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ORIGINAL ARTICLE

The *KMO* allele encoding Arg^{452} is associated with psychotic features in bipolar disorder type 1, and with increased CSF KYNA level and reduced *KMO* expression

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The kynurenine pathway metabolite kynurenic acid (KYNA), modulating glutamatergic and cholinergic neurotransmission, is increased in cerebrospinal fluid (CSF) of patients with schizophrenia or bipolar disorder type 1 with psychotic features. KYNA production is critically dependent on kynurenine 3-monooxygenase (KMO). *KMO* mRNA levels and activity in prefrontal cortex (PFC) are reduced in schizophrenia. We hypothesized that *KMO* expression in PFC would be reduced in bipolar disorder with psychotic features and that a functional genetic variant of *KMO* would associate with this disease, CSF KYNA level and *KMO* expression. *KMO* mRNA levels were reduced in PFC of bipolar disorder patients with lifetime psychotic features ($P = 0.005$, $n = 19$) or schizophrenia ($P = 0.02$, $n = 36$) compared with nonpsychotic patients and controls. *KMO* genetic association to psychotic features in bipolar disorder type 1 was studied in 493 patients and 1044 controls from Sweden. The *KMO* Arg^{452} allele was associated with psychotic features during manic episodes ($P = 0.003$). *KMO* Arg^{452} was studied for association to CSF KYNA levels in an independent sample of 55 Swedish patients, and to *KMO* expression in 717 lymphoblastoid cell lines and 138 hippocampal biopsies. *KMO* Arg^{452} associated with increased levels of CSF KYNA ($P = 0.03$) and reduced lymphoblastoid and hippocampal *KMO* expression ($P \leqslant 0.05$). Thus, findings from five independent cohorts suggest that genetic variation in *KMO* influences the risk for psychotic features in mania of bipolar disorder patients. This provides a possible mechanism for the previous findings of elevated CSF KYNA levels in those bipolar patients with lifetime psychotic features and positive association between KYNA levels and number of manic episodes.

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INTRODUCTION

Bipolar disorder type 1 is characterized by episodes of mania and depression, usually followed by symptom-free intervals (euthymia). Severe manic and depressive episodes in bipolar disorder type 1 often include psychotic features, for example, hallucinations and delusions, which are main characteristics in schizophrenia.[1] Both schizophrenia and bipolar disorder clearly aggregate in families, and quantitative genetic analyses highlight a strong, in part overlapping, inherited component to both disorders.[2–4] In accord with the dopamine hypothesis of schizophrenia, symptoms of mania and psychosis in bipolar episodes are believed to involve dopaminergic hyperactivity.[5]

There is evidence for increased levels of pro-inflammatory markers in plasma of bipolar disorder patients (reviewed in Goldstein *et al.*[6]). Recently, elevated cerebrospinal fluid (CSF) levels of the pro-inflammatory cytokine interleukin-1β were found in euthymic patients with bipolar disorders having had a recent manic or hypomanic episode.[7] Interestingly, the kynurenine pathway (Figure 1a) is activated by pro-inflammatory cytokines.[8,9] Brain kynurenic acid (KYNA) is produced primarily within astrocytes as an end metabolite of tryptophan degradation within the kynurenine pathway.[10] KYNA tonically modulates glutamatergic and cholinergic neurotransmission by blocking the glycine site of the *N*-methyl-D-aspartate receptor[11–14] and the cholinergic α7 nicotinic receptor,[15] respectively. Briefly, animals with elevated brain levels of endogenous KYNA are characterized by having increased spontaneous midbrain dopamine activity,[16–19] disruption in sensory gating[20] and prepulse inhibition of the acoustic startle reflex,[21] as well as impairments in contextual learning and spatial working memory.[22] Elevated KYNA levels have been found in the CSF from both euthymic males with bipolar disorder[23] and patients with schizophrenia,[24–26] as well as in postmortem prefrontal cortex (PFC) of schizophrenia patients.[27,28] Interestingly, within bipolar type 1 males and females, CSF KYNA levels were positively associated with both the lifetime history of psychotic features, and the recent (last year) occurrence of a manic episode (odds ratios (OR) = 4.9 and 4.4, respectively); furthermore, CSF KYNA was

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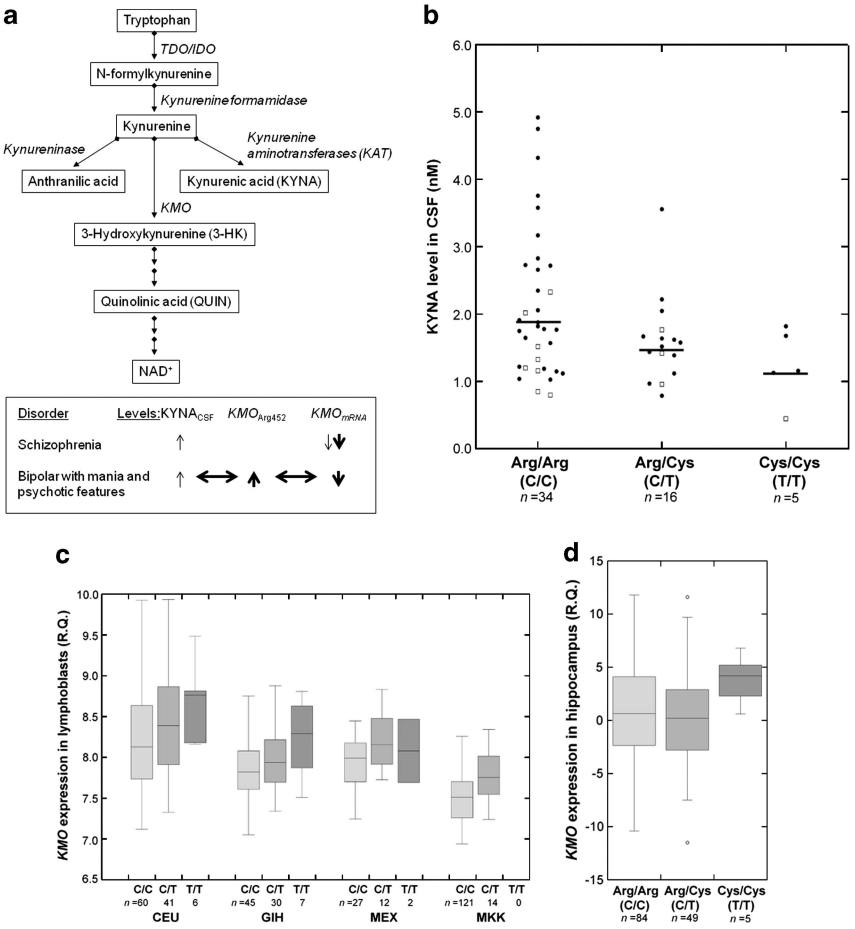

Figure 1. Kynurenine 3-monooxygenase (*KMO*) rs1053230 allele C encoding Arg^{452} is associated with increased level of kynurenic acid (KYNA) in cerebrospinal fluid (CSF) and reduced expression of *KMO* in lymphoblastoid cell lines and hippocampus. (**a**) (Upper panel) The kynurenine pathway including KMO and KYNA. (Lower panel) Previously reported dysregulations (thin arrows) and study hypotheses (thick arrows). Double-sided arrow indicates association. (**b**) Linear increase of CSF KYNA level by number of Arg^{452} (rs1053230 C) alleles in bipolar disorder type 1 patients (sample III). Patients with psychotic feature are indicated by filled circle, whereas those without psychotic feature are indicated by open square. The KYNA value corresponding to the mean of the natural logarithm-transformed KYNA level is indicated by horizontal lines. (**c**) Lower *KMO* expression level by increasing the number of Arg^{452} (rs1053230 C) alleles in lymphoblastoid cell lines from individuals within the HapMap3 project (sample IV): Caucasians (CEU), Indians (GIH), Mexicans (MEX) and Maasai (MMK). (**d**) Lower *KMO* expression level in hippocampal biopsies from patients with one or two Arg^{452} (rs1053230 C) alleles (sample V). Dot in box plot indicates an outlier defined by $>1.5\times$ IQD from 25th or 75th percentile; IQD = distance between the 25th and the 75th percentile (that is, the box length). R.Q. = relative quantification.

borderline significantly positively associated with number of manic episodes.[29] Bipolar type 1 individuals without a lifetime history of psychosis had CSF KYNA levels similar to those reported in healthy volunteers.[23,29] Moreover, CSF KYNA levels are known to correlate positively with the dopamine metabolite homovanillic acid in healthy volunteers[30] and schizophrenia patients.[31]

KYNA is one of the three products of three parallel enzymatic modifications of kynurenine (Figure 1a). Kynurenine 3-monooxygenase (KMO) has a high affinity for kynurenine, suggesting that KMO metabolizes most of the available kynurenine into the neurotoxic agent 3-hydroxykynurenine and downstream metabolites.[32] Pharmacological inhibition of KMO consequently leads to a reduced formation of 3-hydroxykynurenine and increased kynurenine availability, thus shunting metabolism of kynurenine towards KYNA.[33–36] In agreement with increased KYNA in the CSF and postmortem brain of schizophrenia patients, *KMO* gene expression and KMO enzyme activity are reduced in postmortem PFC (Brodmann areas (BA) 9 and 10) and frontal eye field (BA 6) of schizophrenia patients.[28,37]

We therefore hypothesized that *KMO* expression would be reduced in PFC of bipolar patients with psychotic features, that genetic variation in *KMO* would associate with psychotic features, particularly during manic episodes in bipolar disorder type 1, and that the same genetic variation in *KMO* would show functionality by associating also with elevated CSF KYNA levels of bipolar disorder type 1 patients and with reduced *KMO* expression in human lymphoblastoid cell lines.

MATERIALS AND METHODS

Ethics statement

The studies of Swedish samples (samples II and III) and German sample (sample V) were approved by the Regional Ethical Review Boards. All patients gave written informed consent, bipolar patients consented when they were in euthymic phase. The *KMO* expression results (sample I) reported here from Stanley Medical Research Institute (SMRI) Online database were obtained from previous analyses.[38,39]

KMO expression in brain of bipolar disorder and schizophrenia (sample I)

Level of *KMO* RNA in postmortem brains was studied using data obtained from the SMRI On-Line Database (www.stanleygenomics.org). A prior meta-analysis[39] of 105 dorsolateral PFC (DLPFC; BA 46) samples using the SMRI microarray collection, the 'Array Collection,' was queried for *KMO*

Table 1. The study groups sample I, sample II and sample III

Group	n	% Males	Age at tissue sampling[a]
Sample I-KMO expression			
Bipolar disorder (type 1 + type 2/NOS)	34 (26 + 8)	47.1	45 (39, 55)
Bipolar disorder with psychotic features (1 + 2/NOS)	20 (18 + 2)	40.0	47 (36, 54)
Bipolar disorder without psychotic features (1 + 2/NOS)	10 (7 + 3)	70.0	42 (34, 56)
Bipolar disorder, psychotic features unknown (1 + 2/NOS)	4 (1 + 3)	25.0	47 (41, 54)
Schizophrenia	36	74.3	45 (40, 45)
Controls	35	75.0	45 (40, 50)
Sample II-genetics of psychotic features			
Bipolar disorder type 1 (BP1)	493	42.4	52 (39, 64)
BP1 with psychotic features during episode	344	39.8	52 (38, 63)
BP1 with psychotic features during mania	315	40.3	52 (39, 63)
BP1 with psychotic features during depression	98	37.7	52 (38, 63)
BP1 without psychotic features	127	49.6	55 (42, 65)
Anonymous blood donors	1044	59.0	NA
Sample III-genetics of KYNA level in CSF			
Bipolar disorder type 1 (BP1)	55	38.0	34 (28, 49)[b]
BP1 with psychotic feature	43	33.0	38 (28, 49)[b]
BP1 without psychotic feature	12	58.0	33 (22, 46)[b]

Abbreviations: CSF, cerebrospinal fluid; KMO, kynurenine 3-monooxygenase; KYNA, kynurenic acid; NA, not available; NOS, not otherwise specified.
[a]Age; median (25th, 75th percentile).
[b]At lumbar puncture.

expression in bipolar disorder and schizophrenia compared with controls and also comparing bipolar disorder cases with psychotic features to bipolar disorder cases without psychotic features. Diagnosis was made according to DSM-IV (DSM-IV-TR, 2000) for at least a majority of the patients.[38] These data on *KMO* have not previously been reported on a single-gene basis, thus the levels of *KMO* mRNA in this data set has not been published. The SMRI Array Collection was conducted using the same 105 DLPFC RNA samples (extracted at SMRI) at six independent laboratories by microarray analysis (Tony Altar, Sabine Bahn, Seth Dobrin, Tadafumi Kato, Marquis Vawter and L Trevor Young). The cases and controls are described in Table 1. Brain pH and postmortem index for the brains are shown in Supplementary Table S1. Probes used are listed in Supplementary Table S2.

Subjects in study of association between of *KMO* genetic variation and psychosis (sample II)

Patients ($n = 573$) with clinical diagnosis of bipolar disorder type 1 consecutively recruited from specialized outpatient clinics for affective disorders (the Huddinge cohort in Stockholm County: $n = 509$) and from ordinary psychiatric outpatient clinics ($n = 64$). The clinical investigation procedure is previously described.[40] Briefly, lifetime manic and depressive symptoms were assessed based on interviews and medical records focusing on the most severe manic episode using the modules for mania and depression in the Schedules for Clinical Assessment in Neuropsychiatry (SCAN; Table 3).[41] Phenotypes such as lifetime psychotic features during manic or depressive episodes were also assessed. Psychosis was defined as loss of reality and delusions, hallucinations or paranoia during manic or depressive episodes according to DSM-IV (DSM-IV-TR, 2000). A total of 493 bipolar type 1 patients were included in the study. Anonymous blood donors (ABD; $n = 1\ 044$) recruited from Karolinska University Hospital, Stockholm, Sweden, were used as controls. They were between 18–70 years and not allowed to be on sick leave. Patients and controls are described in Table 1 and Supplementary Table S3.

Subjects in study of association between of *KMO* genetic variation and KYNA level in CSF (sample III)

Patients ($n = 55$) were recruited from a long-term follow-up program at a bipolar outpatient unit at the Northern Stockholm psychiatric clinic, Sweden. Consecutive new outpatients referred for treatment and continuing patients at the bipolar outpatient unit were invited to participate in this study if they met the DSM-IV criteria for bipolar disorder type I and were older than 17 years. The clinical investigation procedure is completely described previously.[42] Briefly, the clinical diagnosis of bipolar disorder was established according to the Affective Disorder Evaluation.[43] Psychosis was defined as loss of reality and delusions, hallucinations or paranoia during manic or depressive episodes according to DSM-IV (DSM-IV-TR, 2000). CSF samples of 12 ml were collected from the L4–L5 interspace when the patients were symptom free and in a stable euthymic mood, at 0900 to 1100 hours after an overnight of fast and rest. CSF was inverted to avoid gradient effects and aliquots frozen at $-70\,^{\circ}C$. Patients are described in Table 1 and Supplementary Table S2. KYNA levels in CSF was determined using an isocratic reversed-phase HPLC system.[23,44] The standard curve revealed linear signal of KYNA concentrations from 0.5 to 30 nM. The precision of the assay was determined from the coefficient of variation (CV) of the mean, according to the equation CV (%) = (standard deviation÷mean) × 100. Inter-day and intra-day assay CV of standards were consistently 3–8%. Mean intra-individual CV between duplicates of patient samples was below 5%.

KMO genetic variation association with *KMO* expression in lymphoblastoid cell lines (sample IV)

The relationship between *KMO* rs1053230 (C/T, $Arg^{452}Cys$; allele C (Arg) being ancestral) and *KMO* expression levels was investigated using the *cis*-eQTL feature of the software Genevar (Gene Expression Variation, Wellcome Trust Sanger Institute, Hinxton, UK) (http://www.sanger.ac.uk/resources/software/genevar/).[45] Here, genome-wide gene expression levels (Illumina Sentrix Human-6 Expression BeadChip version 2, Illumina, San Diego, CA, USA) and single nucleotide polymorphism (SNP) genotypes from lymphoblastoid cell lines from 717 individuals of 8 HapMap populations from the HapMap3 project were available. The numbers of individuals of each population included CEU: 107 Caucasians living in UT, USA, of northern and western European ancestry; CHB: 80 Han Chinese from Beijing, China; GIH: 82 Gujarati Indians in Houston, TX, USA; JPT: 82 Japanese in Tokyo, Japan; LWK: 82 Luhya in Webuye, Kenya; MEX: 41 with Mexican ancestry in Los Angeles, CA, USA; MKK: 135 Maasai in Kinyawa, Kenya; YRI: 108 Yoruba in Ibadan, Nigeria.[46]

KMO genetic variation association with *KMO* expression in hippocampal biopsies (sample V)

The relationship between *KMO* rs1053230 and *KMO* expression levels was investigated in biopsies of patients suffering from chronic pharmacoresistant temporal lobe epilepsy ($n = 138$), available from the Epilepsy Surgery Program at the University of Bonn. The patients underwent surgery of the epileptic focus to achieve seizure control. Total RNA and DNA was extracted from fresh frozen hippocampal tissue of these 138 patients using the AllPrep DNA/RNA Micro Kit (Qiagen, Hilden, Germany). For genome-wide genotyping, samples were analyzed with Illumina's Human660W-Quad BeadChips (Illumina). Genome-wide expression analysis was conducted using Illumina HumanHT-12v3 BeadChips.

Genotyping (samples II and III)

Peripheral blood samples were drawn and genomic DNA was extracted by standard procedures. Sixteen SNPs in the *KMO* gene, spanning from the 5′ near gene region to exon 14 next to 3′ UTR, representing a gene coverage of 80–85% were selected for genotyping using the HapMap database. All SNPs were genotyped on a 7900HT Fast Real-Time PCR System Instrument by using allele-specific Taqman MGB probes labeled with fluorescent dyes FAM and VIC (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocols. Allelic discrimination was performed with the ABI PRISM 7900HT SDS and the SDS 2.2.1 program (Applied Biosystems) in 384-well format with nine negative controls distributed in each plate.

Table 2. Allele frequency association results for the 16 *KMO* SNPs to psychotic features during manic episode (PEM) in bipolar disorder type 1 patients vs bipolar disorder type 1 patients without history of psychotic episode (nonPE)

SNP	*Location*	*Alleles*	*MAF: A/U*	*OR [95% CI]*[a]	P-*value*[a]	*Empirical* P-*value*
PEM vs *nonPE*						
rs10926508	5′UTR	A*/G	0.036/0.028	1.22 [0.50–3.0]	0.66	0.70
rs2992642	Intron 1	T*/G	0.22/0.21	1.00 [0.71–1.47]	0.91	1.00
rs3014572	Intron 1	A/G*	0.24/0.24	0.98 [0.68–1.40]	0.89	1.00
rs2050513	Intron 1	C/A*	0.19/0.18	1.11 [0.75–1.64]	0.60	0.86
rs3014569	Intron 1	A*/G	0.13/0.10	1.29 [0.80–2.07]	0.30	0.33
rs10926513	Intron 1	A*/T	0.39/0.42	0.86 [0.63–1.16]	0.32	0.29
rs6661244	Intron 1	C*/T	0.33/0.30	1.15 [0.83–1.60]	0.39	0.38
rs6689793	Intron 3	G/C*	0.08/0.11	0.63 [0.38–1.05]	0.08	0.074
rs3007737	Intron 4	C/T*	0.45/0.41	1.21 [0.89–1.64]	0.23	0.23
rs2065799	Intron 4	C*/T	0.081/0.064	1.33 [0.74–2.39]	0.34	0.46
rs3765806	Intron 5	C*/G	0.33/0.29	1.20 [0.86–1.67]	0.29	0.36
rs12139441	Intron 6	A*/G	0.19/0.18	1.04 [0.71–1.54]	0.82	0.75
rs4660103	Intron 8	G*/A	0.29/0.27	1.11 [0.79–1.55]	0.56	0.46
rs10802971	Intron 9	C*/G	0.33/0.30	1.18 [0.85–1.63]	0.33	0.38
rs850678	Intron 9	A*/T	0.29/0.25	1.20 [0.86–1.68]	0.28	0.27
rs1053230	Exon 14 next to 3′UTR	C*/T $Arg^{452}Cys$	0.18/0.28	0.59 [0.42–0.84]	0.0028	0.0030
PEM vs *ABD*						
rs1053230	Exon 14 next to 3′UTR	C*/T $Arg^{452}Cys$	0.18/0.22	0.80 [0.63–1.01]	0.069	0.048
PE vs *nonPE*						
rs1053230	Exon 14 next to 3′UTR	C*/T $Arg^{452}Cys$	0.19/0.28	0.64 [0.45–0.90]	0.0093	0.0086

Abbreviations: ABD, anonymous blood donors; CI, confidence interval; KMO, kynurenine 3-monooxygenase; LD, linkage disequilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
Association to rs1053230 was thereafter tested in PEM vs ABD and in bipolar disorder type 1 patients with psychotic features during manic or depressive episode (PE) vs nonPE patients. All from sample II. Alleles, major allele first.
*Ancestral allele in CEU population data (CEPH (Utah residents with ancestry from northern and western Europe)) from www.ncbi.nlm.nih.gov Built 136. For all SNPs, but for rs3007737, the minor allele was the same as in 1000 genomes. MAF for the affected (A) and unaffected (U). Odds ratio (OR), the proportion of minor vs major allele among affected (A)/proportion of minor vs major allele among unaffected (U). Threshold for significance in PEM vs nonPE was $P = 0.0042$: Bonferroni correction considering partial LD.[47,48] 0.05/12(six SNP groups [defined by $D' > 0.90$] × two phenotypes [BP vs ABD and PEM vs nonPE]) = 0.0042. Empirical *P*-value (EM*P*1), point-wise *P*-value from 10 000 permutations.
[a]χ^2 test and logistic regression with no covariate.

Table 3. Genotype association for rs1053230 to psychotic features during manic episode in bipolar disorder type 1 patients (sample II)

Control set	*Cases, aa/ab/bb*	*Cases,* n	*Controls aa/ab/bb*	*Controls,* n	*Cochran-Armitage trend* P-*value*[a]	P-*value*[a] *OR [95% CI]*	
						Dominant model[b]	Recessive model[b]
nonPE	14/79/198	291	9/51/65	125	0.0038	0.0019	0.33
ABD	14/79/198	291	45/327/576	948	0.064	0.025	0.96

Abbreviations: ABD, anonymous blood donors; CI, confidence interval; nonPE, bipolar disorder type 1 patients without history of psychotic features during manic or depressive episode.
Alleles, minor allele (a) first. Odds ratio (OR), the proportion of minor vs major allele among affected (A)/proportion of minor vs major allele among nonaffected (U).
[a]χ^2 test and logistic regression with no covariate.
[b]Of minor allele.

Ten percent of the samples were run in duplicates to verify genotyping results. The average genotyping success among the SNPs was 91.5%.

Statistical analyses

KMO expression in postmortem PFC (sample I). In the meta-analysis, expression was compared between disease types and controls using linear regression models, on a gene-by-gene basis, adjusting for covariates being the demographic terms that met the criteria for significance for that gene (pH, sex, age, RNA quality, postmortem interval, disease severity, drug use, smoking and other covariates (up to 23 demographic effects)). The fold change and *P*-values for each gene were condensed, when appropriate, from multiple probesets on the microarray platforms by using all probes from each microarray study independently. The consensus fold changes and associated 95% confidence intervals and *P*-values were reported as a weighted combination of the individual fold changes and standard errors of the six studies.

Genetic association to psychotic features (sample II). Initially, allele frequency difference between bipolar disorder type 1 *per se* (BP) and ABD was tested for each of the 16 *KMO* SNPs. Thereafter, the bipolar type 1 patients with history of psychotic features during manic episode (PEM) were compared with bipolar disorder type 1 patients without psychotic episode (nonPE) with regard to allele frequency difference of the 16 SNPs using logistic regression, and using permutation analyses (10 000 permutations). Nominally significant allelic associations ($P < 0.05$) were further tested for genotypic association in this *case–case* comparison. Thereafter, support for identified genotypic association was tested for by comparing the PEM cases and the ABD controls. Any SNP that showed nominal significance in these three steps (PEM vs nonPE allelic, PEM vs nonPE genotypic and PEM vs ABD genotypic) were analyzed for formation of haploblock with any other of the genotyped SNPs in the ABD controls. Such haploblock was studied for haplotype frequency difference in PEM vs nonPE and in PEM vs ABD. Finally, such SNP was tested for allelic association to bipolar type 1 with history of psychotic features during manic or depressive episode (PE) compared with nonPE cases.

All 16 *KMO* SNPs fulfilled the criteria for HWE (Hardy–Weinberg equilibrium) within each of the three case–controls groups (PEM–nonPE, PEM–ABD, PE–nonPE, $P > 0.05$). Threshold for significance in the allele frequency association screen was calculated using a Bonferroni correction considering the partial linkage disequilibrium (LD) between several markers:[47,48] 0.05/12 (six SNP groups (defined by $D' > 0.90$) × two phenotypes (BP vs ABD and PEM vs nonPE)) = 0.0042. In the PEM vs ABD, SNP analysis $P = 0.05$ was regarded significant (one SNP, one phenotype). In the

Table 4. Haplotype association for *KMO* to psychotic features during manic episode in bipolar disorder type 1 patients (sample II)

SNPs	Haplo-type	Control set	Frequency cases	Frequency controls	OR[a]	P-value[a]
rs850678–rs1053230	AT	nonPE	0.18	0.28	0.59	0.0034
		ABD	0.18	0.22	0.78	0.032
	TC	nonPE	0.29	0.25	1.23	0.23
		ABD	0.29	0.25	1.28	0.015
	AC	nonPE	0.53	0.48	1.21	0.19
		ABD	0.53	0.53	0.96	0.67

Abbreviations: ABD, anonymous blood donors; KMO, kynurenine 3-monooxygenase; nonPE, bipolar disorder type 1 patients without history of psychotic features during manic or depressive episode; SNPs, single nucleotide polymorphisms.
Odds ratio (OR), the ratio-specific haplotype vs all other haplotypes among the cases, relative to the ratio-specific haplotype vs all other haplotypes among the controls.
[a]Logistic regression with no covariate.

haplotype distribution test (2 df), $P<0.05$ was regarded significant (one haplotype block and one phenotype), whereas $P<0.05/3$ (three haplotypes) $=0.01$ was considered significant for the individual haplotype tests. The LD measure D' was calculated between the SNPs using the Haploview program, version 4.2 (Broad Institute of MIT and Harvard, Cambridge, MA, USA).[49] Haplotype blocks were constructed using the LD block parameters and the D' confidence interval algorithm in the Haploview program. HWE test, allele, genotype and haplotype frequency difference tests were calculated using the PLINK program, version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/),[50] and IBM SPSS Statistics version 20.0 (IBM, Armonk, NY, USA). The statistical power to exclude association between PEM and allele frequency of a SNP at $\alpha=0.05$ was calculated according to http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html.

Genetic association to KYNA levels in CSF (sample III). Association between KYNA and sex was tested using nonparametric Mann–Whitney *U*-test, and association between sex and genotype was tested using the χ^2 test. To normalize the distribution of KYNA concentrations, values were transformed with the natural logarithm. KYNA level dependence on rs1053230 was tested using linear regression with rs1053230 and age at lumbar puncture as independent factors, since KYNA was previously shown to be positively linearly dependent on age at lumbar puncture.[23,29] The principal assumptions of linearity, homoscedasticity and normality were checked. Regression analyses were performed using SPSS version 20. A *P*-value <0.05 was regarded as statistically significant.

Genetic association to KMO expression in lymphoblastoid cell lines (sample IV) and in hippcampal biopsies (sample V). Within lymphoblastoid cell lines from populations with three *KMO* rs1053230 genotype groups (CEU, GIH and MEX) association between *KMO* rs1053230 and *KMO* expression was determined using the nonparametric Spearman's correlation test. To construct test statistic distribution under H_0 for permutation test, expression intensities were randomly re-assigned to individuals' genotypes, then correlation coefficient and statistical significance were re-computed for 10 000 times. Permutation-based *P*-values $\leqslant 0.05$ were considered statistically significant. For lymphoblastoid cell lines from MKK with two genotype groups (C/C and C/T), and for hippocampal biopsies, association between rs1053230 and *KMO* expression was determined using *t*-test and $P\leqslant 0.05$ as significance threshold.

RESULTS

KMO expression is reduced in PFC from bipolar disorder patients with psychotic features and from patients with schizophrenia (sample I)

Data on *KMO* expression in DLPFC (BA 46) from bipolar patients, schizophrenia patients and controls (described in Table 1) were retrieved from a meta-analysis[39] deposited in the SMRI Online Database. There was no effect of sex on *KMO* expression (fold change = 1.03 (M/F), $P=0.18$). The data showed that *KMO* expression in DLPFC was reduced (fold change = −1.10, 95% CI (confidence interval): −1.01 to −1.22, $P=0.0046$) in bipolar disorder patients with psychotic features compared with bipolar disorder patients without psychotic features. However, comparing the whole bipolar disorder group with healthy controls, *KMO* expression in DLPFC was not different (fold change = −1.01, 95% CI: −1.05 to 1.03, $P=0.24$). Also DLPFC sections from schizophrenia patients had reduced *KMO* expression compared with corresponding sections from controls (fold change = −1.03, 95% CI: −1.01 to −1.05, $P=0.022$).

The C allele of *KMO* rs1053230 encoding Arg^{452} is associated with bipolar disorder type 1 with psychotic features (sample II)

The bipolar disorder type 1 patient group, that is, including both those with and those without psychotic features, did not have any different allele frequencies of the 16 *KMO* SNPs compared with ABD (rs1053230 had OR = 0.99, $P=0.94$). However, the bipolar disorder type 1 patients with psychotic features during manic episode (PEM) had reduced frequency of the minor allele T (encoding Cys^{452}) of the nonsense SNP rs1053230 compared with the nonPE patients (18% vs 28%, OR = 0.59, $P=0.003$) (Table 2), showing a codominance (trend test, $P=0.004$) and dominance ($P=0.002$) of the T allele in the genotypic association (Table 3). Likewise, the PEM group had reduced rs1053230 T allele frequency compared with the ABD group (22%, OR = 0.80, $P=0.05$) (Table 2) and a dominant mode of genotypic association ($P=0.02$) (Table 3). rs1053230 formed an LD block with upstream SNP rs850678 (Supplementary Figure S1). This block had three haplotypes with rs1053230 allele T present in only the haplotype AT; hence a significant difference in distribution of haplotypes between the psychotic features during mania group (PEM) and the nonpsychotic feature group (nonPE) ($\chi^2=9.4$, df = 2, $P=0.0090$), and between the psychotic features during mania group and the ABD group ($\chi^2=8.4$, df = 2, $P=0.015$) (Table 4). None of the other *KMO* SNPs analyzed here showed nominal allele frequency association to PEM comparing to nonPE or ABD. However, the power to exclude true association of the other SNPs was low (0.10–0.45). Including as cases, all bipolar disorder type 1 patients with a psychotic features during an episode of either mania or depression (PE) showed a weaker allelic association to rs1053230 (OR = 0.64, $P=0.009$) compared with that shown above where only patients with psychotic features during a manic episode (PEM) where studied (PEM, OR = 0.59, $P=0.003$) (Table 2). Hospitalization for episodes, age at onset of mania and number of manic episodes did not influence the rs1053230 association to psychotic features during mania.

The C allele of *KMO* rs1053230 encoding Arg^{452} is associated with higher KYNA levels in CSF from bipolar disorder type 1 patients (sample III)

In the bipolar disorder type 1 patient group, CSF KYNA levels were similar in males and females ($P=0.81$) and proportion of males was similar between genotypes ($P=0.13$). The presence of *KMO* rs1053230 allele C was linearly positively associated with CSF KYNA levels from bipolar disorder type 1 patients, also when correcting for age at lumbar puncture ($\beta=0.20\pm0.088$, $P=0.027$, adjusted $R^2=0.23$). The effect of rs1053230 allele C was slightly lower compared with the effect of age ($\beta_{standardized}=0.27$ vs 0.39). The effect of rs1053230 allele C was present also in those with lifetime history of psychosis ($\beta=0.20\pm0.91$, $P=0.034$, adjusted $R^2=0.26$) (Figure 1b). The patients with bipolar disorder type 1 without psychotic features were too few (12) to analyze KYNA dependence on rs1053230 in that group.

The C allele of *KMO* rs1053230 encoding Arg^{452} is associated with lower *KMO* expression in lymphoblastoid cell lines (sample IV) and in hippocampal biopsies (sample V)

There was an increase in lymphoblastoid *KMO* expression (probe ID: ILMN_1730917) by decreasing number of rs1053230 C alleles

(that is, from CC to CT to TT) in four of the eight HapMap3 populations studied: Caucasian CEU (Spearman correlation coefficient (rho) = 0.19, $P = 0.05$), Indian GIH (rho = 0.22, $P = 0.04$), Mexican MEX (rho = 0.35, $P = 0.02$), Maasai MKK ($t = 2.7$, $P = 0.008$ (no TT genotype present)) (Figure 1c). Hippocampal biopsies from those patients with one or two rs1053230 C alleles had lower *KMO* expression than those with no C allele ($t = 2.7$, $P = 0.04$) (Figure 1d).

DISCUSSION

KYNA is an end metabolite of the kynurenine pathway that inhibits brain glutamatergic and cholinergic transmission and hereby tonically modulates dopaminergic and GABAergic activity.[10,51,52,53] KYNA is elevated in the PFC of schizophrenia patients[27,28] and in the CSF of patients with schizophrenia,[25,26,54] or bipolar disorder.[23] Recently, it was found that the elevated KYNA levels in bipolar disorder type 1 were restricted to those patients with psychotic features and/or last year manic episode.[23,29] The enzyme KMO is indirectly involved in the production of KYNA and reduced KMO enzyme activity will shunt the kynurenine pathway towards KYNA synthesis.[32] In line with an increased KYNA in the CSF and PFC of schizophrenia patients, *KMO* expression and KMO enzyme activity are reduced in the PFC of schizophrenia patients.[28,37] Based on these findings, we hypothesized that *KMO* expression would be reduced in PFC also of bipolar disorder patients with psychotic features, and that genetic variation in *KMO* affecting its expression or KMO enzyme activity would segregate in humans and that the low activity variant would be overrepresented in bipolar disorder type 1 patients with psychotic features in manic episode. Further, we hypothesized that such a low activity variant would associate with increased CSF KYNA levels in these patients, hereby indicating a reduced KMO functionality. Data deposited at the SMRI showed that *KMO* expression in PFC was reduced in bipolar disorder patients with psychotic features compared with bipolar disorder patients without psychotic features, with a fold change point estimate of 1.3, but not different in bipolar disorder PFC compared with PFC from controls. A strength of this *KMO* expression finding is that it is based on six independent microarray experiments of the same RNA samples. Thus, the fold change was based upon six studies, and the effects of multiple covariates were removed. The limited fold change is in line with that individual gene dysregulation in bipolar disorder is generally small in magnitude, in the range of 10–20%.[39] Using a Swedish sample of bipolar disorder type 1 patients, representative of the clinics catchment area, and ABD from the same area, we could demonstrate that the Arg^{452} (C) allele of the $Arg^{452}Cys$ variation rs1053230 was more common among specifically those patients with psychotic features, in particular those with psychotic features during manic episode. Using a different independent sample of bipolar disorder type 1 patients from the same city, we could also demonstrate that this same Arg^{452} allele associated with higher CSF KYNA levels in bipolar disorder patients, in consonance with a reduced KMO activity of the Arg^{452} allele. The Arg^{452} allele-dose pattern was similar in the association with risk for psychotic features as in the association with risk for higher CSF KYNA levels. Moreover, using HapMap3 project data, we could show that this Arg^{452} allele also associated with reduced *KMO* expression in lymphoblastoid cell lines from four different populations: white Americans, Indian Americans, Mexican Americans and Kenyan Maasai. Further, hippocampal biopsies from epileptic patients with at least one Arg^{452} allele had lower *KMO* expression than those with no Arg^{452} allele. The reduced *KMO* expression and increased KYNA level by Arg^{452} allele is consistent with the fact that pharmacological inhibition of KMO shunts metabolism of kynurenine towards KYNA.[33–36]

KMO is located in the outer mitochondrial membrane.[55] The $Arg^{452}Cys$ variation is at the extracellular C-terminal side of the KMO peptide, which is likely the site for interaction with its substrate kynurenine. A change between the hydrophilic Arg^{452} and the hydrophobic Cys^{452} may affect protein conformation and consequently substrate binding or catalysis rate.[56] Given the reduced *KMO* expression with Arg^{452} allele and reduced *KMO* expression in PFC of bipolar disorder with psychotic features, one speculation is that KMO protein function may affect *KMO* expression through feed-back mechanisms. Alternatively, the $Arg^{452}Cys$ variation may be in LD with polymorphism affecting *KMO* expression; rs1053230 is close to the 3′ untranslated region where polymorphisms may influence stability of the mRNA, for example, the degradation of the mRNA by microRNAs expression. In agreement to previous reports from other brain collections,[28,37] we here found reduced *KMO* expression also in the PFC of patients with schizophrenia. Genetic variation in *KMO* has previously been tested for association to schizophrenia in the Japanese population with an initial indication of an Arg^{452}-containing risk-haplotype, but that could not be confirmed.[57] Likewise, neither a study of Swedish material nor a study of American material found genetic association between *KMO* and schizophrenia,[37,58] although *KMO* is located in schizophrenia susceptibility linkage locus 1q42.[59,60] However, a modest effect of *KMO* intron 9 rs2275163 was found on schizophrenia oculomotor endophenotypes,[37] and *KMO* was among the top candidate genes for schizophrenia using a translational convergent functional genomic approach.[61] We recently published that in a cohort of primarily Swedish healthy controls those homozygous for the rs1053230 Arg^{452} allele had reduced CSF KYNA levels compared with carriers of the Cys^{452} allele.[56] It may be that the $Arg^{452}Cys$ has different effect on KYNA level in bipolar patients compared with healthy individuals, indirectly through for example another functional polymorphism.

Using five independent cohorts: *KMO* expression data from SMRI,[39] *KMO* eQTL data from HapMap3 using Genevar,[46] and from German hippocampal biopsies, as well as *KMO* and KYNA data from two independent Swedish samples, we show findings on gene expression, gene sequence and metabolite level that collectively suggest that functional genetic variation in *KMO* influences the risk for psychotic features in mania of bipolar disorder patients. Clearly, these findings highlight the potential that elevated brain levels of KYNA could account for psychotic behavior, whether the basic diagnosis is schizophrenia or bipolar disorder. The observations that other *N*-methyl-D-aspartate receptor antagonists such as phencyclidine or ketamine induce psychotomimetic effects in healthy individuals lend further support to this pathophysiological model of psychosis. However, a genetic variation in the KMO enzyme may not solely account for the pathophysiologically elevated brain KYNA observed in psychotic diseases. Mechanisms extrinsic to the kynurenine pathway may participate in triggering synthesis of KYNA. Thus, recent studies from our laboratory show a brain immune activation, expressed by elevated CSF levels of interleukin-1β, both in patients with schizophrenia and with bipolar disorder.[7,62] Such an inflammatory response is specifically associated with the induction of TDO in human astrocytes,[63] an enzyme responsible for the rate limiting step of kynurenine production,[32] hereby resulting in increased synthesis of KYNA. Indeed, an increased density and intensity of glial cells stained for TDO has previously been observed postmortem in cortex from individuals with schizophrenia or bipolar disorder.[64] Genetic aberrations within the kynurenine pathway and inflammatory components may thus synergistically elevate brain KYNA levels in psychotic disorders.

There are some limitations in this study that should be considered. For the expression data in sample I, a minor part of the patients (18%) had bipolar disorder type 2 or NOS and not bipolar disorder type 1. There was no information for sample I on whether the psychotic features occurred during mania or during depression. The numbers of bipolar disorder patients without psychotic features in the samples I, II and III are limited. However,

the allele frequency association in sample II survived correction for the multiple testing. The statistical power to exclude true genetic association for the *KMO* SNPs showing no association in sample II was low. However, the Swedish patients (in samples II and III) were almost all recruited from specialized affective disorder units, all the medical records were studied by two investigators and most of the patients were also interviewed, resulting in a thorough phenotype assessment process. Further, all Swedish participants were white and the absolute majority were sampled from the same area of Sweden, the Stockholm County. Excluding the 9% of the bipolar patients who had a non-Swedish family name did not affect the rs1053230—psychotic features during mania association. Even if Sweden is not entirely homogeneous, the current Swedish population has no strong internal genetic borders[65] and especially the southern/middle parts of Sweden (from where the participants of this study are derived from) are more genetically homogeneous.[66] Another limitation is that most of the patients sampled for CSF (sample III) were on psychotropic drugs, for example, lithium, lamotrigine, valproate and antipsychotics, during the CSF sampling. However, experimental studies show that brain KYNA concentration is not affected when drugs, such as valproate and lamotrigine, are administered to animals at clinically relevant concentrations.[67] Furthermore, according to postmortem findings in patients with schizophrenia[64] and experimental studies in the rat,[16,68] brain KYNA concentrations are unaffected or even reduced following chronic treatment with antipsychotics. Also, only mirtazapine treatment was associated to KYNA level in sample III,[29] and the CSF KYNA level association to rs1053230 remained after excluding the six patients on mirtazapine ($P = 0.039$). This argues against an influence of treatment in the current study. Lymphoblastoid cell lines may not be the optimal tissue for studying regulation of genes to elucidate pathology within the brain. However, KMO is previously reported to be highly active in other mononuclear leukocytes, monocytes/macrophages, upon cytokine stimulation.[69] KMO is reported to be active also in neurons (nonpyramidal neurons of PFC),[9] whereas QUIN, a product several steps downstream KMO, was detected only in microglia.[70] The immune cell-microglia-astrocyte-neuron cross-talk with regard to the kynurenine pathway remains to be further elucidated.[9] Finally, the hippocampal data must be interpreted with caution; here, the Arg^{452} allele had a dominant and not an additive effect, true eQTL effects may be hidden by seizure-induced transcription in the biopsies, and the number of Cys^{452} homozygots was small.

In conclusion, we report several lines of support for *KMO* dysregulation in bipolar disorder with psychotic features. This genetic aberration may provide an underlying mechanism for the previous findings of elevated CSF KYNA levels in bipolar patients with lifetime psychotic features and/or manic episode. The findings reported here motivate further studies to elucidate the role of KMO in psychotic disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)