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ORIGINAL RESEARCH ARTICLE

Dopa decarboxylase gene polymorphisms and attention deficit hyperactivity disorder (ADHD): no evidence for association in the Irish population

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Dopa decarboxylase (DDC) is an enzyme which catalyses the decarboxylation of both dopa to dopamine and L-5 hydroxytryptophan to serotonin. Both catecholamines are major neurotransmitters of the mammalian nervous system. It has been suggested that genes involved in the dopaminergic system play a primary role in predisposing to attention deficit hyperactivity disorder (ADHD). In this study, the 4-bp insertion/deletion variant mapped to the first neuronally expressed exon 1 at the dopa decarboxylase gene and two microsatellite markers flanking the gene were investigated for possible association with ADHD. Using HHRR, we observed an increased transmission (though not significant) of the 4-bp insertion (allele 1) to ADHD cases (χ^2 = 2.72, P = 0.1, RR = 1.25). However marginally significant excess transmission of allele 10 (213 bp) of the 3′ microsatellite D7S2422 (~0.75 cM distal to dopa decarboxylase gene) was found (χ^2 = 4.2, P = 0.04, RR=1.48). Interestingly, a haplotype containing both alleles is transmitted more frequently (χ^2 = 5, P = 0.025). Analysing data by the sex of transmitting parent showed a greater relative risk for paternal transmission of the 4-bp insertion allele and allele 10 of the D7S2422 (RR = 1.48 and 1.63 respectively). This provides preliminary evidence that this locus or a closely mapped DNA variant may be involved in the genetic susceptibility to ADHD. However, further studies are required to either confirm or refute these observations.
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Keywords: attention deficit hyperactivity disorder (ADHD); dopamine; dopa decarboxylase; association; haplotype based haplotype relative risk (HHRR)

Introduction

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder affecting 3–6% of children worldwide. Characteristic symptoms are hyperactivity, inattention and impulsivity. It causes marked educational, social and family difficulties for sufferers and their relatives. Although the biological basis of ADHD is not known, a strong genetic component has been implicated through family,[1,2] twin[3] and adoption studies.[4] Concordance rates of 66% for MZ twins and 28% for DZ twins have been reported by Gjone *et al*[5] and Levy *et al*[6] with estimates of heritability (h^2) ranging from 50% to 98%.

Pharmacological evidence has suggested a role for dopaminergic neurotransmission systems in the pathology of ADHD.[7,8] Medications which inhibit the dopamine transporter and thus increase dopamine availability in the synaptic cleft such as methylphenidate were found to ameliorate the symptoms of ADHD. Studies of animal models have lent further support to these findings. Mice with a disrupted dopamine transporter (DAT1 knockout mice) have high extracellular striatal dopamine levels and markedly increased locomotive and stereotypic activity compared to normal (wild-type) mice.[9,10] In addition, brain imaging studies have shown abnormalities in the frontal lobe and subcortical structures which are known to be rich in dopamine and important in the control of attention.[11,12] Positron emission tomography (PET) studies in adolescents and adult ADHD showed different patterns of cerebral metabolic rates of glucosein.[13,14] Recently, Dougherty *et al*[15] investigated dopamine transporter density *in vivo* and found a 70% increase in ADHD adults compared to healthy controls. This finding lends support to a functional hypodopaminergic hypothesis of ADHD. Several recent allelic association studies have implicated the dopamine transporter (DAT1), the dopamine receptors DRD4, DRD5 and dopamine beta hydroxylase (DBH) in the aetiology of ADHD.[16–19]

Dopa decarboxylase, an enzyme involved in the synthesis of the neurotransmitter dopamine, catalyses the formation of functional dopamine through decarboxylation of a precursor tyrosine derivative. In addition, it participates in the synthesis of trace amine compounds suggested to act as modulators of central neurotransmission.[20] The dopa decarboxylase gene (dopa gene) maps to chromosome 7p12.1–p12.3[21,22] and contains 15 exons spanning over 85 kb.[20,21] The

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expression of the gene is controlled by two promoters located between positions −106 and −38 relative to the transcription initiation site. Expression in nerve and glial cells requires a CNS specific activator located between nucleotides −59 and −83 which bind to the transcription factor Elf1 in a tissue-specific manner.[23,24] Mutations in the coding or promoter regions of this gene could alter either the function or the quantity of the enzyme and therefore lead to the production of abnormal levels of dopamine. Thus the dopa decarboxylase gene may be considered as a candidate gene for ADHD susceptibility.

In this study we used the haplotype based haplotype relative risk (HHRR) approach to analyse the 4-bp insertion/deletion (at the neuronal exon 1 of the dopa gene) for possible association with ADHD in a sample of probands and their parents. As the extent of LD within and surrounding the gene is unknown, we also genotyped two flanking microsatellite markers (D7S2561 and D7S2422).

Materials and methods

ADHD cases and available parents were recruited from child psychiatric clinics and schools in west County Dublin and from the Hyperactive and Attention Deficit Children's Support Group of Ireland. Twenty-one families consisted of mother and affected child, and 64 families consisted of mother, father and affected child (trios). The age range of the probands was between 4 and 14 years, with males accounting for 85%. The families were ethnically Irish with the exception of one family in which the father was Croatian. Details of diagnosis and clinical criteria can be found in Daly *et al*.[19]

Consensus diagnoses were made according to DSM-IV ADHD or UADD either with or without comorbidity. These diagnoses were based on all available clinical information and the Child Behaviour Checklist (CBCL), the Conners Parents and Teachers Rating Scales, and the Comprehensive Teachers Rating Scale (ACTeRS). The 25-item Wender–Utah Rating Scale (WURS) was applied to all parents. Familiality was defined as the presence of one or more parents with a score on the WURS of >36 (96% sensitive and specific for a retrospective diagnosis of childhood ADHD.[25]

DNA amplification

DNA was extracted from EDTA blood using the standard phenol chloroform procedure. The PCR primer sequences and conditions used to amplify the 4-bp insertion/deletion at the untranslated exon 1 of dopa were those used in Speight *et al*.[26] Standard PCR reactions were carried out in reaction volumes of 25 μl containing 60 ng of genomic DNA, 20 pmol of each primer, 200 μM of each dNTP, 50 mM of KCl, 10 mM of Tris-HCl (pH 9), 1.5 mM of $MgCl_2$, 0.01% of gelatine and 1 U of Taq pol. Genotyping was achieved by incorporating pα^{32} in the PCR mix and separating the product on 6% denaturing polyacrylamide gels. Two microsatellite markers (D7S2561 and D7S2422) flanking the dopa decarboxylase gene at the 5′ and 3′ ends were also genotyped using the semi-automated florescent methods on an ABI 377 DNA sequencer. All genotypes were scored independently by two investigators.

Statistics

We used the haplotype based haplotype relative risk (HHRR) design to avoid any potential population stratification. The chi-square test and Fisher's exact *P* were used to assess the significance of the transmission of alleles from parents to their affected children. All chi-square tests and *P*-values are presented without correction for multiple testing. We also used the programme TRANSMIT to examine haplotypes that might be transmitted more frequently to the ADHD cases.

Results

Alleles and frequencies of the dopa decarboxylase exon 1 and two microsatellite flanking polymorphisms and numbers of alleles transmitted or not are presented in Table 1. The results of HHRR analysis of 85 ADHD families are presented in Table 2. Increased transmission of allele 1 (presence of insertion) of the dopa gene polymorphism was observed but did not attain statistical significance (χ^2 = 2.72, *P* = 0.1, RR=1.25). In addition, no significant difference was observed on dividing the sample into family history positive and negative (Table 2). However, when the transmission from parents was considered separately, the trend of association observed in the total sample was enhanced in the paternal group (χ^2 = 2.65, *P* = 0.1, RR = 1.44).

Two flanking markers mapping ~1.8 cM proximal and ~0.75 cM distal (D7S2561 and D7S2422 respectively) (Http://cedar.genetics.soton.ac.uk/) to the dopa gene were also analysed (Table 2). This analysis showed increased transmission of allele 10 of the marker D7S2422 (χ^2 = 4.2, *P* = 0.04, RR = 1.48). Increased transmission of the allele 10 was observed in the family history positive group, but was not significant at the *P* = 0.05 level. Analysing the data by sex of transmitting parent showed a greater relative risk for paternal transmission of allele 10 (χ^2=3.01, *P* = 0.08, RR = 1.63). Haplotype analysis of the three polymorphisms (considering all possible combinations) using the programme TRANSMIT showed a significant association of the haplotype containing allele 1 of the dopa gene and allele 10 of D7S2422 and with ADHD (χ^2 = 5, *P* = 025). None of the other haplotypes was significant.

Discussion

In this current investigation, we observed increased (though not significant) transmission of 4-bp insertion allele of the neuronal exon 1 of the dopa gene to ADHD cases. Analysing data by sex of transmitting parent showed a greater relative risk for paternal transmission of this allele (RR = 1.44). In addition, analysis of microsatellite marker D7S2422 (~0.75 cM distal to the dopa gene) showed significant association between allele 10 and the ADHD phenotype (χ^2 = 4.2, *P* = 0.04,

Table 1 Alleles and frequencies of dopa decarboxylase exon1 and two microsatellite flanking polymorphisms and numbers of alleles transmitted or not transmitted to ADHD children

Allele (frequency)	*T*	*NT*	*Allele (frequency)*	*T*	*NT*	*Allele (frequency)*	*T*	*NT*
D7S2561			**Exon 1**			**D7S2422**		
1 (5.9)	9	8	1 (67.3)	116	103	1 (0.76)	0	1
2 (51.1)	62	69	2 (32.7)	37	50	2 (32.1)	50	42
3 (8.1)	17	11				3 (10.7)	12	14
4 (7.4)	8	10				4 (0.0)	Nd	Nd
5 (20.0)	27	27				5 (1.53)	2	2
6 (7.4)	12	10				6 (3.8)	4	5
						7 (2.3)	1	3
						8 (3.1)	0	4
						9 (2.3)	0	3
						10 (4.6)	15	6
						11 (6.1)	2	8
						12 (4.6)	5	6
						13 (11.5)	18	15
						14 (7.6)	12	10
						15 (6.1)	4	8
						16 (1.5)	6	2
						17 (1.5)	0	2

T = Transmitted, Nt = not transmitted, Nd = not detected.

Table 2 HHRR analysis of D7S2561, dopa 4-bp insertion/deletion and D7S2422 in 64 ADHD trios and 21 parent probands

Marker	*Associated allele*	*RR (95% CI)*	χ^2	*P*
	Allele 3 (214 bp)			
D7S2561	Total	1.25 (0.90–1.72)	1.43	0.23
	FH+ve	1.0 (0.55–1.82)	0.0	1.0
	FH–ve	1.44 (0.99–2.1)	2.51	0.1
	Maternal	1.29 (0.85–1.96)	1.12	0.29
	Paternal	1.2 (0.71–2.1)	0.44	0.5
	Allele 1 (insertion)			
Dopa exon 1	Total	1.25 (0.95–2.6)	2.72	0.1
	FH+ve	1.3 (0.83–2.1)	1.49	0.22
	FH–ve	1.21 (0.86–1.71)	1.29	0.26
	Maternal	1.15 (0.82–1.6)	0.66	0.41
	Paternal	1.44 (0.89–2.33)	2.65	0.1
	Allele 10 (213 bp)			
D7S2422	Total	1.48 (1.1–2.0)	4.2	0.04
	FH+ve	1.63 (1.1–2.4)	3.01	0.08
	FH–ve	1.37 (0.89–2.1)	1.45	0.23
	Maternal	1.38 (0.89–2.1)	1.45	0.23
	Paternal	1.63 (1.1–2.4)	3.01	0.08

'Associated' allele(s) are those showing excess transmission. RR = relative risk, FH+ve = family history positive, FH–ve = family history negative.

RR = 1.48). A greater relative risk for paternal transmission (RR = 1.63) was also observed. The haplotype containing allele 1 of the 4-bp insertion/deletion and allele 10 of D7S2422 was also transmitted more often to the cases (χ^2=5, P = 0.025).

Dopa decarboxylase plays a central role in the synthesis of dopamine as well as serotonin. It also participates in the synthesis of trace amines—compounds suggested to act as endogenous modulators of central neurotransmission. Direct evidence of altered dopa decarboxylase activity in children with ADHD was reported by Ernst *et al*[14] using positron emission tomography with the tracer [(^{18})fluorine] fluorodopa [(^{18})F]dopa. This technique allows a measurement of dopa decarboxylase activity to be obtained by quantifying the accumulation of labelled dopamine in dif-

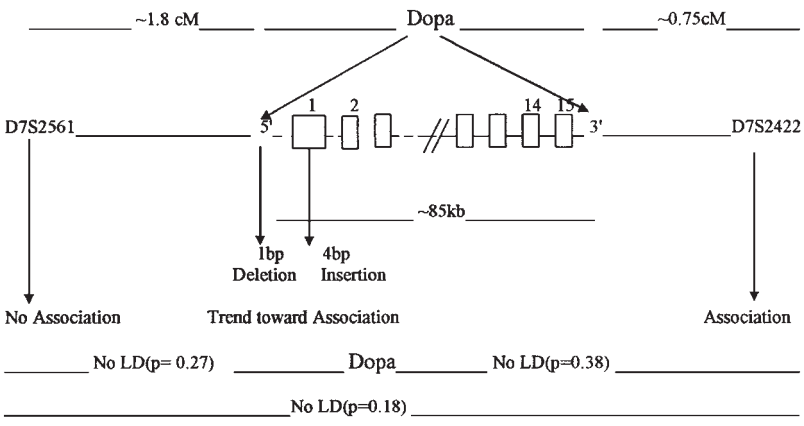

Figure 1 Diagrammatical representation of the dopa gene and two microsatellite flanking markers. The diagram is not to scale. Empty squares represent exons. LD = linkage disequilibrium. 'Association' is with the disease phenotype.

ferent regions of the brain. They observed lower (by 51%) F18-dopa ratios in the medial and left prefrontal areas in ADHD cases compared to controls.

Increased transmission of allele 1 (the 4-bp insertion variant) was observed in our sample but was not statistically significant (Table 2). This particular variant is known to be in perfect linkage disequilibrium with the 1-bp deletion at the promoter region of the gene.[20] The latter is known to affect a binding site (GCGGGGGCG) for the NGFI-A and NGFI-C transcription factors known to be expressed in the neuronal tissue.[27] The G (pos 7) deletion in the binding site is the most critical change and was found to drop the binding capacity of these factors to zero for all members of the NGFI-family.[20,27] The functional impact of this deletion on the expression of dopa decarboxylase has not yet been assessed. However, it seems likely that the 1-bp deletion may have an impact by either reducing or ablating the expression of dopa decarboxylase. The consequence of this would be a reduction in the amount of dopamine in brain areas important for attention and locomotion. Thus the trend for association of the 4-bp insertion would concur with the hypodopaminergic hypothesis for ADHD.[14]

The haplotype analysis also showed significantly increased transmission of the haplotype containing allele 1 (4-bp insertion/deletion) and allele 10 of the microsatellite. However, we did not find evidence for LD between the alleles at dopa (4-bp insertion/deletion) and those at the microsatellite flanking markers at the 5′ and 3′ end of the gene (Figure 1). The significant association of the allele 10 of the 3′ flanking microsatellite marker D7S2422 may be a false positive finding especially since the frequency of this allele is low in the general population (4.5%). However, it is possible that the associated allele is in linkage disequilibrium with a functional polymorphism or a causative mutation in the vicinity of the D7S2422 which might predispose to ADHD. The 1-bp deletion in the promoter is a possible candidate.

The increased paternal transmission of the 4-bp insertion and allele 10 of D7S2422 to ADHD cases may represent a chance finding or might suggest genomic imprinting at or near this locus. Evidence of imprinting has been reported at chromosome 7q32, genes of which may contribute to the development of the Silver–Russell syndrome phenotype.[28] However, dopa decarboxylase maps to chromosome 7p12.1–p12.3. Since imprinted genes in general tend to cluster at a chromosomal region, it is unlikely that the imprinted region at chromosome 7 influences the development of ADHD. However, our sample size is relatively small and our results are speculative at best. The parental origin of expressed alleles needs to be determined in order to provide convincing evidence of imprinting.

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