an odds ratio of 1.5 at alpha-level of 0.05 for the promotor, intron 2 and 3′UTR polymorphisms respectively, we estimated 78%, 80%, and 78% power in the UK sample and 79%, 49%, and 68% in the Taiwanese sample. Power estimations were made using the Genetic Power Calculator for TDT analysis of discrete traits (http://statgen.iop.kcl.ac.uk/gpc/) and population frequencies for the three markers estimated from parental genotypes.

Analysis of haplotypes demonstrated significant preferential transmission of the long-allele/9-repeat/T-allele haplotype in the UK sample, however, the observed haplotype is rare and therefore unlikely to have an etiological role in ADHD susceptibility in most cases. Furthermore, previous studies had not identified evidence of association to the 9-repeat allele and haplotypes containing more common alleles of HTT-VNTR2 were not found to be associated with ADHD. In conclusion, this study provides no evidence for the allelic associations of the serotonin transporter gene previously reported in two independent samples that have previously shown association to other genetic loci. Formal meta-analyses of these and other available datasets is now required to clarify whether the serotonin transporter gene has an etiological role in ADHD susceptibility.

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