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Downregulated Kynurenine 3-Monooxygenase Gene Expression and Enzyme Activity in Schizophrenia and Genetic Association With Schizophrenia Endophenotypes

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Abstract

Context—Kynurenic acid, a metabolite of the kynurenine pathway of tryptophan degradation, is an antagonist at *N*-methyl-D-aspartate and α7 nicotinic acetylcholine receptors and modulates glutamate, dopamine, and acetylcholine signaling. Cortical kynurenic acid concentrations are elevated in the brain and cerebrospinal fluid of schizophrenia patients. The proximal cause may be an impairment of kynurenine 3-monooxygenase (KMO), a rate-limiting enzyme at the branching point of the kynurenine pathway.

Objectives—To examine *KMO* messenger RNA expression and KMO enzyme activity in postmortem tissue from the frontal eye field (FEF; Brodmann area 6) obtained from schizophrenia individuals compared with healthy control individuals and to explore the relationship between *KMO* single-nucleotide polymorphisms and schizophrenia oculomotor endophenotypes.

Design—Case-control postmortem and clinical study.

Setting—Maryland Brain Collection, outpatient clinics.

Participants—Postmortem specimens from schizophrenia patients (n=32) and control donors (n=32) and a clinical sample of schizophrenia patients (n=248) and healthy controls (n=228).

Main Outcome Measures—Comparison of quantitative *KMO* messenger RNA expression and KMO enzyme activity in postmortem FEF tissue between schizophrenia patients and controls and association of *KMO* single-nucleotide polymorphisms with messenger RNA expression in postmortem FEF and schizophrenia and oculomotor endophenotypes (ie, smooth pursuit eye movements and oculomotor delayed response).

Results—In postmortem tissue, we found a significant and correlated reduction in *KMO* gene expression and KMO enzyme activity in the FEF in schizophrenia patients. In the clinical sample,

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KMO rs2275163 was not associated with a diagnosis of schizophrenia but showed modest effects on predictive pursuit and visuospatial working memory endophenotypes.

Conclusion—Our results provide converging lines of evidence implicating reduced KMO activity in the etiopathophysiology of schizophrenia and related neurocognitive deficits.

Impairment of the kynurenine pathway (KP) of tryptophan metabolism has been suggested to play a role in the pathophysiology of schizophrenia and related cognitive deficits.[1-5] The KP generates 3 neuroactive metabolites with purported links to neuropsychiatric diseases.[6-8] These compounds—kynurenic acid (KYNA), 3-hydroxykynurenine, and quinolinic acid—are downstream products of the regulatory enzymes tryptophan 2,3-dioxygenase (TDO), indoleamine 2,3-dioxygenase, and kynurenine 3-monooxygenase (KMO) (Figure 1). The levels of KYNA, an endogenous antagonist of the glycine coagonist ($glycine_B$) site of the glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) and the α7 nicotinic acetylcholine receptor (α7nAChR),[6,9,10] are elevated in the prefrontal cortex and cerebrospinal fluid of schizophrenia patients.[1,11-13] This unique receptor affinity profile of KYNA is particularly interesting when viewed against accumulated evidence implicating NMDAR hypofunction in the pathophysiology of schizophrenia[14-19] and α7nAChR hypofunction in schizophrenia-related cognitive deficits.[20,21] In rats, endogenous KYNA controls extracellular levels of cortical acetylcholine, dopamine, and glutamate[22-24] and the firing rate and burst activity of midbrain dopaminergic neurons.[25] Remarkably, experimentally increased brain KYNA levels in rats induce deficits in visuospatial working memory, contextual learning, sensory gating, and prepulse inhibition of the acoustic startle reflex.[26-29] These neurocognitive deficits are of particular relevance to schizophrenia because they are highly frequent in schizophrenia individuals and their unaffected first-degree relatives and are considered endophenotypes.[30,31] Taken together, these findings suggest that cognitive deficits in schizophrenia could be causally related to elevated cortical KYNA concentrations.[5]

Recent genetic studies provide support for a role for abnormal KP metabolism in the etiopathophysiology of schizophrenia. These studies include the demonstration of upregulated brain *TDO2* (OMIM 191070) messenger RNA (mRNA) (encoding the TDO enzyme) in postmortem tissue from schizophrenia patients[32,33] and in the brains of newborn mice experimentally infected with influenza virus (a relevant animal model of schizophrenia[34,35]), possibly signifying an enhanced ability of the tissue to generate KYNA downstream (Figure 1). Moreover, an association between polymorphisms in the *KMO* gene (OMIM 603538) (which encodes the KMO enzyme) and schizophrenia was demonstrated in a Japanese cohort.[36] This finding was not replicated in an independent sample drawn from the same population,[36] nor was an association with schizophrenia demonstrated in a recent report on a European sample.[37] These findings are of particular interest because the *KMO* gene maps to chromosome 1q42-q44, a region that has shown linkage in schizophrenia samples.[38]

Difficulties replicating genetic findings are not uncommon in schizophrenia, likely due to the polygenic and heterogeneous nature of this complex disorder. This phenotypic and genetic heterogeneity has hindered the search for schizophrenia liability genes using approaches from classical genetics, yielding associations with candidate loci and genes that are rarely replicated.[39] As an alternative to using schizophrenia as an end point, one popular approach is to focus on genes that are directly or indirectly related to neurochemical pathways associated with pathogenesis, thus reducing neurochemical and genetic heterogeneity.[3,40] Another approach aims to decrease phenotypic and genetic heterogeneity by focusing on disease-associated heritable quantitative traits (ie, endophenotypes).[41,42] Using the latter approach, we have studied abnormal smooth pursuit eye movements (SPEMs), an established schizophrenia endophenotype, [43-45] and found that the predictive smooth pursuit component (hereafter referred to as predictive pursuit) is highly

heritable.[31,46,47] A phylogenetically recent function fully preserved only in primates, SPEM (or eye tracking) is a highly developed behavioral response subserved by a known neuronal network[48] and is an important component of the oculomotor response to smoothly moving objects in visual space. In combination with saccadic eye movements, SPEMs capture and maintain the image of a moving object on the fovea as the eyes track it in space and time. In the course of our studies, we have used predictive pursuit to parse differences in small gene effects between schizophrenia groups and healthy control individuals that otherwise would have gone unnoticed using traditional global SPEM measures, clinical diagnosis, or behavioral symptoms.[49,50]

In this study, we integrated these 2 alternative approaches and investigated the KP and the predictive pursuit endophenotype. Initially, we compared *KMO* gene expression and KMO enzyme activity in postmortem brain tissue, focusing on the frontal eye field (FEF; Brodmann area 6). On the basis of neuroimaging data from monkeys and humans, this cortical area is a key region associated with SPEMs, and FEF deficits have been consistently found in schizophrenia patients.[51-56] Comparisons between postmortem FEF specimens from schizophrenia patients and control samples revealed a significant and correlated downregulation of *KMO* gene expression and KMO enzyme activity in the tissues from the former compared with the latter. Subsequently, we examined the association of 2 *KMO* single-nucleotide polymorphisms (SNPs) with gene expression in the FEF tissues and 2 schizophrenia oculomotor endophenotypes in a clinical sample.

METHODS

BRAIN TISSUE FOR GENE EXPRESSION AND BIOCHEMICAL STUDIES

Human postmortem brains (n=64 total), stored at −80°C, were obtained from the Maryland Brain Collection (http://www.mprc.umaryland.edu/mbc.asp). The brains were slowly warmed to −20°C, and the FEF was removed via dissection with a Stryker bone saw from the location denoted by Rosano and colleagues.[57]

Specimens were obtained from 32 schizophrenia donors (15 had taken first-generation antipsychotic medications, 5 had taken second-generation antipsychotics, 2 had taken combined first-generation antipsychotics and second-generation antipsychotics, 5 had not taken antipsychotics for 6 months or more before death, and 5 were of unknown antipsychotics status at the time of death). Normal control specimens (n=32) were from individuals with no history of psychosis, mood disorders, drug dependence, psychiatric treatment, or hospitalization, as determined by medical examiner documentation and telephone screening of the next of kin. Specimens were selected as case-control pairs and were matched on sex, age, postmortem interval, pH, hemisphere, and freezer storage time (Table 1). Clinical information was obtained through family interviews, including the Structured Clinical Interview for *DSM-IV* Axis I Disorders (SCID-IV). Senior psychiatrists performed best-estimate diagnoses using the Diagnostic Evaluation After Death[58] in conjunction with review of past medical and psychiatric records. Postmortem clinical diagnoses were established according to the method developed by Roberts and colleagues.[59] All specimens were obtained with informed consent of the legal next of kin with approval from the office of the chief medical examiner of the state of Maryland.

ASSOCIATION AND PHENOTYPIC STUDY PARTICIPANTS

Schizophrenia individuals (n=248) were recruited from outpatient research programs at the Maryland Psychiatric Research Center and Baltimore area mental health centers (Table 2). Enrollment was based on consensus diagnosis of schizophrenia derived from all available information, including past medical and psychiatric histories and the SCID-IV (patient

version).[60] We attempted to match healthy controls (n=228) with the demographic characteristics of our outpatient population via 2 methods. Using public databases, we conducted random telephone screenings to recruit controls by matching them to the age (SD, 3 years), sex, ethnicity, and zip code of recruited patients. The zip code was determined by the residence at the time of the patient's first psychotic episode. Alternately, if zip codes were unavailable, we used targeted local community media advertisements based on patients' county of residence. The design was intended to re-create, in the controls, the environment in which patients resided during the premorbid period that led to the first psychotic break. Thus, comparison subjects were drawn from similar geographic areas as the patient group. Because many neurophysiologic measures may be affected by age, particularly in individuals 60 years or older,[61] endophenotypic studies were restricted to individuals between ages 18 and 58 years. Healthy controls did not meet *DSM-IV* criteria for Axis I disorders (confirmed by SCID-IV, nonpatient version) and had no family history of psychosis based on the Family History Research Diagnostic Criteria interview that extended through 3 generations. Respondents were excluded if they had general medical or neurologic conditions that could affect measurement of oculomotor movements, reported substance dependence within 6 months before study enrollment or current substance abuse, or had mental retardation.

The study sample of 476 individuals included 256 self-identified European Americans and 220 who self-identified as African American/of mixed ethnicity. Completed endophenotypic measurements were available in subgroups as predictive pursuit subcomponent of SPEM (n=306) and oculomotor delayed response (ODR; also known as memory-guided saccade, a visuospatial working memory measure) (n=156).[62] All participants gave written informed consent in accordance with the University of Maryland Institutional Review Board guidelines. In schizophrenia patients, the evaluation of the capacity to sign consent was performed to assess their understanding of the study before signing consent.

LABORATORY PROCEDURES

RNA Isolation and Reverse Transcription—We performed RNA isolation and reverse transcription, as previously described.[50] Briefly, tissues were homogenized and total RNA extracted using TRIZOL Reagent (Life Technologies Corporation, Carlsbad, California), purified using Qiagen RNeasy mini spin columns (Qiagen, Hilden, Germany), and treated with DNase. The quality of the RNA was confirmed by high-resolution capillary electrophoresis (Agilent Bioanalyzer 2100; Agilent Technologies, Santa Clara, California) and assessed by RNA integrity number (RIN), obtained from the entire Agilent electrophoretic trace with RIN software algorithm (on a scale of 1 to 10, with 1 being the lowest and 10 the highest RNA quality).[63] The RNA samples that showed clearly defined, sharp 18S and 28S ribosomal peaks, 28S/18S ratios greater than 1.7, and an RIN of 6.0 or higher were included. Total RNA (1 μg) was used for 20 μL of reverse transcriptase reaction to synthesize complementary DNA (cDNA) (iScript cDNA Synthesis Kit; Bio-Rad Laboratories Inc, Hercules, California).

TaqMan Gene Expression Assays and Real-Time Quantitative Polymerase Chain Reaction—Commercially available assays were used for *KMO* (Hs00175738_m1) and *GAPDH* (Hs99999905_m1) (Gene Expression Assays; Life Technologies Corporation). Expression levels of *KMO* mRNA transcripts were measured with real-time quantitative polymerase chain reaction (PCR) using relative quantitation by comparative C_T assay in an ABI 7900HT Fast System with standard 96-well reaction plates normalized to *GAPDH* (Life Technologies Corporation). The PCR cycle parameters were 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 59°C or 60°C for 1 minute. The PCR data were acquired with the Sequence Detection Software, version 2.3 (Life Technologies

Corporation), and quantified by relative quantitation analysis. Amplification efficiency was quantified by a standard curve method with serial dilutions of pooled cDNA from the FEF of 4 control samples. For all experiments, the R^2 values of the curves, the slopes, and amplification efficiencies were 0.96 to 0.99, −3.48 to −3.78, and 91% to 95%, respectively. Negative controls (ie, those having no-template cDNA) resulted in no detectable signal. All assays were performed in single wells with the target gene and endogenous control genes as triplicates on the same 96-well plate.

KMO Activity—On the day of the assay, tissues were thawed and homogenized (1:5, wt/vol) in ultrapure water. After further dilution (1:5, vol/vol) in 100 mM Tris hydrochloride buffer (pH 8.1) containing 10 mM potassium chloride and 1 mM editic acid, 80 μL of the tissue preparation was incubated for 40 minutes at 37°C in a solution containing 1 mM nicotinamide adenine dinucleotide phosphate, 3 mM glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehydrogenase, 100 μM L-kynurenine, 10 mM potassium chloride, and 1 mM editic acid, in a total volume of 200 μL. Blanks were obtained by including the specific enzyme inhibitor Ro 61-8048 (100 μM; provided by W. Fröstl, MD, Novartis AG, Basel, Switzerland[64]) in the incubation solution. After centrifugation (16 000*g* for 15 minutes), 20 μL of the supernatant was applied to a 3-μm high-performance liquid chromatography column (HR-80; 80×4.6 mm; ESA Biosciences Inc, Chelmsford, Massachusetts), using a mobile phase consisting of 1.5% acetonitrile, 0.9% triethylamine, 0.59% phosphoric acid, 0.27 mM editic acid, and 8.9 mM sodium heptane sulfonic acid and a flow rate of 1.0 mL/min. In the eluate, the reaction product, 3-hydroxykynurenine, was detected electrochemically using an HTEC 500 detector (Eicom Corporation, San Diego, California; oxidation potential, +0.5 V). The retention time of 3-hydroxykynurenine was approximately 11 minutes. The protein content of the samples was measured spectrophotometrically using the method developed by Lowry et al.[65]

Genotyping—The SNP genotyping was performed on genomic DNA isolated with the QIAamp DNA Maxi Kit (Qiagen) from postmortem tissue for the gene expression association studies and from leukocytes obtained from the clinical sample for the endophenotype association studies. Context sequences and assay identification numbers of genotyped *KMO* SNPs are given in Table 3. For each PCR reaction, 2 ng of genomic DNA was used in a 5-μL reaction mixture (1.3 mM magnesium chloride, 200 μM deoxyribonucleotide mix, 0.25 μM of each primer, 5% dimethyl sulfoxide, and 1.5 U Taq polymerase). The PCR program included denaturation of DNA (5 minutes at 94°C), 30 cycles of 30 seconds at 94°C, 30 seconds at 62°C, and 30 seconds at 72°C. This sequence was followed by a final extension of 10 minutes at 72°C. All PCR reactions were performed using the Bio-rad Multiplatform Thermocycler, which has 4 blocks of 384 wells (Bio-Rad Laboratories Inc).

SNP Selection—We selected 2 *KMO* SNPs for analysis: 1 that results in a nonsynonymous change in *KMO* gene sequence (*KMO* rs1053230) and 1 (*KMO* rs2275163) that was previously reported to be associated with schizophrenia.[36] Both SNPs have minor allele frequencies of 0.15 or more in European American and African American/mixed ethnicity populations (http://hapmap.ncbi.nlm.nih.gov/)[66] (Table 4).

PHENOTYPIC MEASURES

Oculomotor Measures—Oculomotor measures were scored by investigators masked to the identity and group membership of study participants. Self-reported smokers were requested to refrain from smoking for at least 1 hour before testing.

Smooth Pursuit Eye Movement Task—In the smooth pursuit task, a target starts from a center fixation and moves back and forth across the screen. Participants were instructed to follow the moving target (velocity of 18.7° per second; amplitude, greater or less than 12°) across the computer monitor with their eyes. After 4 to 6 half-cycles, the target was unpredictably masked (ie, made invisible) for 500 milliseconds. Of the 25 trials presented, 15 had the masking appear at some point during a half-cycle and the rest at the change in ramp direction. Participants were instructed to follow the moving target even when it became briefly invisible. Pursuit gain was defined as the ratio of pursuit eye velocity to target velocity. Pursuit gain measures from the masked period from these 2 types of trials were averaged to obtain a measure of predictive pursuit gain. The scoring algorithm of the measures has been fully described elsewhere.[46]

Oculomotor Delayed Response Task—In this task, participants fixated on a centrally located set of crosshairs. A target was flashed for 250 milliseconds between 2.5° and 10.0° to the left or right of the central fixation point. Participants continued to fixate until the fixation cross was turned off 10 seconds after the target was briefly shown. With this offset of the center crosshairs, participants were instructed to move their eyes to the location where the target had flashed. Feedback was provided by a small circle that appeared after 1.5 seconds, indicating where the target had flashed at the beginning of the trial. Absolute error in degrees was the primary dependent measure.

Statistical Analysis

Postmortem Brain: KMO mRNA Expression, KMO Activity, and SNP Analyses: Measurements of *KMO* mRNA expression and KMO enzyme activity were performed masked to diagnostic groups. The *KMO* mRNA expression data were approximately normally distributed (skewness, 0.10; median, 0.83; standard error of skewness, 0.3) and were compared between diagnosis groups using 1-way analysis of variance (ANOVA). Comparisons via ANOVA were performed for KMO activity. Pearson correlational analysis examined the correlation between *KMO* mRNA expression and KMO activity. In an exploratory framework, we examined the relationship between *KMO* SNPs and *KMO* mRNA expression in the FEF tissue samples. Because the sample size was small, we collapsed all minor allele carriers into 1 group (ie, CT/TT for rs2275163 and AG/AA for rs1053230) and compared them with participants with the homozygous major allele (ie, CC for rs2275163 and GG for rs1053230) genotype to assess the effects of genotype on *KMO* mRNA expression using ANOVA.

Clinical Sample: Endophenotype and Single-Nucleotide Polymorphism Association Analyses: Before analysis, the distribution of genotypes was evaluated for their fit to Hardy-Weinberg equilibrium expectations using the χ^2 test. On the basis of the results of the exploratory analyses of *KMO* SNPs on *KMO* mRNA expression in the postmortem brain samples, we used multiple logistic regression to assess the association of the *KMO* rs2275163 SNP genotype with schizophrenia endophenotypes. Cognizant of ethnic differences in allelic frequencies, we determined the minor allele frequency (MAF) in both groups in our sample. Consequently, MAFs were 0.23 and 0.14 in the European American and African American/mixed ethnicity groups, respectively. The MAFs based on case-control status in each group were 0.21 and 0.18 in the European American cases and controls, respectively, and 0.10 and 0.08 in the African American/mixed ethnicity cases and controls, respectively. Thus, the MAFs were in the same direction in cases and controls in both ethnic groups. Analysis of variance was used to compare mean endophenotype scores (ie, predictive pursuit and ODR-visuospatial working memory) between different genotype classes. A subgroup analysis was conducted limited to SNP-endophenotype comparisons between healthy controls and schizophrenia patients who displayed poor predictive pursuit.

For these analyses, we considered schizophrenia patients who had mean predictive pursuit values below 0.4025 to have poor predictive pursuit.[67] This exploratory analysis was motivated by an attempt to apply this endophenotype in an extreme trait design framework. Smooth pursuit shares a similar neurocognitive construct to working memory,[68] which involves higher-order mnemonic processing of transient information in service of a response.[69] The FEF neurons controlling predictive pursuit maintain firing when the target sensory information is removed, and their firing rates directly correlate with predictive eye velocity.[70] During predictive pursuit, the patient relies on an internal representation of the target velocity information, as occurs during target masking.[71] Indeed, preliminary analyses showed that schizophrenia patients who displayed poor eye tracking performed significantly worse than schizophrenia patients who displayed good eye tracking on 4 of 5 neuropsychological domains of cognitive function (data not shown, available on request from the authors). We adjusted the *P* values for multiple correlated tests using methods described by Conneely and Boehnke,[72] using their software program (http://csg.sph.umich.edu/boehnke/p_act.php).

RESULTS

KMO mRNA EXPRESSION

Expression of *KMO* mRNA in the FEF was significantly reduced (−33%) in schizophrenia compared with control samples ($F_{1,63}$=8.59; *P*=.005; Figure 2A). The mean (SD) *KMO* mRNA expression values observed in FEF tissue from patients who had not been taking medications for 6 months or more before death (0.78 [0.47]; n=5) were not different from those in patients who had been taking medications at the time of death (0.94 [0.36]; n=27).

KMO ENZYME ACTIVITY

KMO activity was also significantly reduced (−30%) in schizophrenia compared with control FEFs ($F_{1,61}$=5.64; *P*=.02; Figure 2A). The *KMO* gene expression and enzyme activity in the FEF were significantly correlated (Pearson correlation: combined group *r*=0.66, *P* < .001; within groups: schizophrenia, *r*=0.43; *P* < .01; and controls, *r*=0.63; *P* < .001; Figure 2B). The mean (SD) KMO activity in samples from patients who had not been taking medications for 6 months or more before death (15.70 [8.44] pmol/h per milligram of protein; n=5) was not different from that in patients who had been taking medications (15.62 [8.35] pmol/h per milligram of protein; n=27).

EFFECTS OF *KMO* SNPs ON *KMO* mRNA EXPRESSION IN FEF

KMO mRNA levels were slightly higher in schizophrenia samples carrying the rs2275163 minor allele (TT/CT) compared with the major allele homozygotes (ie, CC) (nominal *P* = .05; effect size=0.61; Figure 3), although this difference did not achieve our threshold for statistical significance. No significant differences were found in expression levels between samples with and without the minor allele at rs1053230 (*P* = .50; effect size=0.30). No significant genotype effect on KMO activity was found.

ENDOPHENOTYPE AND SNP ASSOCIATION STUDIES IN THE CLINICAL SAMPLE

Results of SNP genotyping were available for 286 of the 306 study participants who completed predictive pursuit testing and for all 156 study participants who completed ODR testing to measure visuospatial working memory (Table 2 and Figure 4). The *KMO* rs2275163 genotype was significantly associated with predictive pursuit in the combined group of patients and controls ($F_{2,279}$=4.32; corrected *P* < .05). Post hoc comparisons showed lower predictive pursuit in individuals with the CC genotype (mean [SD], 0.47 [0.13]; n=157) compared with those with the CT genotype (0.53 [0.15]; n=105; *P* < .001;

effect size=0.46) and no difference regarding those with TT genotypes (0.51 [0.15]; n=24; effect size=0.31; Figure 4A). The SNP rs2275163 also was associated with ODR performance ($F_{2,149}$=3.78; corrected $P < .05$), with post hoc comparisons revealing more visuospatial errors in participants with the CC genotype (2.46° [1.65°]; n=84) than in those with the CT genotype (1.77° [0.78°]; n=60; $P < .009$; effect size=0.47; Figure 4B) but not different from those with TT genotypes (1.99° [2.46°]; n=12; effect size=0.28).

No association was found between rs2275163 and the diagnosis of schizophrenia ($P = .62$). Subanalyses based on poor and good predictive pursuit in patients revealed that schizophrenia patients who had poor predictive pursuit (n=53) were less likely to be minor allele carriers than were healthy controls (n=126) (25.0% vs 50.0%; $P = .03$). In contrast, the proportion of schizophrenia patients who had good predictive pursuit and were minor allele carriers was virtually identical to the proportion of controls with those qualities (49% vs 50%). Age did not differ significantly ($P = .70$) between healthy controls (43.0 [14.0] years) and schizophrenia individuals who had poor eye tracking (44.3 [11.3] years).

NEUROLEPTIC EXPOSURE

Neuroleptic medication dosing in schizophrenia patients was converted to chlorpromazine equivalent doses, as previously described.[73-75] The mean chlorpromazine equivalents for schizophrenia with good eye-tracking performance (760.3 [448.2] mg; n=114) were not significantly different ($P = .14$) from schizophrenia with poor eye-tracking performance (819.6 [410.2] mg; n=53).

COMMENT

The present study provides the first demonstration, to our knowledge, of reduced *KMO* gene expression in postmortem cortical samples from schizophrenia patients. Moreover, the correlated reduction of *KMO* gene expression and KMO activity in FEF samples from schizophrenia patients shown here complements the recent report by some of us[76] of significantly decreased KMO activity in the prefrontal cortex of patients and preliminary observation[77] of a similar reduction in enzyme activity in the basal ganglia. For several reasons, the differences between patients and controls reported here are unlikely to be artifacts of antemortem antipsychotic medication exposure. First, similar *KMO* gene expression and KMO enzyme activity were observed in the FEF of schizophrenia patients who had not been taking medications for 6 or more months before death. Second, long-term (ie, 28-day) treatment of rats with risperidone does not affect cortical *KMO* gene expression (I. Wonodi, K.V. Sathyasaikumar, and R. Schwarcz, unpublished data, available on request from the authors) or KMO enzyme activity.[78] Moreover, in postmortem brain studies of schizophrenia patients, total RNA quality rather than exposure to antipsychotic drugs was identified as the most critical factor affecting gene expression,[79] and the RNA quality of the tissues used was high (RIN ≥ 6.0; Table 1).[62] Finally, although an effect of excessive nicotine exposure due to the high prevalence of smoking in schizophrenia patients[80-82] cannot be discounted categorically, it is notable that no effect on *KMO* gene expression was found in a study[83] in which differential expression of more than 200 genes was observed in schizophrenia smokers compared with schizophrenia nonsmokers.

In functional terms, our data suggest that KP flux toward 3-hydroxykynurenine and quinolinic acid might be compromised in several brain regions and possibly throughout the brain of schizophrenia individuals. This implies that the substrate of KMO (ie, kynurenine) might eventually accumulate in hyperphysiologic concentrations, resulting in a shift toward enhanced KYNA formation (Figure 1). Notably, elevations in brain kynurenine and secondary increases in KYNA formation may be accentuated further by increased synthesis of the metabolite in the brain and/or increased kynurenine influx from the circulation

secondary to peripheral KP activation.[3,32-34,84] Of particular relevance to schizophrenia, redirected cortical KP metabolism toward increased KYNA production is positioned to play a critical role in NMDAR and α7nAChR hypofunction, which are presumed to be causally related to cognitive deficits in the disease (Figure 1).[14-21] We are currently in the process of examining KYNA and several other KP metabolites and enzymes as part of a comprehensive follow-up study.

Our SNP association analyses revealed that the *KMO* CC genotype was associated with neurocognitive endophenotypic deficits (ie, in predictive pursuit and visuospatial working memory) in the clinical sample and revealed a trend toward reduced *KMO* mRNA expression in FEF tissue from schizophrenia patients in the postmortem sample. Although no significant difference was observed in CC genotype frequencies between healthy controls and the total sample of schizophrenia patients, patients with good predictive pursuit had similar proportions of the CC genotype as controls. Differences only emerged when CC genotype frequencies were compared between healthy controls and schizophrenia patients who displayed poor eye tracking. These results merit further exploration. For example, in a genetic background that includes *KMO* risk variants, additional environmental and/or genetic "hits" could lead to increased cortical KYNA levels and KYNA-related cognitive impairments. We also note that in a previous study[36] that examined a Japanese sample, the CC genotype was nominally less frequent in schizophrenia.

The mechanism by which *KMO* rs2275163, which maps to intron 9 of the *KMO* gene, might affect *KMO* gene expression is unclear. Because it is neither a nonsynonymous coding nor a promoter SNP,[85] our findings might indicate susceptibility mutation(s) in linkage disequilibrium with *KMO* rs2275163 or multiple functional rare variants in synthetic association with the *KMO* CC genotype.[86,87] Extensive molecular characterization, including gene resequencing, is currently underway and may identify the putative causative variant(s) captured by the SNP association in this study. Alternatively, *KMO* rs2275163 might influence *KMO* function by posttranscriptional and posttranslational modifications possibly involving micro-RNAs (miRNAs). The latter provides a particularly plausible mechanism because 50% of miRNA genes are located in intronic regions of protein-coding genes and control gene expression post-transcriptionally by regulating mRNA translation or stability via interactions with the 3′ UTRs of the mRNAs.[88] So far, however, no miRNAs from the *KMO* genomic region have been deposited in the miRBase database for chromosome 1 (www.mirbase.org).[89]

In summary, the present study provides the first evidence of a significantly correlated reduction in *KMO* gene expression and KMO enzyme activity in postmortem tissue from schizophrenia patients obtained from a cortical region associated with SPEMs and *KMO* variation affecting predictive pursuit eye movements in a clinical schizophrenia sample. Although the results from our clinical population could provide a plausible explanation for the failure of Aoyama and colleagues[36] to replicate a *KMO* SNP association with schizophrenia, our results also could be explained by ascertainment strategies and/or cryptic population stratification across samples. More generally, our data support the concept that schizophrenia-related endophenotypes could be used to dissect the effects of susceptibility genes of small effect.[30,90-93] It follows that extreme-trait designed studies[87] focusing on subgroups identified by endophenotypic markers might help resolve inconsistencies across genetic data sets. Therefore, future studies probing the complex interactions between molecular genetic and specific neurochemical perturbations using endophenotypes may advance our understanding of the etiopathophysiology of schizophrenia. This, in turn, could facilitate the development of novel, targeted treatments for cognitive deficits in schizophrenia and possibly in other neuropsychiatric diseases.

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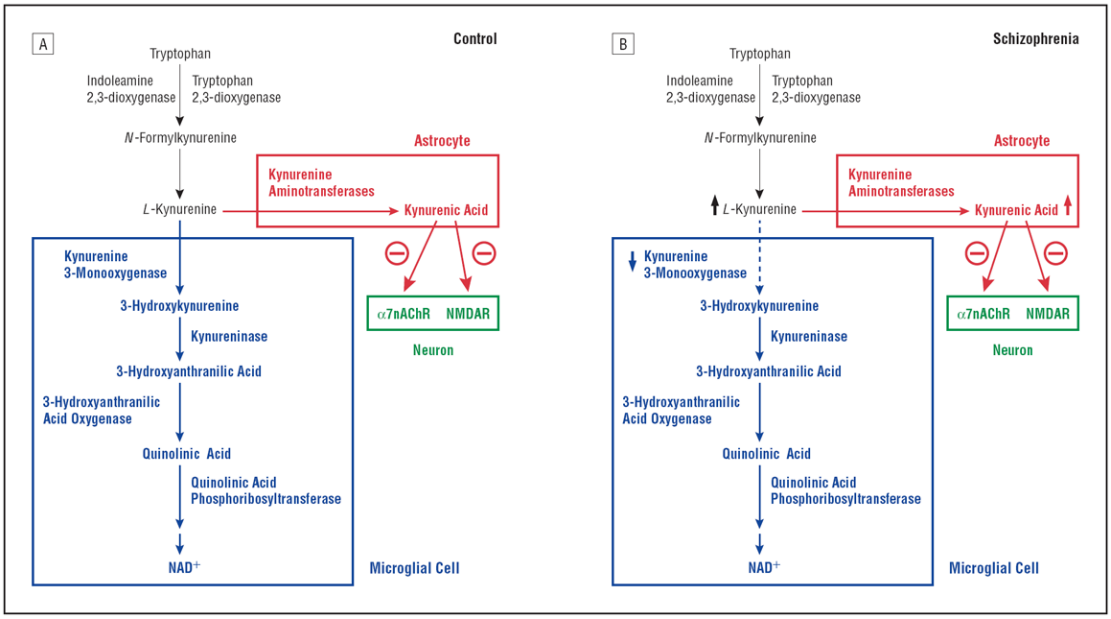

Figure 1.

The kynurenine pathway of tryptophan degradation. A, Metabolism is initiated by the oxidative ring opening of tryptophan by indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase. In the brain, the pivotal metabolite kynurenine is enzymatically converted to 3-hydroxykynurenine and kynurenic acid in microglial cells and astrocytes, respectively. B, In schizophrenia, a persistent reduction of microglial kynurenine 3-monooxygenase activity would result in increased kynurenic acid formation in, and release from, astrocytes. This could cause increased inhibition of neuronal α7 nicotinic receptors (α7nAChRs) and *N*-methyl-D-aspartate receptors (NMDARs) (modified from Wonodi and Schwarcz[5]). NAD^+ indicates nicotinamide adenine dinucleotide.

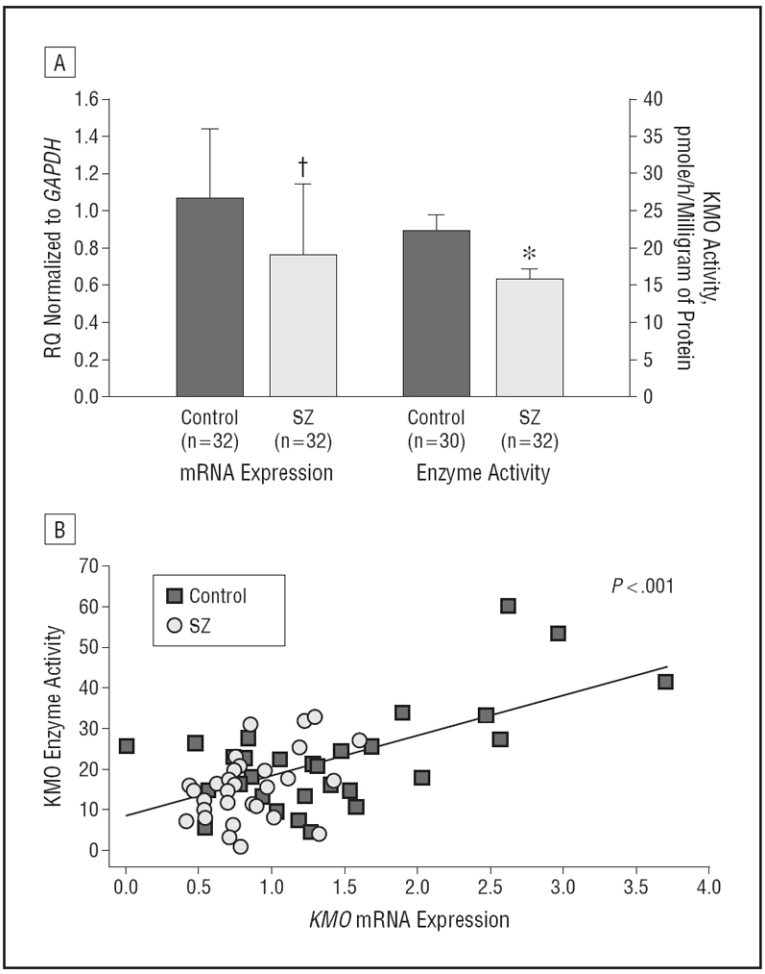

Figure 2.

KMO messenger RNA (mRNA) expression and kynurenine 3-monooxygenase (KMO) enzyme activity. A, Mean *KMO* mRNA expression and KMO enzyme activity in frontal eye field (FEF) tissues obtained post mortem from schizophrenia (SZ) patients and healthy control individuals. Both measures are significantly reduced in the FEF of SZ patients: **P*<. 05, †*P*=.005 (analysis of variance). Error bars indicate SD; RQ, relative quantitation. B, Scatterplot of Pearson correlation (*r*= 0.66) between *KMO* gene expression (RQ values, normalized to *GAPDH*) and KMO enzyme activity in FEF tissue from 30 healthy controls and 30 SZ patients.

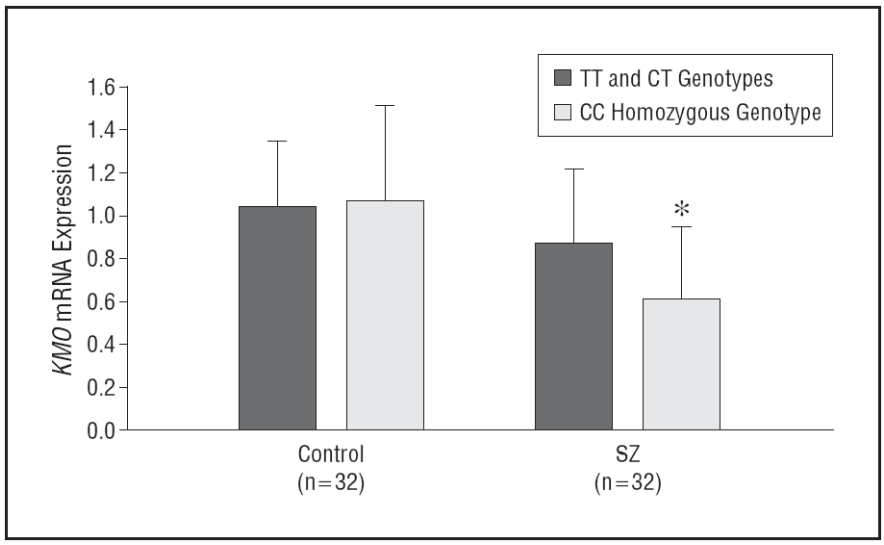

Figure 3.

Mean effects of kynurenine 3-monooxygenase (*KMO*) rs2275163 genotype groups on *KMO* gene expression (ie, relative quantitation values normalized to *GAPDH*) in postmortem frontal eye field (FEF) samples. *KMO* gene expression is compared between carriers of the minor allele (TT and CT) (black bars) and carriers that are homozygous for the major allele (CC) (gray bars) in control and schizophrenia FEF tissue specimens: **P*<.05, nominal (analysis of variance). Error bars indicate SD. TT and CT: control specimens: n=18, schizophrenia specimens: n=17; CC: control specimens: n=14, schizophrenia specimens: n=15.

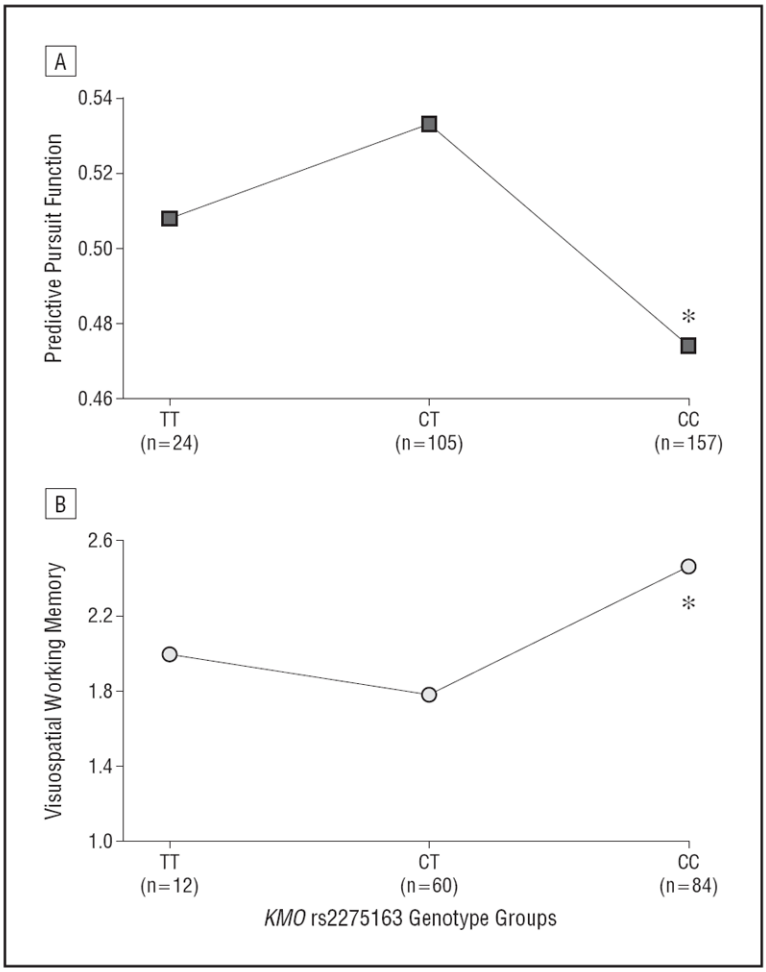

Figure 4.

Results of single-nucleotide polymorphism (SNP) genotyping. A, Effect of kynurenine 3-monooxygenase (*KMO*) SNP rs2275163 CC genotype on predictive pursuit function in the combined clinical sample of schizophrenia patients and healthy control individuals. Participants with the CC genotype had significantly worse predictive pursuit function compared with participants with CT or TT genotypes: **P*<.003 (analysis of variance post hoc test). B, Effect of *KMO* SNP rs2275163 CC genotype on visuospatial working memory in the combined clinical sample of schizophrenia patients and healthy control participants. Participants with the CC genotype made significantly more visuospatial (spatial) working memory errors compared with participants with the CT or TT genotypes: **P*<.009 (analysis of variance post hoc test).

Table 1

Postmortem Frontal Eye Field Tissue Specimens[a]

Study Group	No. of Specimens	Ethnicity of Specimens (EA/AA) No.[b]	Sex (F/M), No.	Mean (SD)			
				Age, y	PMI, h	pH	RIN
Healthy control individuals	32	20/12	5/27	46.9 (13.3)	14.5 (5.8)	6.63 (0.2)	6.6 (0.8)
Schizophrenia patients	32	21/11	5/27	44.7 (12.7)	14.0 (6.9)	6.70 (0.2)	7.1 (0.6)

Abbreviations: AA, African American/mixed ethnicity; EA, European American; PMI, postmortem interval; RIN, RNA integrity number (scale, 1-10).

[a] None of the characteristics differed significantly between the schizophrenia and control groups.

[b] Designates postmortem specimens from donors from the control group and from schizophrenia patients.

Table 2

Demographic and Endophenotypic Measures of Study Participants

Demographics	Mean (SD)[a]		*P* Value
	Healthy Control Participants[b]	Schizophrenia Patients[c]	
Age, y	43.3 (14.6)	45.2 (12.4)	.14
Female sex, %	47.8	26.2	<.001
Ethnicity (EA/AA), %	57.9/42.1	50.0/50.0	.19
Predictive pursuit gain	0.57 (0.22) (n = 144)	0.48 (0.21) (n = 162)	<.001
Visuospatial working memory, absolute error in degrees[d]	1.57 (0.58) (n = 73)	2.64 (1.83) (n = 82)	<.001

Abbreviations: AA, African American/mixed ethnicity; EA, European American.

[a] Data are presented as mean (SD) unless otherwise indicated.

[b] n = 228 unless otherwise indicated.

[c] n = 248 unless otherwise indicated.

[d] On the basis of the Oculomotor Delayed Response Task.

Table 3

Effect of *KMO* Genotype on Endophenotypes in Healthy Control Individuals and Schizophrenia Patients Combined

	***KMO* rs2275163 Genotype, Mean (SD)**		
Variable	**TT**	**CT**	**CC**
Predictive pursuit gain[a]	0.51 (0.15) (n = 24)	0.53 (0.15) (n = 105)	0.47 (0.13) (n = 157)
Visuospatial working memory, absolute error in degrees[a,b]	1.99 (2.46) (n = 12)	1.77 (0.78) (n = 60)	2.46 (1.65) (n = 84)

[a] Significant main effects of genotype corrected $P < .05$.

[b] Based on the Oculomotor Delayed Response Task.

Table 4

SNP Genotyping

Gene Symbol	Chromosome	ABI Assay Identification No.	SNP	Function	RefSNP Alleles	MA	Context Sequence
KMO	1q42-q44	C_8856260_10	rs1053230	Nonsyn	A/G	A	CTACATGTCACCACGATCTTTCCTC[C/T]GCTTGAGAAGACCATGGAACTGGAT
KMO	1q42-q44	C_16183814_10	rs2275163[a]	Intron	C/T	T	CAGAAACCTACATTAGAGCAAAAGT[C/T]TAAGTGGATATTGTGCTGTGAGCAG

Abbreviations: ABI, Applied Biosystems Inc; MA, minor allele; Nonsyn, nonsynonymous; RefSNP Alleles, National Center for Biotechnology Information reference SNP alleles; SNP, single-nucleotide polymorphism.

[a]Haplotype-tagged SNP is based on a minor allele frequency of ≥0.15.