American Journal of Medical Genetics (Neuropsychiatric Genetics) 114:269–271 (2002)

Association Study of a SNAP-25 Microsatellite and Attention Deficit Hyperactivity Disorder

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Several lines of evidence implicate synaptosomal-associated protein of 25 kDa (SNAP-25) in the etiology of attention deficit hyperactivity disorder (ADHD). Most notably, the *coloboma* mouse mutant, considered to be a good animal model of hyperactivity, has a deletion spanning this gene. Introducing a SNAP-25 transgene into these animals alleviates hyperlocomotion. We have identified a novel microsatellite repeat in SNAP-25 located between the 5′UTR and the first coding exon, and tested for association with ADHD. Case-control analyses suggest there may be a role of this polymorphism in ADHD, with one allele over-represented in controls and another over-represented in probands. Within-family tests of linkage and association confirmed these findings. Further work is needed to ascertain the role of SNAP-25 in ADHD and assess the functional significance of this polymorphism.

KEY WORDS: attention deficit hyperactivity disorder (ADHD); SNAP-25; genetics; association study

DATABASES: bA416N4 (GenBank no. AL354824); HS1068F16 (GenBank no. AL023913)

INTRODUCTION

SNAP-25 (synaptosomal-associated protein of 25 kDa) is a presynaptic plasma membrane protein with an integral role in synaptic transmission. It forms a complex with syntaxin and the synaptic vesicle proteins (synaptobrevin and synaptotagmin) that mediates the Ca^{2+}-mediated exocytosis of neurotransmitter from the synaptic vesicle into the synaptic cleft. Expression studies suggest that SNAP-25 is differentially expressed throughout the brain, and present primarily in the neocortex, hippocampus, anterior thalamic nuclei, substantia nigra, and cerebellar granule cells [Oyler et al., 1989]. During development, SNAP-25 appears to be involved in synaptic plasticity and axonal growth [Ossen-Sand et al., 1993], but in the mature nervous system expression is generally only seen in presynaptic terminals [Oyler et al., 1989].

Evidence for the role of SNAP-25 in attention deficit hyperactivity disorder (ADHD) comes primarily from the *coloboma* mouse strain, which is a good putative model of hyperkinesis and ADHD [Wilson, 2000]. *Coloboma* mice display a number of behavioral and developmental deficits including hyperlocomotion, head bobbing and ocular defects. Furthermore, the high activity levels in these mice can be reduced to normal with D-amphetamine, paralleling the use of stimulants to treat ADHD in humans, although methylphenidate appears to have no effect in the *coloboma* mice [Hess, 1996]. The extreme hyperactivity exhibited by these mice results from a 2-cM deletion on mouse chromosome 2, in a region containing the mouse SNAP-25 gene (*Snap*) [Hess et al., 1992]. Hess [1996] replaced the deleted *Snap* gene with a homologous transgene and observed amelioration of the hyperlocomotion normally exhibited by *Coloboma* mice, suggesting it was the cause of the *coloboma* phenotype.

Relatively little work has been done on the human homologue of the *Snap* gene, SNAP-25. Hess et al. [1995] looked at several markers on chromosome 20 in the region syntenic to mouse chromosome 2, but found no evidence of linkage in five family pedigrees using an autosomal dominant model. Several limitations to this study were raised by Barr et al. [2000] who carried out a linkage study of the 3′ untranslated region (UTR) of the human SNAP-25 gene and observed biased transmission using the transmission disequilibrium test (TDT) on haplotypes derived from two single nucleotide

Grant sponsor: Medical Research Council.

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Received 27 July 2001; Accepted 15 November 2001

DOI 10.1002/ajmg.10253

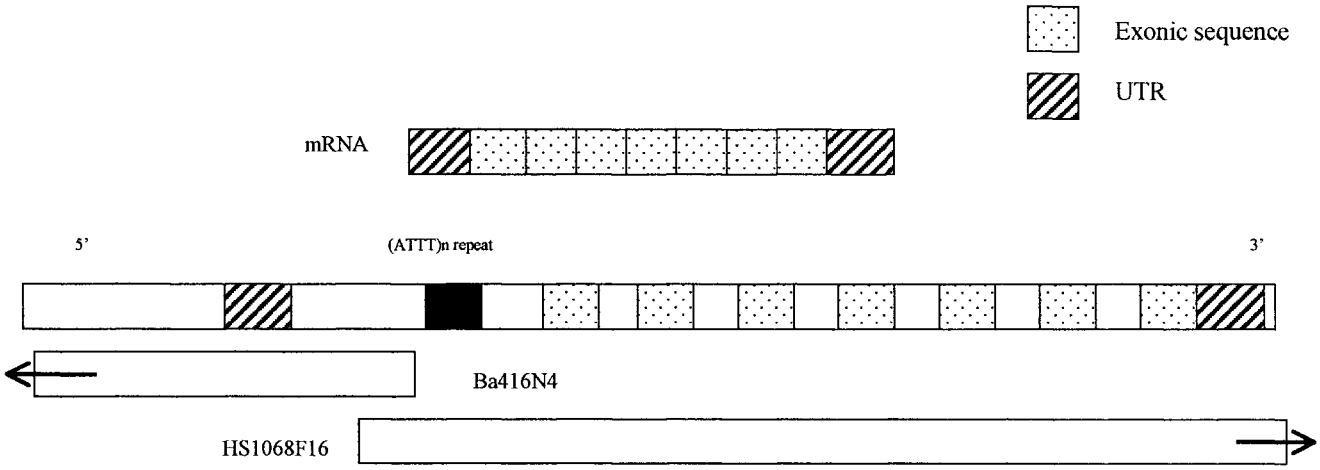

Fig. 1. Structure of the SNAP-25 gene.

polymorphisms (SNPs). The human SNAP-25 cDNA was cloned by Zhao et al. [1994], although the full genomic sequence of the gene has only just become available. Using genomic information available in web-based sequence databases we have identified a novel tetranucleotide repeat polymorphism (ATTT)n located between the 5′UTR and the first transcribed exon of the SNAP-25 gene. In this study we examine the role of this polymorphism in the etiology of ADHD in a clinically ascertained sample.

MATERIALS AND METHODS

Samples

'Cases' were clinically-referred individuals diagnosed using semi-structured research interviews and rating scales, from two centres (the Institute of Psychiatry and University of Birmingham) and all fulfilled criteria for a diagnosis ADHD-combined subtype (DSM IV) with no significant Axis I co-morbidity apart from oppositional defiant disorder (ODD) and conduct disorder (CD). 'Controls' were drawn from two independent epidemiological samples, matched for ethnicity. A more detailed description of the subjects can be found in Mill et al. [2001]. In total 159 clinical subjects and 177 control individuals were included in this study. For family-based analysis we used DNA from 107 parent-child trios.

Identification of Novel Microsatellite Polymorphism

The structure of the SNAP-25 gene can be seen in Figure 1. The genomic sequence spans clones bA416N4 (GenBank accession number AL354824) and HS1068F16 (GenBank accession number AL023913). We screened the sequence using a web-based program called Tandem Repeats Finder (Benson, 1999), and detected an (ATTT)n repeat between the first transcribed exon and the 5′UTR of the SNAP-25 gene.

Amplification of SNAP-25 Intron 1 Microsatellite

The SNAP-25 microsatellite was amplified using the primers : FAM-5′-TGG AGG GAT GTG GTT TGG-3′ and 5′-AAG TTG TAC ACT TCA AAT ATG TGG-3′ with an initial 5 min denaturing step at 95°C followed by 35 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 1 min, and a final extension phase of 72°C for 10 min. The fluorescently-tagged products were separated on an ABI 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) and analyzed using GENOTYPER (PE Applied Biosystems) software.

Statistical Analysis

Case-control association was assessed using CLUMP [Sham and Curtis, 1995], a program that tests for the overall allele frequency differences between two groups as well as testing one allele against the rest clumped together. The transmission disequilibrium test (TDT) was applied to the sub-set of probands with DNA available from both parents for genotyping.

RESULTS

Case-Control Association Analysis

Seven alleles of the SNAP-25 microsatellite were detected in our samples, and the allele frequencies can be seen in Table I. There was no significant difference in allele frequencies between the two control samples so they were analyzed as one complete group. Comparing the allele frequencies between the probands and

TABLE I. Allele Frequencies and Clumped χ^2 Values for ADHD Probands and Controls

Allele	Proband alleles (%)	Control alleles (%)	Clumped chi-square	2-tailed *P*
1	0 (0)	1 (0)	0.00	1.00
2	31 (10)	58 (16)	6.42	0.01
3	10 (3)	5 (1)	2.30	0.13
4	58 (18)	68 (19)	0.10	0.75
5	196 (62)	193 (55)	3.48	0.06
6	20 (6)	24 (7)	0.07	0.791
7	3 (1)	5 (1)	0.31	0.578
Total	318	354	—	—

TABLE II. TDT Analysis on SNAP-25 Alleles 2 and 5

Allele	Transmitted	Non-transmitted	TDT (2-tailed *P*)
2	19	33	3.77 (0.05)
5	65	44	4.05 (0.04)

controls gave a near significant difference ($\chi^2 = 10.64$, 6 *df*, 2-tailed $P = 0.08$). Using CLUMP we found a significant difference between probands and controls when one allele was taken compared to the rest clumped together ($\chi^2 = 6.42$, 1 *df*, 2-tailed $P = 0.01$). As shown in Table I, when a similar analysis was performed for each allele individually, a significant difference was found for allele 2 ($\chi^2 = 6.42$, 1 *df*, 2-tailed $P = 0.01$) with this allele being at a lower frequency in probands as compared to controls (16.4% vs. 9.7%) and a near-significant difference was found for allele 5 ($\chi^2 = 3.48$, 1 *df*, 2-tailed $P = 0.06$) that was more prevalent in probands as compared to controls (61.6% vs. 54.5%). The odds ratio for allele 2 is 0.54, highlighting its protective effect, and that for allele 5 is 1.34 suggesting it is a weak risk allele.

Within-Family TDT Analysis

Table II shows the results from TDT analysis on the two alleles nominated from the case-control analysis. Both are marginally significant (allele 2: $\chi^2 = 3.77$, 1 *df*, 2-tailed $P = 0.05$; allele 5: $\chi^2 = 4.05$, 1 *df*, 2-tailed $P = 0.04$), and transmissions are in the direction predicted from case-control analysis.

DISCUSSION

The data from both analyses suggest that SNAP-25 may play a role in the genetic etiology of ADHD, although further work is required to confirm or reject this hypothesis. Allele 2 of the reported microsatellite may confer a protective effect, being significantly lower in frequency in the control sample compared to the ADHD probands, and transmitted significantly less to affected individuals. Allele 5 may be a risk allele, being more common in probands compared to controls, and transmitted significantly more to affected individuals. Future work will focus on typing this polymorphism in a larger set of trios and a population sample rated using quantitative measures of hyperactivity that will provide an independent replication of these findings and increase our power to confirm this putative association.

The association reported in this study is with a polymorphism at the other end of the SNAP-25 gene to the SNPs analyzed by Barr et al. [2000]. It would be interesting to ascertain the level of linkage disequilibrium across the gene, and localize precisely any functional variants that may be the cause of our putative finding. Taken together with results obtained by Barr et al. [2000] and experiments on the *coloboma* mouse strain, these findings suggest that more work should be done on the role of SNAP-25 in the etiology of ADHD. Future investigations should focus on the detection of further polymorphisms within the SNAP-25 gene and other genes involved in synaptic transmission.

ACKNOWLEDGMENTS

This research was funded by a Medical Research Council (MRC) component grant to the Social, Genetic, and Developmental Psychiatry Research Centre. Jonathan Mill is an MRC funded PhD student. Control samples were collected with funds from a Wellcome Training Fellowship to Sarah Curran and University of Birmingham samples were collected with funds from a Wellcome Training Fellowship to Lindsey Kent. We wish to thank all clinicians and families who helped in this research. Particular thanks go to Dr. Geof Kewley, Dr. Mark Berelowitz, Professor Peter Hill, Fiona McNicholas, Mary Cameron, and Saama El Abd.

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