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Running head: DDC gene variants in autism

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Though genetic risk factors are important for the development of autism, no specific risk alleles have yet been identified. DOPA decarboxylase (*DDC*) is involved in both the catecholaminergic and serotonergic pathways and may be considered a functional candidate gene for autism. The present study is the first to test if two new variants of possible functional significance in the *DDC* gene increase the susceptibility to autism. A total of 90 parent-offspring trios recruited in Denmark and France were investigated using the transmission disequilibrium test (TDT). We found no evidence of linkage disequilibrium between autism and either of the two polymorphisms. Nor did we find linkage disequilibrium between autism and haplotypes of the two variants using a multiallelic TDT. These findings suggest that the *DDC* gene is unlikely to play a major role in the development of autism in our data set.

KEY WORDS: ***DDC*; polymorphisms; association; autism; TDT**

INTRODUCTION

Infantile autism is a pervasive developmental disorder with onset of symptoms before the age of three and impairment in the areas of social interaction and communication together with restricted, stereotyped behavior and interests. A prevalence estimate of 5/10,000 children has been reported (Fombonne, 1999) but some studies have found a prevalence as high as 1/1,000 (Gillberg and Wing, 1999). Family and twin studies point to a strong genetic influence (Bolton et al., 1994; Bailey et al., 1995) which most likely is oligo- or polygenic, possibly with epistasis, for the majority of cases (Pickles et al., 1995; Risch et al., 1999). No specific disease genes for autism have yet been identified, but interesting regions have been suggested on chromosome 7q31-35 and 15q11-13 (Cook et al., 1998; International Molecular Genetic Study of Autism Consortium, 1998; Ashley-Koch et al., 1999; Barrett et al., 1999; Vincent et al., 2000; Warburton et al., 2000; International Molecular Genetic Study of Autism Consortium, 2001).

The gene for DOPA decarboxylase (DDC) is localized on chromosome 7p12.2 (http://genome.cse.ucsc.edu). DDC is a regulated enzyme involved in two different metabolic pathways (Christenson et al., 1972). DDC decarboxylates L-dihydroxyphenylalanine (L-DOPA) to form dopamine which besides being a neurotransmitter is also the precursor of two other catecholamines, noradrenaline and adrenaline. In addition, DDC decarboxylates 5-hydroxytryptophan to form serotonin. DDC is thus unique in being involved in two neurotransmitter pathways which have been central to neuropsychiatric research for more than three decades.

DDC is a functional candidate gene for autism as abnormalities in the serotonin neurotransmitter system have been reported in some autistic individuals. Elevated levels of whole blood or platelet serotonin have been repeatedly observed in autistic subjects as well as among their non-affected relatives (Anderson et al., 1987; Abramson et al., 1989; Leboyer et al., 1999). Moreover, it has been

hypothesized that serotonin dysregulation may be involved in self-injury (Winchel and Stanley, 1991). This hypothesis has been supported by studies showing that serotonin reuptake inhibitors are partially successful in suppressing autistic symptoms such as aggression, self-mutilation and repetitive and ritualistic behaviors (McDougle et al., 1996). Candidate genes involved in monoaminergic neurotransmission may separately or together be involved in susceptibility to autism. A few linkage and association studies have suggested variants in the serotonin transporter gene as functional candidate genes of autism (Cook et al., 1997; Klauck et al., 1997; Yirmiya et al., 200); Tordjman et al., 2001; International Molecular Genetic Study of Autism, 2001). However, this has not been supported by all studies (Maestrini et al., 1999, Persico et al., 2000). Apart from the genome-wide scan by Risch et al. (1999), which reported a rather low MLS of 1.01 near marker D7S2564 at chromosome 7p, no other linkage studies of autism has supported the region around DDC.

The purpose of this study was to search for association between infantile autism and two recently identified possibly functionally important variants in *DDC* (Borglum et al., 1999). The 1-bp deletion variant affects a G corresponding to nucleotide number g.-601 relative to the transcription start site and may alter a possible binding site for a family of NGF1-A transcription factors. The 4-bp deletion comprises a GAGA sequence at position 722-725 in exon 1 which may be a binding site for a transcription factor named GAF (Borglum et al., 1999). Both polymorphisms may thus be of functional importance.

MATERIALS AND METHODS

Family Recruitment and Diagnostic Assessments

Danish families with a child diagnosed with infantile autism according to ICD-10 (World Health Organisation, 1993) were recruited at child psychiatric hospitals in the western part of Denmark (Jutland). The families were contacted by letter and asked to participate in the study. All children of the participating families were assessed by the same diagnostician (M.B.L.) both by interview of one or usually both parents, using the Danish version of Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994), and by observation, using the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (Lord et al., 2000) in order to obtain a research diagnosis of infantile autism. ADOS-G was videotaped and ADI-R was recorded on tape. The child had to satisfy the ADI-R and ADOS-G algorithm criteria for autism in the three behavioral domains (qualitative impairments in reciprocal social interaction; qualitative impairments in communication; restricted, repetitive and stereotyped patterns of behavior, interests and activities) with onset before three years of age. Both ADI-R and ADOS-G are based on ICD-10 (World Health Organisation, 1993) and DSM-IV (American Psychiatric Association, 1994) criteria for the diagnosis of autism. The final diagnosis of infantile autism based on ADI-R and ADOS-G was made in collaboration with an independent expert, Lennart Pedersen, National Centre for Autism, Copenhagen, Denmark. Only children diagnosed with infantile autism by both diagnosticians were included in the study. The study was approved by The Central Scientific Ethical Committee of Denmark and The Danish Data Protection Agency. Informed consent to

participate was given by all parents.

French families with a child fulfilling the DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organisation, 1993) criteria for autistic disorder were recruited at an outpatient university clinic for children with autism in Paris (Hôpital Robert Debré). Both parents were interviewed using the French version of the ADI-R (Lord et al., 1994). Subjects were included after a complete clinical and neuropsychological evaluation, including karyotyping and molecular genetic testing for fragile-X; patients with known etiologies of autism were excluded. The study was approved by the local ethical committee. Informed consent forms were completed by the parents.

A total of 90 families consisting of one child with infantile autism and both parents were included. Of these families, 17 were Danish with a male-female ratio of 3.3:1 and 73 were French with a male-female ratio of 2.5:1. The age range among Danish cases was from three to 30 years (mean age 11 years) and the age range among French cases was from four to 28 years (mean age 12 years). All patients were of Caucasian descent.

Laboratory Procedures

Blood samples were collected from children and their parents, and DNA was extracted from lymphocytes by standard procedures. Genotyping of the two DDC variants was performed essentially as described previously (Borglum et al., 1999). Briefly, a multiplex PCR amplification with radioactive ^{33}P end-labeled primers was carried out under standard conditions in the presence of 150 ng genomic DNA, 0.4 μM of each of the 1-bp deletion primers, and 1.2 μM of each of the 4-bp deletion primers. The primers for the 1-bp deletion variant were DDCprom-up (5'-GCACCCATCAACCAGAAGT-3') and DDCprom-low1del (5'-GCCTATCAGCATCTAAAACAT-3'), amplifying products of 118-bp and 117-bp. The primers for the 4-bp deletion variant were DDCprom-up4del (5'-GCTTCGGGGAGGCAGACAC-3') and DDCprom-low (5'-GGATGAGGACAAAGAGCAGTA-3'), amplifying products of 143-bp and 139-bp. PCR products were heat denatured in 50% formamide for 5 min before separation on a 6% polyacrylamide/6M urea gel. After vacuum drying the gel was autoradiographed for five days.

Statistical Analysis

In order to assume random mating cases were checked for Hardy-Weinberg equilibrium using chi-square statistics. The transmission disequilibrium test (TDT) was used to search for linkage disequilibrium of the 1-bp and 4-bp deletion variants of the DDC gene (Spielman and Ewens, 1998). Heterozygous parents were classified according to the allele they transmitted to the affected child. Only families with both parents genotyped were included in the study. The extended transmission disequilibrium test (ETDT) was used on haplotype-based genotypes (Sham and Curtis, 1995). Transmission disequilibrium was also investigated for maternal and paternal alleles separately.

RESULTS

Table I shows the distribution of alleles, genotypes, and haplotypes of the two variants. Twenty-three and 49 of the 180 parents were heterozygous for the 1-bp or 4-bp deletion variants, respectively, and for these transmission of alleles could be scored. Of nine possible two-locus genotypes based on the haplotypes including both polymorphisms, five are heterozygous but we observed only four heterozygous two-locus genotypes (Table I). Transmission data for each allele of the 1-bp and 4-bp deletion in the DDC gene and for the haplotypes containing the two markers are shown in Tables II and III.

For the 1-bp deletion no deviation from Hardy-Weinberg equilibrium was found among Danish and French patients when investigated separately ($p = 0.69$ and $p = 0.53$, respectively). Similarly, no deviation from Hardy-Weinberg equilibrium was detected for the 4-bp deletion ($p = 0.58$ and $p= 0.44$, respectively). We found no significant difference between the allele frequencies at the 1-bp or 4-bp polymorphism in the Danish or French subsample. We therefore analyzed the two subsamples together.

No significant transmission distortion of any alleles of the 1-bp deletion or 4-bp deletion polymorphisms was found (Table II). Moreover, no linkage disequilibrium was observed at either loci when paternal and maternal transmissions or Danish and French subsamples were analyzed separately. Using the ETDT (Sham and Curtis, 1995), which allows simultaneous testing of the importance of several alleles in one test, no distortion of haplotype transmission was found (Table III).

DISCUSSION

In the search for risk alleles involved in infantile autism relatively few candidate genes have been investigated. These candidate genes have mainly been chosen because of their function and possible significance for the pathophysiology of infantile autism. Especially, genes involved in dopaminergic or serotonergic neurotransmission or related to the HLA system have been investigated (Lauritsen and Ewald, 2001). Only very few studies have investigated positional candidates implicated by genetic mapping (Vincent et al., 2000) or cytogenetic abnormalities (Lauritsen et al., 1999). The present study is the first to test if two new variants of possible functional significance in the *DDC* gene affect the susceptibility to infantile autism.

The TDT has been advocated to detect weaker risk alleles for complex disorders (Risch and Merikangas, 1996) and it is therefore suitable to detect susceptibility genes for infantile autism as the disorder is most likely oligogenic. We have only included cases with both parents available in order to reduce possibilities of bias when one parent is missing (Curtis and Sham, 1995).

For the 1-bp deletion polymorphism we did not find preferential transmission of any allele. Because only 23 of the parents in the present study were heterozygous, the power to detect linkage disequilibrium was limited. Similarly, no transmission disequilibrium of the 4-bp deletion was found based on 49 heterozygous parents. Finally, no transmission disequilibrium was found for the haplotype

based on 68 heterozygous parents. Moreover, considering paternal and maternal haplotype transmissions simultaneously, no linkage disequilibrium was found. Conclusively, the present results do not show preferential transmission of any of the variants of the *DDC* gene, suggesting that these variants are not important risk alleles in infantile autism in our data set.

ACKNOWLEDGEMENTS

The authors wish to thank psychologist Lennart Pedersen, National Centre for Autism, Copenhagen, for performing the diagnostic evaluations. Also, Torben Mikkelsen and Anette B. Kvistgaard, Institute of Human Genetics, Aarhus, are thanked for excellent laboratory work. This study was supported by grants from Assistance Publique-Hôpitaux de Paris (CRC and PHRC to M.L.), Cure Autism Now Foundation (to C.B.), Fondation pour la Recherche Médicale (to A.P.), The Danish Medical Research Council (grant no. 9902685 to A.D.B.) and Hermansens Mindelegat (to M.B.L. and H.E.).

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TABLE I. Allele, Genotype and Haplotype Distribution of Two Deletion Variants in the *DDC* Gene Among Patients With Infantile Autism

	All patients (n=90)	Danish patients (n=17)	French patients (n=73)
Alleles			
1-bp deletion			
1-bp del	13	3	10
WT	167	31	136
4-bp deletion			
4-bp del	34	4	30
WT	146	30	116
Genotypes[a]			
1-bp deletion			
WT/WT	77	14	63
WT/1-bp del	13	3	10
1-bp del/1-bp del	0	0	0
4-bp deletion			
WT/WT	58	13	45
WT/4-bp del	30	4	26
4-bp del/4-bp del	2	0	2
Two-locus genotypes			
WT WT/WT WT	48	12	36
1-bp del WT/WT WT	10	1	9
WT 4-bp del/WT WT	27	2	25
WT 4-bp del/ WT 4-bp del	2	0	2
WTWT/1-bp del 4-bp del	3	2	1

[a]The alleles or haplotypes in a genotype are separated by / and the 1-bp deletion polymorphism is mentioned first.

TABLE II. Transmission of Alleles and TDT for the 1-bp and 4-bp Deletion Variants in the *DDC* Gene*

1-bp deletion	Not transmitted		
Transmitted	WT	1-bp del	
WT	155	12	
			TDT χ^2 = 0.04, d.f. = 1, p = 0.84
1-bp del	11	2	

4-bp deletion[a]	Not transmitted		
Transmitted	WT	4-bp del	
WT	116	29	
			TDT χ^2 = 1.65, d.f. = 1, p = 0.20
4-bp del	20	13	

*TDT is calculated as $\chi^2 = (T-NT)^2/(T+NT)$, 1 d.f., where T is number of transmitted alleles and NT is number of non-transmitted alleles.

[a]One trio was uninformative with respect to transmission of the parental alleles of the 4-bp deletion and therefore excluded from the TDT.

TABLE III. Transmission of Haplotypes and ETDT for Two-Locus Genotypes

	WT/WT	1-bp del/WT	WT/4-bp del	1-bp del/4-bp del
Transmitted	36	11	21	0
Not transmitted	26	12	30	0

* Allele-wise TDT = 1.79, 2 d.f., p = 0.41; genotype-wise TDT = 1.87, 3 d.f., p = 0.60. The 1-bp deletion polymorphism is mentioned first.