Kynurenine 3-monooxygenase polymorphisms: relevance for kynurenic acid synthesis in patients with schizophrenia and healthy controls

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Background: Patients with schizophrenia show increased brain and cerebrospinal fluid (CSF) concentrations of the endogenous *N*-methyl-D-aspartate receptor antagonist kynurenic acid (KYNA). This compound is an end-metabolite of the kynurenine pathway, and its formation indirectly depends on the activity of kynurenine 3-monooxygenase (KMO), the enzyme converting kynurenine to 3-hydroxykynurenine. **Methods:** We analyzed the association between *KMO* gene polymorphisms and CSF concentrations of KYNA in patients with schizophrenia and healthy controls. Fifteen single nucleotide polymorphisms (SNPs) were selected covering *KMO* and were analyzed in UNPHASED. **Results:** We included 17 patients with schizophrenia and 33 controls in our study. We found an association between a *KMO* SNP (rs1053230), encoding an amino acid change of potential importance for substrate interaction, and CSF concentrations of KYNA. **Limitations:** Given the limited sample size, the results are tentative until replication. **Conclusion:** Our results suggest that the nonsynonymous *KMO* SNP rs1053230 influences CSF concentrations of KYNA.

Introduction

In recent years, the general view of the pathophysiology of schizophrenia (i.e., disturbances in dopamine [DA] transmission) has been expanded to also involve a glutamatergic dysfunction of the brain. Thus, clinical observations show that systemic administration of *N*-methyl-D-aspartate (NMDA) receptor antagonists (e.g., phencyclidine [PCP] and ketamine) evokes schizophrenia-like symptoms in healthy individuals and provokes symptoms in patients with schizophrenia.[1–3] Furthermore, the glutamate deficiency theory has gained some support from genetic findings.[4] A hypoglutamatergic state of the brain can also be achieved by elevation of the endogenous NMDA receptor antagonist kynurenic acid (KYNA).[5] Indeed, increased concentrations of KYNA have been found in the cerebrospinal fluid (CSF) and in the postmortem brains of patients with schizophrenia.[6–8] Kynurenic acid is a metabolite of tryptophan (Fig. 1) and acts as an antagonist at the glycine coagonist site and the glutamate recognition site of the NMDA receptor.[9–12] Additionally, KYNA blocks the α7* nicotinic receptor at low concentrations.[13] Elevated levels of KYNA in the rat brain are associated with

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increased midbrain DA firing[14–17] and disrupted prepulse inhibition,[18] a deficit that has also been observed in patients with schizophrenia.[19] In this regard, KYNA has important similarities to other NMDA receptor antagonists.[20,21]

Formation of KYNA indirectly depends on the activity of kynurenine 3-monooxygenase (KMO), the enzyme converting kynurenine to 3-hydroxykynurenine.[22] Thus, pharmacologic inhibition of this enzyme will shunt the metabolism of kynurenine to KYNA. A functional polymorphism of the gene encoding the enzyme KMO, possibly resulting in a reduction of the expression of *KMO* and/or its enzyme activity, may contribute to the elevated levels of KYNA in patients with schizophrenia. The *KMO* gene is located on chromosome 1q42 and, interestingly, several genetic analyses of families densely affected with schizophrenia and schizoaffective disorder have reported linkage to this region.[23,24] Furthermore, genes in this region are suggested to affect susceptibility to these disorders.[25,26] However, to our knowledge, no association between *KMO* polymorphisms and schizophrenia has yet been reported.[27,28]

In the present study, we analyzed whether polymorphisms in the gene encoding the enzyme KMO have an impact on CSF concentrations of KYNA in a Swedish sample of patients with schizophrenia and healthy controls.

Methods

Samples

For association analysis between *KMO* polymorphisms and CSF concentrations of KYNA, we recruited participants with schizophrenia and healthy controls who had been previously included in a case–control study in which our group analyzed *KMO* polymorphisms.[28] Cerebrospinal fluid concentrations of KYNA in both controls and patients with schizophrenia have previously been published.[8,28]

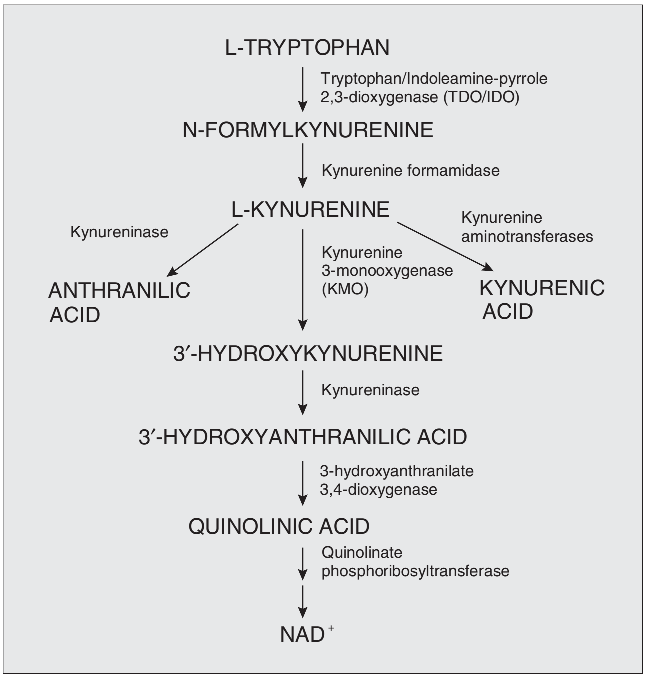

Fig. 1: The kynurenine pathway.

We invited patients with schizophrenia to participate in the study. None of them was subjected to involuntary treatment. We obtained informed consent from patients and controls after providing written and verbal information about the procedure and the purpose of the study. All patients included in the study were competent to give informed consent according to the opinion of psychiatrists familiar with the patients. We recruited healthy controls among age-matched students and hospital staff members. All controls were found to be free from current signs of psychiatric morbidity or difficulties in social adjustment at the time of sampling according to an interview performed by a psychiatrist. Patients and controls included in the present study are those from whom both CSF and blood were collected. The study was approved by the ethical committees of the Karolinska Institutet.

Genotyping

We selected 15 *KMO* single nucleotide polymorphisms (SNPs) spanning 60 kb from the 5′ near gene region to intron 15 for genotyping, including at least 2 in each of the 4 haplotype blocks of the gene (Appendix 1, available at www.cma.ca/jpn), representing gene coverage of 79%.[28] Genomic DNA was extracted from whole blood samples. The selected SNPs were genotyped at the SNP Technology Platform in Uppsala, Sweden (www.genotyping.se) using the Illumina BeadStation 500GX and the 1536-plex Illumina Golden Gate assay (Illumina Inc.). All SNPs were in Hardy–Weinberg equilibrium. The sample success rate was on average 99.4% for the genotyped SNPs, and the reproducibility of the genotyping was 100%, as determined from a sample of 873 broad-spectrum patients with schizophrenia and 1473 unrelated Scandinavian controls, including those enrolled in this study.[28]

Cerebrospinal fluid sampling

We obtained CSF by lumbar puncture after participants had a minimum of 8 hours of observed bedrest and abstained from food and smoking before sampling. For a more detailed description of the procedure, see Nilsson and colleagues[8] and Holtze and colleagues.[28]

Kynurenic acid analysis

We detected KYNA using an isocratic reversed-phase high-performance liquid chromatography (HPLC) system, including a fluorescence detector (Jasco FP-2020) with an excitation wavelength of 344 nm and an emission wavelength of 398 nm (18-nm bandwidth), as previously described.[8] Samples of 25 µL were manually inserted into a Rheodyne injector (Rhonert Park), and the retention time of KYNA was about 13 minutes. The precision of the HPLC method used in the present study was routinely tested within days (intra-assay) and between days (interassay). For the determination of intra-assay precision, aliquots ($n = 10$) of KYNA standards at concentrations of

0.3125 nM and 5 nM were analyzed. The precision of the assay was calculated from the percent coefficient of variation (CV) of the mean, according to the equation CV (%) = (standard deviation ÷ mean) × 100. The CV (%) for 0.3125 nM was 6.44% and that for 5 nM was 1.49%. Interassay precision was calculated by analyzing aliquots of the same KYNA standard (1 nM) on 10 consecutive days. The CV (%) for interassay precision was 2.83%. We measured all samples in a single assay.

Statistical analysis

Cerebrospinal fluid concentrations of KYNA were treated as a quantitative trait and allele associations with *KMO* SNPs were tested in UNPHASED.[29] Back length, age at the time of lumbar puncture, sex and affection state are factors that have previously been associated with CSF concentrations of KYNA.[8,30,31] Consequently, we used these variables as confounders in the analysis. To test whether the allele association differed between individuals with schizophrenia and controls, we treated affection state as a modifier in a separate UNPHASED analysis. The number of individuals homozygous for the minor allele was typically below 4 for each SNP, and thus did not allow for meaningful genotype association tests. Correction for multiple testing was completed using a permutation test in UNPHASED (1000 permutations).

Results

We enrolled 50 individuals from Sweden (17 patients with schizophrenia and 33 healthy controls) in our study. The demographic and clinical characteristics of participants are summarized in Table 1. At the time of lumbar puncture, 3 of the patients were drug-free but had previously received antipsychotic drugs, whereas the remaining patients were prescribed the following neuroleptics: chlorpromazine ($n = 2$), perphenazine ($n = 3$), thioridazine ($n = 2$), raclopride ($n = 1$), cisflupenthixol ($n = 1$), zuclopenthixol ($n = 2$), a combination of clozapine and perphenazine ($n = 1$), a combination of haloperidol and sulpiride ($n = 1$), and a combination of perphenazine and thioridazine ($n = 1$).

We found an association between the *KMO* SNP rs1053230 and CSF concentrations of KYNA (likelihood ratio $\chi^2_1 = 10.0$, $p = 0.002$). The additive value was 1.1 (95% confidence interval 0.34–1.79), and a copy of the T-allele was associated with a 45% increase in CSF concentrations of KYNA (least square means were 1.0 nM for individuals with the CC genotype and 1.49 nM for those with the CT genotype; Fig. 2). This association was observed in both patients and controls and was significant after correction for multiple testing (adjusted $p = 0.023$, empirical 5% quantile = 0.003). Although there was a tendency toward a stronger association in affected individuals (Fig. 2), this difference was not statistically significant ($p = 0.73$ for affection state as modifier).

Table 1: Demographic and clinical characteristics of the study participants

	Group; mean (SD)*	
Characteristic	Schizophrenia	Control
No.	17	33
Sex, % women	17.6	27.3
Age, yr†	33.2 (7.5)	27.9 (9.8)
Age at onset, yr	22.7 (4.2)	—
KYNA, nM	1.4 (0.7)	1.3 (0.6)

KYNA = kynurenic acid; SD = standard deviation.
*Unless indicated otherwise.
†Age at the time of lumbar puncture.

Discussion

We found that the minor allele (T) of the *KMO* SNP rs1053230 was strongly associated with increased CSF concentrations of KYNA. To our knowledge, this is the first study showing an association between a *KMO* SNP and a putative phenotype of schizophrenia (i.e., elevated levels of KYNA concentrations). This SNP is located in exon 15 and results in a shift of the amino acid sequence from arginine to cysteine. The association was evident in both healthy controls and patients with schizophrenia, and it tended to be stronger in patients.

The KMO enzyme is located at the outer membrane of the mitochondria.[32] Although the major part of the enzyme is located inside of the membrane, the *KMO* polymorphism rs1053230 is situated in the part of the gene sequence coding for positions outside of the mitochondria membrane (www.predictprotein.org), likely the site for substrate interaction. Thus, an exchange of amino acids in this part of the enzyme may directly influence substrate binding,[33] for example, affecting the hydropathy index from –4.5 (arginine, the most hydrophilic amino acid) to 2.5 (cysteine, a moderate hydrophobic amino acid). The increased levels of KYNA, seen in individuals with the minor T allele, may thus follow a reduction of kynurenine binding to KMO. In support of this theory, it has been shown that concentrations of brain kynurenine, the precursor of KYNA, are elevated[6,34] and that KMO activity is

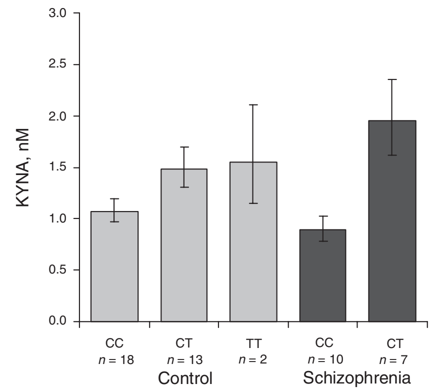

Fig. 2: The concentrations of kynurenic acid (KYNA) increase with the T-allele of rs1053230 ($p = 0.023$ after correction for multiple testing). Least square means and standard errors are given for controls and patients with schizophrenia with the CC, CT and TT genotypes, respectively, adjusting for effects of back length, age and sex.

decreased[35] in patients with schizophrenia. The metabolism of kynurenine would thus be shunted toward KYNA, similar to the outcome of administering pharmacologic compounds that block KMO.[36] The availability of kynurenine is suggested to be the determinant of KYNA synthesis.[22]

One might speculate that the SNP rs1053230 is affecting the function of the KMO enzyme, as it is associated with CSF concentrations of KYNA. The functionality of this SNP is, however, not explored in the present study. An in vitro enzymatic assay, overexpressing the different *KMO* variants, including either the C allele or the T allele of this SNP as the only genetic difference, would have been a desirable approach to analyze functionality. However, since KMO is a mitochondria–membrane bound enzyme, estimating its activity in an artificial environment might be problematic.

Participants in the present study represent a smaller fraction of those included in the study by Nilsson and colleagues,[8] in which CSF concentrations of KYNA were found to be significantly higher in patients than controls. Possibly, the lack of a difference in CSF concentrations of KYNA between patients and controls in the present study was related to the restricted number of samples analyzed, and the less disparate CSF concentrations of KYNA in the 2 groups might mainly be explained by higher KYNA concentrations in controls in the present analysis compared with those controls in the larger study for whom DNA was not available. Despite this limitation, it is of interest to note that the association between the *KMO* (rs1053230) T allele and increased KYNA concentration tended to be stronger in patients compared with controls (Fig. 2). Notably though, *KMO* SNPs per se do not confer major susceptibility to schizophrenia.[28]

Synthesis of KYNA is not only affected by the activity of the enzyme KMO, but is also critically regulated by indoleamine 2,3-dioxygenase (IDO) and/or tryptophan 2,3-dioxygenase (TDO), enzymes responsible for the rate-limiting step of the kynurenine pathway (Fig. 1). Notably, CSF concentrations of KYNA as well as brain IDO and TDO activity are induced during infections or immune activation,[37–39] and numerous studies suggest that brain KYNA is a biologic marker of neuroinflammation.[40,41] In support of an activation of the brain immune system in patients with schizophrenia, the CSF concentration of interleukin-1β, a proinflammatory cytokine, is elevated in patients with first-episode schizophrenia.[42] Indeed, gene expression of TDO and the density of TDO-immunopositive cells are found to be elevated in the postmortem brains of patients with schizophrenia.[43] A change in the *KMO* codon sequence from arginine to cysteine in combination with increased IDO and/or TDO activity may thus be responsible for the elevated KYNA concentrations seen in patients with schizophrenia.[6–8,34,44]

Limitations

One limitation of the present study is the relatively small sample size for a genetic study. To reduce the influence of genetic variation, all participants were white and sampled from the same area of Sweden. Still, replication in additional samples is needed to confirm the relation. Another limitation stems from the use of antipsychotic drugs during CSF sampling among most of the patients. Generally, treatment with antipsychotic drugs should be taken into consideration as a confounding factor when evaluating biologic aberrations in the brains of patients with schizophrenia. However, chronic treatment with antipsychotics in rats has been shown to decrease brain KYNA concentrations,[45] a finding also supported by postmortem findings in patients with schizophrenia.[46] These findings argue against an influence of treatment in the present study. In addition, the observed association between the *KMO* SNP rs1053230 and KYNA concentrations was similar among the larger group of drug-free healthy participants.

Conclusion

The present findings indicate that increased levels of CSF concentrations of KYNA, as previously reported in patients with schizophrenia, are influenced by a nonsynonymous missense polymorphism in *KMO*.

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