Dopamine DRD2 Taq I polymorphism associates with caudate nucleus volume and cognitive performance in memory impaired subjects

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We studied the relationship among dopamine receptor D2 (DRD2) Taq I genetic polymorphism, caudate nucleus volumetry as measured using MRI and neuropsychological functions in 49 memory impaired older people. Compared with DRD2 AI carriers, subjects homozygous for the DRD2 A2 allele performed poorer in a measure of general cognitive functioning (MMSE) and in long term verbal memory, and presented reduced left caudate nucleus volumes. Caudate nucleus atrophy correlated with cognitive measures influenced by the genetic polymorphism and with visual memory performance. Our findings suggest that among the aged with cognitive impairments, the homozygous status for the A2 allele of the DRD2 Taq I polymorphism is associated with diminished cognitive performance and increased atrophy in the striatum. *NeuroReport* 13:1121–1125 © 2002 Lippincott Williams & Wilkins.

Key words: Aging; Cognitive; Genetics; Dopamine; Caudate

INTRODUCTION

The highest concentrations of the D_2. dopamine receptor (DRD2) are found in the caudate nuclei. The *Taq I* genetic polymorphism of the DRD2 gene characteristically presents two alleles named A1 and A2. Previous reports have suggested that this polymorphism may influence certain brain characteristics: *in vivo* and *in vitro* studies have shown that normal subjects with the A1 allele exhibit low DRD2 density and few binding sites in the striatum [1–3]. Additionally, humans who carry the A1 allele showed low relative glucose metabolic rates in brain regions that are rich in dopamine receptors and participate in a variety of complex cognitive and motivational processes [4].

No study is available addressing a possible effect of this polymorphism on cognitive performance and cerebral characteristics in the aged. The dopaminergic system is involved in a number of cognitive functions (in particular the frontal lobe executive functions) which depend on the integrity of the fronto-striate circuitry [5]. These functions are among the first to decline with advancing age in normal elders [6] and are compromised in elders with memory impairment [7]. Molecular genetic investigations have reported that a few allelic variants are associated with the lower range of cognitive performance and increased atrophic brain changes among the aged. For example, the apolipoprotein E (APOE) E4 allele has been related to decreased memory functioning [8,9] and reduced medial temporal lobe structure [10], while the apolipoprotein CI A allele was found to be more frequent in patients presenting hippocampal sulcal cavities and cognitive impairment [11,12].

In this study we investigated the relationship between cognitive functioning and caudate nuclei volumes as measured by MRI in a sample of subjects presenting memory decline who had been genetically assessed for the DRD2 TaqI polymorphism. Given the high density of the DRD2 receptor in the caudate nuclei and the reported influence of dopaminergic system on cognitive functions, we hypothesized that genetic variations in this polymorphism could modulate neuropsychological performance and brain characteristics in these elders.

MATERIALS AND METHODS

Subjects: For the present study we recruited 49 subjects fulfilling the criteria for age-associated memory impairment [13] and for questionable dementia on the basis of a score of 0.5. in the Clinical Dementia Rating Scale (CDR). These persons presented memory complaints and objective memory decline according to standardized neuropsychological tests but did not fulfill criteria for dementia and did not present any other known neuropsychiatric condition (for example, depression) which might have affected cognitive functioning. The procedures used to exclude dementia or other medical conditions are described elsewhere [11]. All subjects gave informed consent to participate in the study

which was approved by the local ethics committee. Sample characteristics are presented in Table 1.

Neuropsychological assessment: Memory performance was assessed by means of two verbal memory tests (Rey Auditory Verbal Learning test (RAVLT) and Logical Memory, Wechsler Memory Scale Revised (WMS-R)) and two visual memory tests (Visual Reproduction and Paired Visual Associates, WMS-R). Immediate and long-term free recall (30 min delay) measures were obtained from all memory assessments. Frontal lobe executive tests included the Trail Making Test (TMT, forms A and B), the Controlled Oral Word Association Test (COWAT, letters F, A and S, 1 min), Category Fluency (Animals 1 min) as well as the Tower of Hanoi Test. Finally, since the DRD2 polymorphism has been associated with visuospatial performance we used the judgment of line orientation (JLO) test to assess this cognitive domain [14].

MRI examinations: MR scans were acquired on a 1.5 T Sigma (General Electric, Milwaukee, WI). The protocol included axial T2Wdual FSE (4000TR/20-100TE/1nex/3 mm slice thickness) and coronal 3D (SPGR 300TR/min full TE/20 flipα/1nex/1 mmslice thickness reconstruction), field of view 24 $\times$ 24 and matrix 256 $\times$ 256.

Image processing: All image processing was performed by the same research fellow (JM. S-G) who was blinded to all clinical information. The region of interest containing the caudate nucleus was manually traced, and its volume automatically calculated by means of the ANALYZE v3.0 software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN). Medial and lateral limits of the caudate nucleus were defined as the presence of the lateral ventricle and of the anterior parts of the internal capsule respectively. Superior boundary of the caudate nucleus was clearly identifiable by the presence of white matter. The inferior limit of caudate nucleus was defined as the presence of the nucleus accumbens. This boundary was the most difficult to determine, since caudate and accumbens nuclei are very close ventromedially. To overcome these difficulties, we used the Orthogonal sections tool of ANALYZE. This tool gives the co-ordinates in any spatial localisation simultaneously in coronal, sagital and transversal views. In a coronal view, the caudate nucleus was delimited from the accumbens tracing a horizontal line from the more basal limit of lateral ventricle. This can be considered a conservative criteria, since a little portion of caudate region was excluded from the ROI to avoid the inclusion of parts of the accumbens. In more posterior coronal slices, caudate nucleus was easily distinguishable from other structures.

For each subject, caudate volumes were adjusted for his or her whole brain volume obtained using a voxel-by-voxel comparison procedure from T2 weighted images with the Statistical Parametric Mapping software (SPM99, Wellcome Department of Cognitive Neurology, London). MRI acquisitions were transformed into standard MNI (Montreal Neurological Institute) space using an automated spatial normalization (12-parameter affine transformation followed by nonlinear iterations using $7 \times 8 \times 7$ basis functions), using the SPM-T2 template image and the sinc interpolation ($9 \times 9 \times 9$) option to adapt them to a standardised space, with equal orientation (anterior commisure– posterior commisure), equal number of slices ($79 \times 95 \times 68$) and equal size of voxel ($2 \times 2 \times 2$). The normalised whole-brain images were automatically segmented into separate images representing probability maps for grey matter, white matter, and CSF using the combined pixel intensity and *a priori* knowledge approach integrated in SPM99, and supplemented by the lots of inhomogenety corrections option. We used an automatic Matlab routine to obtain volumetric measurements of the above mentioned three tissue compartments. Total brain measures were obtained by adding the values obtained from white, grey and CSF compartments. The automatic segmentation process used in the present report comprises the whole brain including the cerebellum and the brain stem. Adjustments of caudate volumes were performed according to the following procedure: (caudate volume in mm^3/total brain volume in mm^3) $\times$ 100.

Genetic analyses: Genomic DNA was isolated from peripheral blood leukocytes. DRD2 Taq I genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as described previously [15]. By using this method the A1 allele is characterized by a failure to digest with Taq I resulting in a fragment of 310 bp whereas the A2 allele is digested into 130 bp and 180 bp fragments. Among the 49 subjects, we found 21 subjects carrying the A1 allele, who were compared to 28 cases homozygous for the A2 allele.

Table 1. General characteristics of the sample of the 49 subjects studied. Values are expressed in means $\pm$ s.d.

Age	65.73 $\pm$ 9.23
Gender (% women)	69.4
Years of formal education	6.86 $\pm$ 4.01
Inferred QI	12.39 $\pm$ 3.79
MMSE	27.24 $\pm$ 1.39
Depression	5.50 $\pm$ 3.58

Inferred QI: score obtained in the Vocabulary subtest of the Wechsler Adult Intelligence Scale Revised, a score $\geq$ 9 is indicative of normal general intellectual functions. MMSE: Mini mental state examination. Depression: score obtained in the Hamilton's depression scale, the cutoff score for depression is 13.

RESULTS

Subjects carrying the DRD2 A1 allele were comparable to those DRD2 A2 homozygous in terms of age ($t = 0.95$, $p < 0.35$), years of formal education ($t = 0.75$, $p < 0.46$), premorbid inferred intelligence (Vocabulary scores from the WAIS-R, $t = 0.48$, $p < 0.64$), depression scores (Hamilton's depression scale, $t = 0.85$, $p < 0.39$) and gender distribution ($\chi^2 = 0.128$; $p < 0.72$).

A1 carriers exhibited larger brain-corrected left caudate volumes than A2 homozygous subjects (Fig. 1). Right caudate values (mean $\pm$ s.d.) were also larger for A1 carriers (A1 carriers 3121.54 $\pm$ 432.7; A2 homozygous 2896.84 $\pm$ 417.1) but the difference did not reach statistical significance after adjusting for whole brain volume ($t = 1.80$, $p < 0.08$). Caudate size asymmetry did not differ between the two genetic subgroups ($t = 0.21$, $p < 0.83$). To investigate whether the effect of DRD2 polymorphism on brain

morphology was specific for caudate nucleus or it also influenced other brain regions, subjects carrying the DRD2 A1 allele were further compared to A2/A2 homozygous on volumetric measurements of frontal lobe and hippocampus adjusted for whole brain volumes. These two regions were selected because previous evidence indicated that AMAE subjects exhibit neuropsychological or neuroanatomical abnormalities in both brain structures [7,11]. No significant differences could be found for any of these brain areas in relation to the DRD2 genotype status (frontal lobe $t = 1.11$, $p < 0.27$; left hippocampus $t = 0.83$, $p < 0.41$; right hippocampus $t = 1.5$, $p < 0.14$).

Direct comparison of genetic groups on neuropsychological tests indicated that the presence of the A1 allele was related to better performance in RAVLT long term recall (A1/A− 7.81 ± 2.34 *vs* A2/A2 5.96 ± 2.46; $t = 2.66$, $p < 0.01$) and in the Mini Mental Status Examination (A1/A− 27.71 ± 1.55 *vs* A2/A2 26.89 ± 1.17; $t = 2.12$, $p < 0.04$).

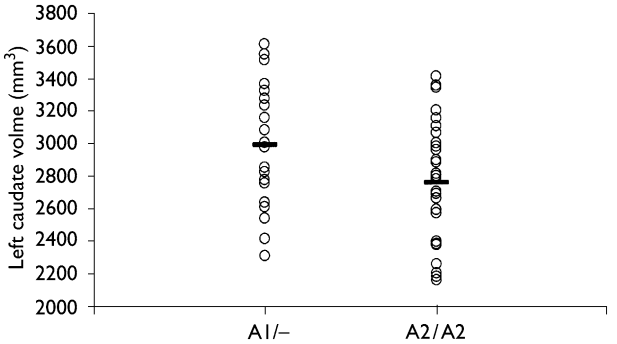

Fig. 1. Differences in left caudate nuclei volumetry between subjects carrying the A1 allele of the DRD2 *Taq I* polymorphism compared to those A2 homozygous. Black horizontal bars indicate mean volumes (mean difference: $t = 2.12$; $p < 0.04$).

Caudate volumetry correlated positively with several neuropsychological functions (Table 2). In particular, left caudate volume, which presented statistically significant differences between the genetic subgroups, correlated positively with the cognitive functions that presented differences according to DRD2 polymorphism (i.e. long term RAVLT and MMSE).

To identify the best predictors of cognitive performance in our subjects we performed a multiple regression analyses. Multiple regression analyses considers all independent variables simultaneously and allows to study the relationship between several independent or predictor variables and a dependent criterion variable. We used a forward stepwise regression approach in which independent variables are entered one after another to analyse how much each one adds to the explanation of the dependent variable (cognitive status). In the analyses we included demographic variables (age, gender, years of formal education, inferred premorbid intelligence), depression scores (Hamilton's depression scale), the DRD2 genetic polymorphism and the corrected caudate.

As can be seen from Table 3, age, years of formal education and estimated intelligence scores were strongly related to cognitive performance. However, both DRD2 Taq I polymorphism and caudate measurements explained part of the variance of cognitive scores: the Taq I polymorphism was clearly related to general cognitive function and verbal memory, and caudate measurements to visual memory.

DISCUSSION

Our results suggest that the DRD2 Taq I genetic variation has an influence on caudate brain morphology and

Table 2. Pearson coefficient correlations of right and left caudate volumes and neuropsychological performance in cognitively impaired subjects.

	Right caudate	Left caudate
MMSE	0.23	0.29*
Memory		
Verbal		
Immediate RAVLT	0.13	0.13
Delayed RAVLT	0.21	0.30*
Immediate logical memory	0.12	0.12
Delayed logical memory	0.01	0.14
Visual		
Immediate visual reproduction	0.37**	0.41***
Delayed visual reproduction	0.35**	0.41***
Immediate visual paired associates	0.22	0.15
Delayed visual paired associates	0.23	0.23
Frontal lobe		
Trail Making Test-A	−0.43**	−0.45***
Trail Making Test-B	−0.31*	−0.26
COWAT	0.34*	0.34*
Category fluency	0.19	0.24
Tower of Hanoi	−0.16	−0.22
Visuospatial function		
Judgement of line orientation	−0.36**	−0.27

RAVLT: Rey Auditory Verbal Learning Test; COWAT: Controlled Oral Word Association Test. In Trail Making Tests and in Tower of Hanoi test higher scores (time) indicate worse performance. *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$.

Table 3. Neuropsychological variables predicted by the multiple regression analyses model including demographic, genetic and volumetric brain measures.

	Regressors	β	t	Sign
MMSE	Inferred IQ	0.50	4.02	0.0002
	DRD2 polymorphism	0.35	2.78	0.007
Immediate RAVLT	Age	−0.44	−3.36	0.002
Delayed RAVLT	Age	−0.43	−3.47	0.001
	DRD2 polymorphism	0.29	2.33	0.02
Immediate logical memory	Inferred IQ	0.39	3.16	0.003
	Age	−0.37	−3.03	0.004
Delayed logical memory	Inferred IQ	0.46	3.80	0.0004
	Age	−0.47	−3.78	0.0005
	Right caudate volume	−0.29	−2.35	0.02
Visual				
Immediate visual reproduction	Left caudate volume	0.35	2.78	0.007
	Education	0.35	2.77	0.008
Delayed visual reproduction	Left caudate volume	0.38	2.95	0.005
	Education	0.27	2.03	0.04
Immediate visual paired associates	Inferred IQ	0.43	3.35	0.002
Delayed visual paired associates	Inferred IQ	0.34	2.58	0.01
	Age	−0.28	−2.01	0.04
Frontal lobe				
Trail Making Test-A	Inferred IQ	−0.44	−3.60	0.0008
	Age	0.34	2.77	0.008
Trail Making Test-B	Age	0.42	3.56	0.001
	Inferred IQ	−0.35	−2.88	0.007
	Gender	0.31	2.64	0.01
	Depression	0.24	2.03	0.05
COWAT	Inferred IQ	0.64	6.80	0.00001
	Gender	0.38	4.14	0.0002
	DRD2 polymorphism	0.25	2.71	0.009
Category fluency	Age	−0.44	−3.41	0.002
	Gender	0.35	2.72	0.009
	Depression	−0.26	−2.03	0.05
Visuospatial function				
Judgement of line orientation	Gender	0.45	3.42	0.001

RAVLT: Rey Auditory Verbal Learning Test; COWAT: Controlled Oral Word Association Test.

neuropsychological performance in cognitively impaired elders. We found that subjects with questionable dementia homozygous for the A2/A2 allele exhibit reduced left caudate volumes as well as diminished performance in verbal memory and general cognitive ability (i.e. MMSE). Additionally, our results indicate that the effect of DRD2 polymorphism on brain morphology do not extend to other brain regions that are probably relevant to the neurobiology of the AAMI condition such as the hippocampus and the frontal lobes [7,11].

Volumetric reductions and pathological changes in the striatum are observed with advancing age [16,17] and are especially evident in neurodegenerative conditions associated with cognitive impairment such as Alzheimer's disease [18]. We found that the variables associated with reduced caudate volumetry were low cognitive performance and the homozygosity for the A2 allele. One of the mechanisms by which the DRD2 gene influences caudate morphology may be by modulating the receptor density in this structure. In animal models a loss of DRD2 receptors has been shown in the striatum in aged rats [19]. Knock-out studies of the DRD2 gene showed that it plays a role in regulating terminal density in the dorsal striatum after lesions in the substantia nigra pars compacta [20]. In humans there is evidence that the DRD2 A1 allele is associated with lower receptor density in the striatum [2,3]. Unfortunately, none of these reports studied whether the changes in receptor density correlated with variations in macroscopic brain morphology. Taking previous and present results together, our findings suggest that receptor density is not a valid indicator of the macroscopic differences in these brain regions, as detected by means of MRI volumetry, since we found that it was the absence of the A1 allele, not its presence, that was associated with reduced caudate volumes. However, these assumptions should be considered as merely speculative until direct evidence on the issue is available.

We found that subjects bearing the A1 allele exhibit better performance in long term verbal memory and a measure of general cognitive function. The A1 allele of the DRD2 Taq I polymorphism has been previously associated with a variety of behaviors or neuropsychiatric conditions such as alcoholism, smoking, cocaine consumption, pathological gambling or attention deficit hyperactivity disorder [21]. However, the possible association of this polymorphism with cognitive functioning in humans remains inconsistent, and has only been tested in children. Berman *et al*. [22] first indicated that boys aged 10–14 carrying the A1 allele performed worse on a visuospatial task than A2 children. However, a recent study comparing high-IQ children (mean 136) with average-IQ children (mean 105) found no

significantly different allelic or genotype frequencies between groups [23].

To our knowledge this study is the first attempt to relate this polymorphism to cognitive performance using a battery of neuropsychological tests to evaluate general cognitive function, memory, frontal lobe-executive measures and the visuospatial function. Former studies proved that the dopaminergic system is critically involved in several cognitive functions including learning and memory [24]. Our data indicate that at least part of the influence of the DRD2 Taq I polymorphism on cognition is related to the modulatory effect of this gene on caudate atrophy.

We found that caudate morphometry predicted visual memory scores even when demographic and genetic variables were also included in the model. Our results suggest a compromise of visual memory functions associated with increased caudate atrophy. These findings are in accordance with previous results in the aged demonstrating that visual memory is severely impaired in degenerative disorders of the basal ganglia such as Huntington's disease [25]. Other findings in patients who underwent surgery due to ateriovenous malformations also reinforce the association of caudate nucleus with cognitive impairment including visual memory [26].

CONCLUSION

Findings from the present report indicate that among subjects presenting cognitive impairment, those homozygous for the A2 allele of the DRD2 Taq I polymorphism exhibit reduced cognitive performance and increased atrophic changes in the caudate nuclei according to MRI measurements. Some of the cognitive influence of the polymorphism on cognitive functioning may be explained by the genetic effects on the morphology of the striatum. Further studies using other methodological approaches should help to clarify the molecular and neurobiological characteristics underlying this association.

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