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Failure to replicate an association between a rare allele of a tyrosine hydroxylase gene microsatellite and schizophrenia

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Abstract An association between schizophrenia and a rare perfect ten-repeat allele, $K1_p$, of a tetranucleotide microsatellite polymorphism in the tyrosine hydroxylase gene has recently been reported. The rare allele was found only in schizophrenic patients. During treatment with antipsychotic drugs patients with the rare allele displayed lower plasma homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) levels than those without. We examined Swedish schizophrenic patients (n = 117) and healthy control subjects (n = 76) for the same polymorphism. In contrast to the previous studies, the $K1_p$ frequency in patients (4 of 117) tended to be lower than among controls (9 of 76). With all six alleles ($K1_p$, $K1_i$, K2–5) considered there was a significant difference between schizophrenic patients and control subjects. There was no significant difference in HVA and MHPG levels in cerebrospinal fluid from a subset (n = 64) of control subjects with and without the rare allele. The discrepant results warrant further investigation of the tyrosine hydroxylase gene.

Key words Schizophrenia · Association study · Tyrosine hydroxylase gene · Cerebrospinal fluid · Homovanillic acid · 3-methoxy-4-hydroxyphenylglycol · 5-hydroxyindoleacetic acid

Introduction

Tyrosine hydroxylase (TH) is the rate limiting enzyme regulating the synthesis of the catecholamines dopamine and noradrenaline in the brain (Nagatsu et al. 1964). An association between schizophrenia and a rare allele of a TH tetranucleotide repeat polymorphism has recently been reported (Meloni et al. 1995). This polymorphism, giving rise to six different alleles, is located in intron 1 of the TH gene and may be involved in its regulation and transcription (Meloni et al. 1997). In French and Tunisian samples the rare perfect ten-repeat allele ($K1_p$) was found only among schizophrenic patients in a case-control study (Meloni et al. 1995). Also in a British sample the rare $K1_p$ allele was only present in schizophrenic patients (P. McGuffin, pers. commun.). The French patients were also studied with regard to plasma levels of the dopamine and noradrenaline metabolites homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) during neuroleptic treatment. Patients with the $K1_p$ allele displayed lower HVA and MHPG levels than patients without this allele (Thibaut et al. 1997).

Previously, we examined Swedish schizophrenic and control subjects for the same TH polymorphism. A tentative negative association was found between the K1 allele and schizophrenia (Jönsson et al. 1996a). Tentative associations indicated lower HVA and MHPG concentrations in cerebrospinal fluid (CSF) in subjects with the K1 and K3 alleles, respectively (Jönsson et al. 1996b). However, the method used did not discriminate the rare $K1_p$ allele from a common imperfect allele ($K1_i$), differing in length by only one base pair. Therefore, in the present study the DNA sequence for the polymorphic region was reexamined in cases and controls with the K1 allele.

Subjects and methods

Swedish patients treated for schizophrenic symptoms (n = 139; 118 meeting DSM-III-R criteria for schizophrenia; 21 with other diagnoses, mainly schizophreniform disorder, schizoaffective disorder, psychosis not otherwise specified and schizotypal personality disorder) and control subjects (n = 108; 78 without any lifetime DSM-III-R disorder; 30 with DSM-III-R diagnoses, mainly alcohol abuse/dependence, depressive and anxiety diagnoses) were assessed through personal interviews and case note examinations as previously described (Jönsson et al. 1996a; Jönsson 1997). Sixty-

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six of the controls had previously been examined for concentrations of monoamine metabolites in the CSF (Jönsson et al. 1996b).

DNA available from subjects with the K1 ($K1_p$+$K1_i$) alleles (n = 138; 64 patients and 74 controls) were sequenced with an automated Laser Fluorescent Applied Biosystems. DNA sequencer (Perkin Elmers, Stockholm, Sweden) with AutoRead 1000 sequencing kit (Pharmacia Biotech, Sollentuna, Sweden) for this locus with the following PCR primers: CAGCTGCCCTAGTCAGCAC (TCAT strand) and GCTTCCGAGTGCAGGTCACA (AGTA strand).

The allele frequencies were compared by χ^2-test using Yates' correction for 2×2 tables. Control subjects with or without the TH $K1_p$ polymorphism were compared (unpaired t-test) for levels of HVA, MHPG, and 5-hydroxyindoleacetic acid (5-HIAA) in CSF. Power was estimated with the program GPower (Erdfelder et al. 1996).

Results

The DNA sequence was successfully estimated in 136 individuals. Four of 117 schizophrenic patients were found to have the $K1_p$ allele, whereas 9 of the 77 control subjects carried this marker (Table 1). All were heterozygotes for the $K1_p$ marker. When the distribution was compared for all six alleles between schizophrenic patients and control subjects without lifetime DSM-III-R diagnosis, there was a statistically significant difference, with a tendency for lower $K1_p$ frequency in patients as compared with controls (Table 1; $\chi^2 = 11.27$, $df = 5$, $p = 0.046$). When all patients and controls were compared a statistically significant overall difference in allele distribution emerged, with lower $K1_i$ and a trend for lower $K1_p$ frequencies in patients (Table 1; $\chi^2 = 12.90$, $df = 5$, $p = 0.024$).

Healthy control subjects with (n = 8) or without (n = 56) the $K1_p$ allele did not differ significantly with regard to CSF levels of HVA ($t = 0.99$, $df = 62$, $p = 0.326$), MHPG ($t = 1.20$, $df = 62$, $p = 0.235$), or 5-HIAA ($t = 1.52$, $df = 62$, $p = 0.134$). Correction for back length, a confounding variable in the measurement of monoamine metabolites in CSF (Jönsson et al. 1996b), failed to change the results (data not shown).

Table 1 Tyrosine hydroxylase allele frequencies (%) and counts (in parentheses) in psychotic patients and control subjects

Allele[a, b]	Patients (n = 138)[b]		Control subjects (n = 106)[b]	
	DSM-III-R schizophrenia (n = 117)[a]	Other psychosis diagnosis (n = 21)	No DSM-III-R diagnosis (n = 76)[a]	Any DSM-III-R diagnosis (n = 30)
$K1_p$[c, d]	1.7 (4)	2.4 (1)	5.9 (9)	3.3 (2)
$K1_i$[e, f]	25.2 (59)	21.4 (9)	32.2 (49)	36.7 (22)
K2	17.5 (41)	21.4 (9)	13.8 (21)	11.7 (7)
K3	10.3 (24)	11.9 (5)	5.3 (8)	8.3 (5)
K4	19.2 (45)	21.4 (9)	21.7 (33)	18.3 (11)
K5	26.1 (61)	21.4 (9)	21.1 (32)	21.7 (13)

[a] $\chi^2 = 11.27$, $df = 5$, $p = 0.046$, for the comparison of allele distribution between DSM-III-R schizophrenic patients and control subjects without any DSM-III-R diagnosis

[b] $\chi^2 = 12.90$, $df = 5$, $p = 0.024$, for the comparison of allele distribution between all patients and all control subjects

[c] $\chi^2 = 3.81$, $df = 1$, $p = 0.051$ (Yates' corrected), for the comparison of the $K1_p$ allele vs the other alleles between DSM-III-R schizophrenic patients and control subjects without any DSM-III-R diagnosis

[d] $\chi^2 = 3.31$, $df = 1$, $p = 0.069$ (Yates' corrected), for the comparison of the $K1_p$ allele vs the other alleles between all patients and all control subjects

[e] $\chi^2 = 1.92$, $df = 1$, $p = 0.166$ (Yates' corrected), for the comparison of the $K1_i$ allele vs the other alleles between DSM-III-R schizophrenic patients and control subjects without any DSM-III-R diagnosis

[f] $\chi^2 = 4.19$, $df = 1$, $p = 0.034$ (Yates' corrected), for the comparison of the $K1_i$ allele vs the other alleles between all patients and all control subjects

Discussion

In the present study, a significant difference in overall tyrosine hydroxylase allele distribution was found between psychotic patients and control subjects, indicating lower frequencies of the rare $K1_p$ allele as well as the common $K1_i$ allele in patients. These results are at variance with results in French, Tunisian, and British populations (Meloni et al. 1995; P. McGuffin, pers. commun.) where the rare $K1_p$ allele was present only in schizophrenic patients. Here, the $K1_p$ allele was present in a substantial number of control individuals. Since the Swedish population is ethnically different from the French, Tunisian, and British populations, it cannot be excluded that the rare $K1_p$ allele may reflect vulnerability for schizophrenia in the latter populations. However, the contradictory results strongly argue against the possibility that the $K1_p$ allele alone is a sufficient factor to cause schizophrenia. The different results instead suggest chance findings, either in the two previous, in the present, or in all three studies. The power to detect an allelic association ($K1_p$ vs other alleles; $\alpha = 0.05$, effect size $w = 0.139$, $df = 1$) was 0.86 in the French sample 0.46 in the Tunisian sample, and 0.78 in the present study. Another remote possibility is that the absence or presence of the $K1_p$ allele reflects susceptibility to different subtypes of schizophrenia, eventually conferred through a functional polymorphism in linkage disequilibrium with the one investigated.

Schizophrenic patients with the rare TH $K1_p$ allele have been found to have significantly lower levels of plasma HVA and MHPG during neuroleptic treatment (Thibaut et al. 1997). We did not find statistically significant differences in CSF monoamine metabolites when healthy controls subjects with or without the TH $K1_p$ allele were compared. Thus, the present results do not support the view that the TH $K1_p$ allele, either by itself or through linkage disequilibrium, exerts major effects on the monoaminergic pathways as reflected by their major metabolites in CSF of healthy volunteers. However, it cannot be excluded that the TH $K1_p$ allele influences the monoaminergic pathways in a way more easily detected in schizophrenic patients treated with neuroleptic drugs, or through assessment of monoamine metabolites in plasma. Although HVA in CSF is of plain central nervous origin, it may only reflect the dopamine turnover of a lim-

ited region of the brain (Amin et al. 1992). Therefore, despite the fact that only 25% of plasma HVA originates from central dopaminergic neurons, assessment of HVA in plasma may reflect a more overall contribution of dopamine turnover in the brain. The power to detect a difference of "large" effect size ($d = 0.80$; one-tailed $\alpha = 0.05$) comparing the $K1_p$ containing genotypes vs all other genotypes was 0.67 in the present study, indicating also the possibility that the result may be falsely negative. In light of our previous findings of tentative associations between some other TH alleles and CSF HVA and MHPG concentrations (Jönsson et al. 1996b), it still seems possible that TH gene variants influence dopaminergic and noradrenergic pathways enough to alter HVA and MHPG concentrations in CSF of healthy volunteers.

Further studies seem warranted to evaluate the possible association between schizophrenia and the tyrosine hydroxylase gene as well as its potential effect on catecholaminergic pathways in humans.

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