Maternal Genotype Determines Kynurenic Acid Levels in the Fetal Brain: Implications for the Pathophysiology of Schizophrenia

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ABSTRACT

Background

Several studies suggest a pathophysiologically relevant association between increased brain levels of the neuroinhibitory tryptophan metabolite kynurenic acid (KYNA) and cognitive dysfunctions in people with schizophrenia. Elevated KYNA in schizophrenia may be secondary to a genetic alteration of kynurenine 3-monooxygenase (KMO), a pivotal enzyme in the kynurenine pathway (KP) of tryptophan degradation. In rats, prenatal exposure to kynurenine, the direct bioprecursor of KYNA, induces cognitive impairments reminiscent of schizophrenia in adulthood, suggesting a developmental dimension to the link between KYNA and schizophrenia.

Aim

To explore the possible impact of the maternal genotype on KP metabolism.

Methods

We exposed pregnant wild-type (*Kmo*$^{+/+}$) and heterozygous (*Kmo*$^{+/-}$) mice to kynurenine (10 mg/day) during the last week of gestation and determined the levels of KYNA and two other neuroactive KP metabolites, 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), in fetal brain and placenta on embryonic day 17/18.

Results

Maternal kynurenine treatment raised KYNA levels significantly more in the brain of heterozygous offspring of *Kmo*$^{+/-}$ than in the brain of *Kmo*$^{+/+}$ offspring. Conversely, 3-HK and QUIN levels in the fetal brain tended to be lower in heterozygous animals derived from kynurenine-treated *Kmo*$^{+/-}$ mice than in corresponding *Kmo*$^{+/+}$ offspring. Genotype-related effects on the placenta were qualitatively similar but less pronounced. Kynurenine treatment also caused a preferential elevation in cerebral KYNA levels in *Kmo*$^{+/-}$ compared to *Kmo*$^{+/+}$ dams.

Conclusions

The disproportionate KYNA increase in the brain of *Kmo*$^{+/-}$ animals indicates that the maternal *Kmo* genotype may play a key role in the pathophysiology of schizophrenia.

Keywords: Cognition, Development, Kynurenine 3-monooxygenase, Prenatal, Tryptophan

Introduction

In both animals and humans, even subtle changes in the intrauterine milieu can lead to permanent alterations in brain structure and cognitive functions in the offspring (Debnath et al., 2015; Seidman et al., 2000; Stolp et al., 2012). These changes are linked to adverse events during gestation and, together with genetic vulnerabilities (Abazyan et al., 2010; Mittal et al., 2008; O'Leary et al., 2014), may be causally related to the emergence of psychiatric illnesses later in life (Brown et al., 2009; Kirkbride et al., 2012). The etiology of schizophrenia (SZ), a devastating brain disease characterized and defined by positive (psychosis), negative and cognitive symptoms (Carpenter and Buchanan, 1994; Owen et al., 2016), can be convincingly traced to a combination of genetic risk factors (Misiak et al., 2017; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Arnedo et al., 2015) and untoward environmental influences, such as stress, infection and inflammation, during critical periods of brain development (Estes and McAllister, 2016; Muller and Schwarz, 2006). One attractive hypothesis posits that the pathophysiology of SZ is triggered specifically by abnormal perinatal increases in the brain levels of kynurenic acid (KYNA), a neuroactive metabolite of the essential amino acid tryptophan, which can function as an endogenous antagonist of α7 nicotinic acetylcholine receptors (α7nAChRs) and N-methyl-D-aspartate receptors (NMDARs). Unrelated to medication, KYNA levels are elevated in the prefrontal cortex and cerebrospinal fluid of patients with SZ (Ceresoli-Borroni et al., 2006; Erhardt et al., 2001; Linderholm et al., 2012; Sathyasaikumar et al., 2011; Schwarcz et al., 2001). Notably, increased brain KYNA levels cause deficits in cognitive functions, including impairments in working memory, sensorimotor gating and attentional processing, in adult rodents (Akagbosu et al., 2012; Chess and Bucci, 2006; Chess et al., 2007; DeAngeli et al., 2014; Erhardt et al., 2004; Pershing et al., 2015; Shepard et al., 2003). These observations are provocative, as fluctuations in KYNA normally control glutamatergic and cholinergic, as well as dopaminergic and GABAergic, neurotransmission in the adult brain (see Pocivavsek et al., 2016 for review) and may therefore also serve critical roles in normal brain development (Ben-Ari et al., 1997; Dwyer et al., 2009).

In rats, prenatal exposure to excessive amounts of kynurenine (the immediate bioprecursor of KYNA) during the last week of gestation causes an elevation of KYNA levels in the fetal brain (Pocivavsek et al., 2014). In adulthood, these animals show cognitive deficits reminiscent of SZ, and these impairments appear to be directly related to a second – and as yet unexplained – surge of cerebral KYNA levels (Pershing et al., 2015; Pocivavsek et al., 2014). These results are especially interesting in light of the fact that KYNA levels in the fetal brain are also increased when

the pregnant dam experiences physical stress (Notarangelo and Schwarcz, 2016) or when it is exposed to lipopolysaccharide (LPS) (Notarangelo et al., 2015), a bacterial toxin known to cause inflammation *in utero (*Meyer*, 2014)*.

Increases in KYNA levels in the fetal brain can also be produced experimentally by inhibition of kynurenine 3-monooxygenase (KMO), diverting metabolism in the kynurenine pathway (KP) of tryptophan degradation away from two other neuroactive metabolites, 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), and toward increased formation of KYNA (Röver et al., 1997; Fig. 1). Recent evidence suggests that KMO, which has a pivotal function in KP metabolism, could play a pathophysiologically significant role in several neurodevelopmental, neurodegenerative and neuropsychiatric disorders (Parrott et al., 2015). Thus, systemic administration of a selective KMO inhibitor to the dam during the final prenatal week results in distinct structural and behavioral impairments in the offspring in adolescence and adulthood (Forrest et al., 2013a; Forrest et al., 2013b; Khalil et al., 2014; Pisar et al., 2014), supporting the potential relevance of prenatal KYNA elevations for the emergence of SZ pathology later in life. Notably, we recently found similar biochemical and behavioral abnormalities reminiscent of SZ in adult mice with a complete deletion of the *Kmo* gene (*Kmo*$^{-/-}$ mice; Erhardt et al., 2017).

Biochemical and genetic studies using specimens from persons with SZ suggest that an impairment in KMO activity may, in fact, be *causally* related to the increased levels and function of KYNA in the brain of patients. Thus, analysis in cortical tissue obtained post-mortem revealed significant reductions in enzyme activity (Sathyasaikumar et al., 2011), and, abnormalities in the *Kmo* gene have been tentatively associated with SZ in three different patient populations (Aoyama et al., 2006; Holtze et al., 2012; Wonodi et al., 2011), as well as in *Kmo*$^{-/-}$ mice (Erhardt et al., 2017).

In order to combine genetic and environmental approaches in this context experimentally, the present study was designed to explore the possible impact of the maternal genotype on KP metabolism. To this end, we exposed pregnant wild-type (WT; *Kmo*$^{+/+}$) and heterozygous (HET; *Kmo*$^{+/-}$) mouse dams to kynurenine during the last week of gestation and investigated the effects on KYNA and other KP metabolites in maternal brain and plasma, as well as in the placenta and the fetal brain.

Materials and methods

Chemicals

L-kynurenine sulfate ("kynurenine"; purity: 99.4%) was obtained from Sai Advantium (Hyderabad, India). KP metabolites were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [$^2H_3$]quinolinic acid was obtained from Synfine Research (Richmond Hill, Ontario, Canada). Other chemicals were provided by various suppliers and were of the highest commercially available purity.

Animals

Wild-type (WT = *Kmo*$^{+/+}$) C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) or bred in-house. Heterozygous (HET = *Kmo*$^{+/-}$) C57BL/6 mice were generated as previously described (Giorgini et al., 2013). Animals were housed in a temperature-controlled, AAALAC-approved animal facility at the Maryland Psychiatric Research Center. The mice were kept on a 12 h:12 h light:dark cycle with free access to food and water. The study was carried out according to the "Principles of Laboratory Animal Care" (NIH publication No. 86-23, 1996) and was approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine.

Breeding and timed pregnancy

Sexually mature female mice (2-4 months-old) were synchronized by exposing them to bedding soaked with male urine (Whitten effect) for 4 days (Dalal et al., 2001). Next, one male was placed with a pair of naturally synchronized females for 48 hours in order to generate timed pregnant females. Two groups of pregnant females, derived from WT/WT and HET/HET breeding, respectively, were used in the study.

Upon removal of males, all females were weighed [embryonic day (ED) 1-2] to determine the baseline body weight. All females were weighed again 9 days after the removal of males.

All biochemical data were obtained from two groups of pregnant mice, generated by WT/WT (*Kmo*$^{+/+}$ dams) and HET/HET *(Kmo*$^{+/-}$ dams) breeding, respectively. Because of the natural distribution of the offspring of HET/HET breeding, and the resulting relative paucity of WT and knocked-out (KO) tissues derived from *Kmo*$^{+/-}$ dams, comparisons of fetal measures were made between offspring of *Kmo*$^{+/+}$ mice (WT *Kmo*$^{+/+}$*)* and the *heterozygous* offspring of *Kmo*$^{+/-}$ mice (HET *Kmo*$^{+/-}$*),* unless specified otherwise.

Treatment

Rodent chow was ground finely in a food processor, and each dam ate approximately 4 grams per day. For the treatment, beginning on ED 10/11, 10 mg of kynurenine was thoroughly mixed into the food daily for kynurenine-treated dams. The dose was chosen based on a previous study in rats (Pocivavsek et al., 2012). Control dams continued to receive wet mash alone. The dams were randomly assigned for the treatment. Animals were fed the diet daily in the morning (between 10:00 and 11:00 a.m.) in the home cage from ED 10/11 to ED 17/18 and were euthanized using CO_2 on ED 17/18. Animals were monitored to ensure that they were healthy, comfortable, well nourished, safe, able to express innate behavior, and not suffering from unpleasant states such as pain, fear, and distress.

Plasma and tissue collection

Trunk blood of the dams was collected in Eppendorf tubes containing 25 µl EDTA (0.5 M) as an anticoagulant. After centrifugation, the resulting supernatant plasma was frozen on dry ice and stored at -80ºC until the day of the assay. Whole brains minus cerebellum (from dams and fetuses) and placenta were rapidly dissected out, frozen on dry ice and stored at -80ºC. Tails from fetal embryos were used for genotyping as previously described (Giorgini et al., 2013).

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Determination of KP metabolites in plasma

On the day of the assay, samples were thawed and processed as follows:

Kynurenine, KYNA, 3-HK: maternal plasma was diluted (1:2, v/v; 1:10, v/v; 1:4, v/v, respectively) in ultrapure water. Next, 100 µl of each preparation was acidified with 25 µl of 6% perchloric acid. After centrifugation (16,000 x g, 15 min), metabolite levels were measured in 20 µl of the resulting supernatant by high performance liquid chromatography (HPLC) analysis, as previously described (Pocivavsek et al., 2012).

QUIN: maternal plasma was diluted in 0.1% ascorbic acid (1:10, v/v). Fifty µl of internal standard ([$^2H_3$]quinolinic acid) were added to 50 µl of the tissue preparation, and proteins were precipitated with 50 µl of acetone. After centrifugation (13,700 x g, 5 min), 50 µl of methanol:chloroform (20:50) were added to the supernatant, and the samples were centrifuged (13,700 x g, 10 min). The upper layer was added to a glass tube and dried down for 90 min. The samples were then derivatized with 120 µl of 2,2,3,3,3-pentafluoro-1-propanol and 130 µl of pentafluoropropionic anhydride at 75ºC for 30 min, dried down and reconstituted in 50 µl of ethyl acetate. One µl was then injected in the gas chromatography/mass spectrometry (GC/MS) apparatus (Notarangelo et al., 2012).

2.8 Determination of KP metabolites in tissue

All tissues were thawed and homogenized 1:5, w/v, in ultrapure water.

Kynurenine: placenta and fetal brain homogenates were further diluted 1:2, v/v, in ultrapure water. Next, 100 µl of each homogenate were acidified with 25 µl of 6% perchloric acid. After centrifugation (16,000 x g, 15 min), 20 µl of the resulting supernatant were subjected to HPLC analysis as above.

KYNA: placenta and fetal brain, but not maternal brain, homogenates were further diluted 1:10, v/v, in ultrapure water, and KYNA was measured as described above.

3-HK: placenta and fetal brain, but not maternal brain, homogenates were further diluted 1:4, v/v, in ultrapure water, and 3-HK was measured as described above.

QUIN: the original tissue homogenate was further diluted in 0.1% ascorbic acid (1:10, v/v, for fetal brain, 1:20, v/v, for placenta and 1:4, v/v, for maternal brain), and QUIN was measured by GC/MS as described above.

HPLC analyses were performed by an investigator who was unaware of the experimental protocol.

Protein determination

Protein was determined according to Lowry et al. (1951), using bovine serum albumin as a standard.

Statistical analyses

Data are expressed as the mean ± standard error of the mean (SEM). Data were analyzed using one-way, or two-way Anova followed by Tukey's post hoc test for multiple comparisons, as described in the text. Statistical significance was determined at $p < 0.05$, $p < 0.01$ and $p < 0.001$. All statistical analyses were performed using Prism software (GraphPad Software Inc.). No statistical methods were used to predetermine the sample size. Data points which qualified as outliers, as defined as differing from the mean by greater than 2 standard deviations, were eliminated from the data set. This accounted for no more than 5% of data points. Power analysis was not used in the present study.

Results

KP metabolism in the brain of Kmo$^{+/-}$ dams

Measurement of brain KMO activity in pregnant *Kmo*$^{+/-}$ mice revealed the expected significant reduction (6.20 ± 0.08 pmoles/h/mg protein vs. 16.97 ± 1.13 pmoles/h/mg protein; $p<0.001$) compared to *Kmo*$^{+/+}$ mice. No significant changes were observed in basal cerebral KYNA, 3-HK or QUIN levels in the brain of *Kmo*$^{+/-}$ mothers (Fig. 2A-C), although KYNA levels were ~2-fold higher in *Kmo*$^{+/-}$ dams than in *Kmo*$^{+/+}$ controls (Fig. 2A).

We next determined the effect of a kynurenine diet (10 mg/day), provided to the dam from ED 10/11 until ED 17/18, on cerebral KP metabolites. After kynurenine treatment, the levels of 3-HK and QUIN were significantly increased in the maternal brain of both genotypes (Fig. 2B, C). On the other hand, whereas KYNA levels in the maternal brain were significantly higher in *Kmo*$^{+/-}$ dams, only a trend towards an increase was observed in *Kmo*$^{+/+}$ mice (Fig. 2A). The tissue concentration of KYNA, but not of 3-HK and QUIN, was significantly higher (~2.5-fold) in the brain of kynurenine-treated *Kmo*$^{+/-}$ mothers than in the *Kmo*$^{+/+}$ controls (Fig. 2A-C).

Basal KP metabolites in the maternal plasma, placenta and fetal brain of HET *Kmo*$^{+/-}$ *mice*

No differences were observed in the levels of kynurenine and KYNA in the maternal plasma and in placental tissue derived from HET *Kmo*$^{+/-}$ mice as compared to WT *Kmo*$^{+/+}$ controls (Figs. 3A-F).

In the fetal brain, basal kynurenine and KYNA levels were similar in both groups of mice. Interestingly, a trend towards a reduction (~1.5-fold) in basal 3-HK and QUIN levels was seen in the fetal brain of HET *Kmo*$^{+/-}$ mice compared to respective WT *Kmo*$^{+/+}$ controls (Figs. 4A-F).

Effect of a maternal kynurenine diet on KP metabolites in the maternal plasma, placenta and fetal brain of WT *Kmo*$^{+/+}$ *and HET Kmo*$^{+/-}$ *mice*

Prenatal kynurenine treatment induced large increases in the levels of all KP metabolites measured in both genotypes (Figs. 3 and 4). KYNA levels in maternal plasma and placenta tended to be higher in the mutant animals (Figs. 3D,E) compared to WT *Kmo*$^{+/+}$ mice, but neither of these differences reached statistical significance ($p = 0.11$ each). Determination of the two other KP metabolites analyzed revealed trends towards lower 3-HK and QUIN levels in the maternal plasma and placentae of mutant mice compared to WT controls (Figs. 4A,B), but none of these differences reached statistical significance.

Significantly higher increases in kynurenine and KYNA levels were observed in the brain of fetuses of HET *Kmo*$^{+/-}$ dams as compared to WT *Kmo*$^{+/+}$ offspring (Figs. 3C,F) (genotype x treatment interaction $F_{1,19} = 5.712$, $p = 0.02$; $F_{1,19} = 5.547$, $p = 0.02$ respectively). 3-HK and QUIN in the fetal brain of HET *Kmo*$^{+/-}$ mice showed a trend toward lower levels without reaching statistical significance (Fig. 4C,F).

KYNA/3-HK ratio

Finally, to examine possible genotypic differences between the offspring of *Kmo*$^{+/-}$ mice, we determined the ratio of KYNA and 3-HK in the placenta and fetal brain of *all 3* genotypes derived from heterozygous pairing (i.e. WT *Kmo*$^{+/-}$, HET *Kmo*$^{+/-}$, KO *Kmo*$^{+/-}$) and compared each value to respective data obtained from the offspring of *Kmo*$^{+/+}$ parents (WT *Kmo*$^{+/+}$). This measure (KYNA/3-HK) provides information regarding the relationship of the two arms of the KP (Fig. 1).

Under basal conditions, the KYNA/3-HK ratio in the placenta of KO *Kmo*$^{+/-}$ and HET *Kmo*$^{+/-}$ showed a trend towards an increase compared to WT *Kmo*$^{+/+}$ mice (Fig. 5B, open bar) as well as in the fetal brain of KO *Kmo*$^{+/-}$ mice where it reached statistical significance ($p < 0.05$ vs. WT *Kmo*$^{+/+}$; Fig. 5C, open bar). The maternal kynurenine diet did not influence the KYNA/3-HK ratio in the fetal tissues (Figs. 5B,C, open bar vs. solid bar). Notably, the brain of WT *Kmo*$^{+/-}$ mice showed a similar trend as HET *Kmo*$^{+/-}$ mice, but the increase did not reach statistical significance compared to WT *Kmo*$^{+/+}$ controls. A non-significant trend towards an increase in the KYNA/3-HK ratio was also observed in the placenta of all mutant mice (Fig. 5C).

Discussion

The present study was based on several convergent premises and findings: 1) the increased KYNA levels seen in the brain of people with SZ may be relevant in the pathophysiology of the disease (Giorgini et al., 2013), although a considerable number of other genetic and environmental factors likely play causative roles as well (Misiak et al., 2018; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014); 2) elevated brain KYNA levels in SZ are accompanied by – and may be the result of – a decrease in cerebral KMO activity (Sathyasaikumar et al., 2011); 3) reduced KMO activity in SZ may be caused by abnormalities in the *Kmo* gene (Aoyama et al., 2006; Holtze et al., 2012; Wonodi et al., 2011); 4) complete elimination of *Kmo* in mice results in several of the phenotypic changes seen in psychiatric disorders (Erhardt et al., 2017; Parrott et al., 2016; Tashiro et al., 2017); and 5) in rats, prenatal treatment with a KMO inhibitor or with the immediate KYNA precursor kynurenine leads to structural and functional impairments reminiscent of SZ later in life (Forrest et al., 2013a; Forrest et al., 2013b; Khalil et al., 2014; Pershing et al., 2015; Pisar et al., 2014; Pocivavsek et al., 2014). Hypothesizing that experimental manipulation of mice deficient in only one *Kmo* allele (*Kmo*$^{+/-}$ mice) would be most relevant and informative with regard to the human disease, we decided here, in a first approach, to examine the effects of prenatal kynurenine treatment in these heterozygous animals.

As shown here, KMO activity is reduced to ~50% of wild-type levels in *Kmo*$^{+/-}$ mice, indicating that a single *Kmo* gene accounts for the expression of the enzyme. In line with the well-established shift of KP metabolism towards the KYNA arm of the KP when KMO is experimentally impaired in rodents (Carpenedo et al., 1994; Giorgini et al., 2013; Röver et al., 1997; Tashiro et al., 2017), we also found that KYNA levels were ~2.5 times higher in the brain of *Kmo*$^{+/-}$ dams, although the increase did not reach statistical significance. Notably, KYNA levels in the maternal plasma were several fold higher than those found previously in adult male mice (Giorgini et al., 2013). The cause and possible functional implications of this difference, which was recently confirmed in our laboratory (FM Notarangelo, unpublished data), ought to be examined further in the future.

Analysis of the major branch of the KP, leading to NAD^+, revealed a slight, non-significant reduction in the basal levels of 3-HK, the primary enzymatic product of KMO, and no changes in its downstream product, QUIN, in the maternal brain. Although these normal 3-HK and QUIN

levels could also be explained by a compensatory increased influx of KP metabolites from the circulation (Eastman et al., 1992; Fukui et al., 1991), it is more likely that the single remaining *Kmo* allele produces sufficient enzyme to maintain quantitatively normal production of 3-HK in the brain of *Kmo*$^{+/-}$ dams under physiological conditions. Regardless, the preferential effect of a partial *Kmo* elimination on KYNA levels in the maternal brain may have translational relevance (see below) and therefore clearly requires further study (see also Giorgini et al., 2013). Finally, and worth noting, no significant differences in any of the measured KP metabolites (kynurenine, KYNA, 3-HK and QUIN) were observed between the plasma of *Kmo*$^{+/+}$ and *Kmo*$^{+/-}$ dams. This indicates that the genotypic differences seen in the maternal brains are likely not the result of differential events in the blood.

Comparison of the placentae of WT *Kmo*$^{+/+}$ and HET *Kmo*$^{+/-}$, too, did not reveal significant differences in the basal levels of kynurenine, 3-HK and QUIN. Although the conversion of kynurenine to 3-HK in the placenta has not been studied so far, this finding was somewhat unexpected since this tissue expresses KMO (Alberati-Giani et al., 1997; Ligam et al., 2005), and KP metabolites downstream from 3-HK are readily recovered from cultured placental explants following exposure to tryptophan (Manuelpillai et al., 2005). In the present study, placental KYNA levels were also not significantly different between the two genotypes, though a tendency toward an increased KYNA/3-HK ratio was observed in HET and KO *Kmo*$^{+/-}$ placentae. Supporting the role of the *Kmo* gene on ambient KP dynamics *within* placental tissue, the ratio of KYNA and 3-HK was, in fact, shifted toward higher (~4.5 fold) KYNA formation in the placenta of KO *Kmo*$^{+/-}$ embryos (cf. Fig. 5B, open bar). Overall, however, our results indicate that a partial reduction of placental KMO has only a relatively moderate influence on local KP metabolism under normal physiological conditions.

In line with observations in other mammalian species (Beal et al., 1992; Cannazza et al., 2001; Walker et al., 1999), the endogenous concentrations of KYNA, 3-HK and QUIN were found to be several-fold higher in the fetal than in the adult mouse brain. Moreover, our results indicate that KMO participates in the control of cerebral KP metabolism *in utero*. Thus, endogenous 3-HK levels in the fetal brain were higher than in the placenta, and there was a trend toward lower 3-HK and QUIN levels in the brain of *Kmo*$^{+/-}$ offspring. As in the placenta (see above), the KYNA/3-HK ratio was substantially higher in the brain of KO *Kmo*$^{+/-}$ mice than in the brain of *Kmo*$^{+/+}$ offspring (Fig. 5A,B), further supporting a role of KMO as a regulator of KP metabolism in the fetal brain.

KP analyses following dietary supplementation of pregnant dams with kynurenine essentially confirmed the conclusions drawn from the study of basal KP metabolites. This treatment mimics the downstream effects of prenatal activation of the tryptophan-degrading enzymes indoleamine-2,3-dioxygenase and tryptophan 2,3-dioxygenase (Fig. 1), which have been repeatedly implicated in the pathophysiology of a variety of (mainly psychiatric) brain diseases later in life. Kynurenine supplementation in rodents is therefore highly informative, resulting in an impressive array of molecular and behavioral changes in treated offspring (see Introduction). As anticipated from similar experiments in rats (Pocivavsek et al., 2014), kynurenine administration from ED 10/11 to ED 17/18 increased the levels of KYNA and 3-HK, as well as QUIN (shown here for the first time), in all compartments examined. Although some trends toward disproportionately elevated KYNA and reduced 3-HK and QUIN levels were observed in the maternal plasma and in the placentae of kynurenine-treated *Kmo*$^{+/-}$ dams compared to their *Kmo*$^{+/+}$ counterparts, none of these differences attained statistical significance. Notably, however, the significantly higher levels of KYNA recovered from the brain of kynurenine-treated *Kmo*$^{+/-}$ dams paralleled the trend in genotype effect seen in basal KYNA levels. Although a small proportion of KYNA can enter the brain from the circulation (Scharfman and Goodman, 1998; Swartz et al., 1990), these results reinforce the conclusion that a partial reduction in cerebral KMO directs *local* KP metabolism towards increased KYNA synthesis – with minimal or no effect on the brain levels of 3-HK and QUIN.

The most pronounced genotypic differences in KP metabolism following prolonged prenatal kynurenine administration were found in the fetal brain. Thus, in the brain of HET *Kmo*$^{+/-}$ offspring of treated *Kmo*$^{+/-}$ dams, the increases in both kynurenine and KYNA levels were significantly greater than in the brain of embryos derived from *Kmo*$^{+/+}$ parents (WT *Kmo*$^{+/+}$*).* On the other hand, maternal kynurenine treatment was *less* effective with regard to 3-HK and QUIN synthesis in embryos derived from HET/HET pairing than in the offspring of *Kmo*$^{+/+}$ mice. As a consequence, the increase in the KYNA/3-HK ratio in the brain of these embryos was different from WT *Kmo*$^{+/+}$ offspring (~ 5 fold in KO *Kmo*$^{+/-}$ offspring and ~2 fold in HET *Kmo*$^{+/-}$ offspring). In addition, the KYNA/3-HK ratio in the brain of fetal WT *Kmo*$^{+/-}$ mice, too, showed a tendency to differ from WT *Kmo*$^{+/+}$ controls (Fig. 5C). Taken together, these data not only confirm the regulatory role of KMO in the fetal brain but also suggest that this role becomes more evident, and therefore possibly more functionally relevant, in situations of increased influx of kynurenine from the mother.

The present study, which focused on events in the prenatal period, has relevance for both physiology and pathology. As expected, and in contrast to homozygous *Kmo* knockout mice

(Erhardt et al., 2017; Parrott et al., 2016; Tashiro et al., 2017), animals with a partial elimination of the *Kmo* gene showed relatively minor impairments in KP metabolism. This reduction in KMO activity caused a shift away from the main KP branch – leading to NAD^+ – toward the enhanced production of KYNA. Interestingly, this effect, which was seen most clearly in the brain and was less pronounced in the placenta and the maternal plasma, more evident after the pregnant dam was fed kynurenine. As even moderate increases in brain KYNA, probably by initially targeting α7 nicotinic receptors (Flores-Barrera et al., 2017), adversely affect cognitive processes in adult animals (see Introduction), the present results imply a direct link between the *Kmo* gene and *maternal* cognitive functions during pregnancy. Moreover, functional impairments may be exacerbated in genetically susceptible females when kynurenine production is enhanced due to untoward environmental influences, including stress (Notarangelo and Schwarcz, 2016), infections (Notarangelo et al., 2015; Williams et al., 2017) or other challenges to the immune system (Larsson et al., 2016). It should be possible to test this hypothesis directly in pregnant women by combining genetic and cognitive testing with the monitoring of kynurenine (and ideally also other KP metabolites) and inflammatory markers in the blood.

Presumably related to the resulting surge in KYNA levels in the fetal brain, prenatal kynurenine feeding or pharmacological KMO inhibition affect neuronal excitability and lead to disruptions in cognitive functions, as well as a reduction in dendritic spine density, in adolescent and adult rats (Alexander et al., 2012; Forrest et al., 2013a; Forrest et al., 2013b; Pershing et al., 2015; Pershing et al., 2016; Pisar et al., 2014; Pocivavsek et al., 2014). The finding that prenatal kynurenine treatment causes disproportionately higher cerebral KYNA levels in a heterozygous fetus derived from a genetically susceptible mother ($Kmo^{+/-}$) now demonstrates that the maternal *Kmo* genotype is likely to significantly affect the structure and function of the brain later in life.

This conclusion may be of special relevance for the pathophysiology of SZ, where both the maternal environment and the maternal genotype are known to play significant etiological roles (Gleason et al., 2011; Martin et al., 2016; Zupan and Toth, 2008). As several adverse events during the perinatal period, such as stress and infection, stimulate KP metabolism (see Introduction), the present results indicate that such environmental influences could increase the likelihood that children of mothers with functional *Kmo* gene abnormalities develop SZ later in life. Ongoing studies in our laboratories are therefore designed to carefully investigate the postnatal development and vulnerabilities of the genetically diverse progeny of $Kmo^{+/-}$ mice.

Conflict of interests

The authors declare no financial or other conflict of interest.

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Figure legends

Figure 1: The kynurenine pathway of tryptophan degradation in mammals.

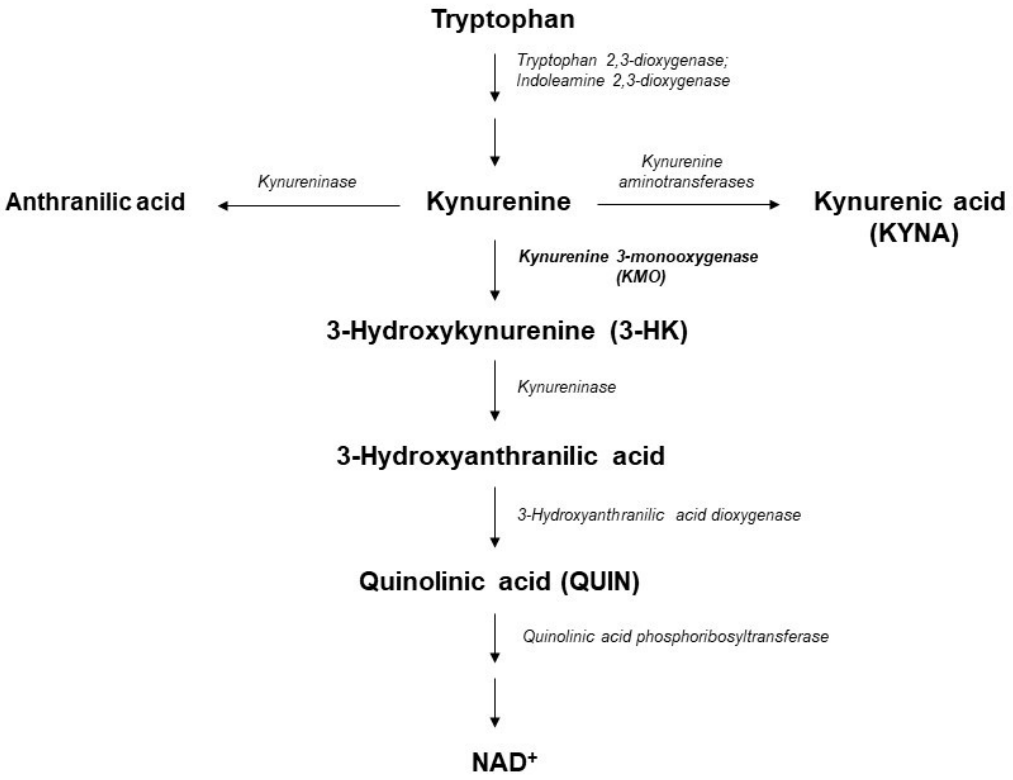
Figure 2: KYNA (**A**), 3-HK (**B**) and QUIN (**C**) levels in the brain of untreated ("Basal") and treated ("Treatment") *Kmo*$^{+/+}$ and *Kmo*$^{+/-}$ dams. Animals were treated with kynurenine (10 mg/day) from ED10/11 until ED17/18. Data are the mean ± SEM (n = 5-6 dams per group). * $p < 0.05$ vs. *Kmo*$^{+/+}$ treatment controls; $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$ vs. the respective basal values; two-way ANOVA followed by Tukey's *post hoc t*-test for multiple comparisons. Panel A: $F_{1,19} = 3.376$ ($p = 0.08$); Panel B: $F_{1,17} = 0.001$ ($p = 0.97$); Panel C: $F_{1,16} = 0.074$ ($p = 0.78$).

Figure 3: Effect of prenatal kynurenine treatment on kynurenine (**A-C**) and KYNA (**D-F**) levels in the maternal plasma, placenta and fetal brain of *Kmo*$^{+/+}$ offspring (WT Kmo$^{+/+}$) and heterozygous offspring of *Kmo*$^{+/-}$ dams (HET *Kmo*$^{+/-}$). Data are the mean ± SEM (plasma values: n = 3-6 dams per group; tissue values: n = 5-6 dams per group, n = 3-4 embryos per dam). * $p < 0.05$ vs. *Kmo*$^{+/+}$ treatment controls; $^{\#}$ $p < 0.05$; $^{\#\#\#}$ $p < 0.001$ vs. the respective basal values; two-way ANOVA followed by Tukey's *post hoc t*-test for multiple comparisons. Panel A: $F_{1,18} = 0.658$ ($p = 0.42$); Panel B: $F_{1,19} = 1.846$ ($p = 0.19$); Panel C: $F_{1,19} = 5.712$ ($p = 0.02$); Panel D: $F_{1,18} = 2.331$ ($p = 0.14$); Panel E: $F_{1,19} = 2.216$ ($p = 0.15$); Panel F: $F_{1,19} = 5.547$ ($p = 0.02$).

Figure 4: Effect of prenatal kynurenine treatment on 3-HK (**A-C**) and QUIN (**D-F**) levels in maternal plasma, placenta and fetal brain of *Kmo*$^{+/+}$ offspring (WT Kmo$^{+/+}$) and heterozygous offspring of *Kmo*$^{+/-}$ dams (HET *Kmo*$^{+/-}$). $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$ $^{\#\#\#}$ $p < 0.001$ vs. the respective basal values; two-way ANOVA followed by Tukey's *post hoc t*-test for multiple comparisons. Panel A: $F_{1,11} = 1.696$ ($p = 0.21$); Panel B: $F_{1,19} = 0.063$ ($p = 0.80$); Panel C: $F_{1,19} = 0.824$ ($p = 0.37$); Panel D: $F_{1,10} = 0.603$ ($p = 0.45$); Panel E: $F_{1,19} = 1.583$ ($p = 0.22$); Panel F: $F_{1,19} = 0.074$ ($p = 0.78$).

Figure 5: KYNA/3-HK ratio in the placenta and fetal brain of WT *Kmo*$^{+/+}$ mice and three genotypes of offspring derived from *Kmo*$^{+/-}$ dams (WT *Kmo*$^{+/-}$, HET *Kmo*$^{+/-}$, KO *Kmo*$^{+/-}$). Data are the mean ± SEM (n = 5-6 dams per group; n = 3-4 embryos per dam). **A:** Breeding chart; **B**: Basal values (open bars); **C**: Kynurenine-treated animals (solid bars). * $p < 0.05$; ** $p < 0.01$; one-way ANOVA followed by Tukey's *post hoc t*-test for multiple comparisons.

Figure 1


Tryptophan
Tryptophan 2,3-dioxygenase;
Indoleamine 2,3-dioxygenase
Kynureninase
Anthranilic acid
Kynurenine
Kynurenine
aminotransferases
Kynurenic acid
(KYNA)
Kynurenine 3-monooxygenase
(KMO)
3-Hydroxykynurenine (3-HK)
Kynureninase
3-Hydroxyanthranilic acid
3-Hydroxyanthranilic acid dioxygenase
Quinolinic acid (QUIN)
Quinolinic acid phosphoribosyltransferase
NAD+

1

Figure 2

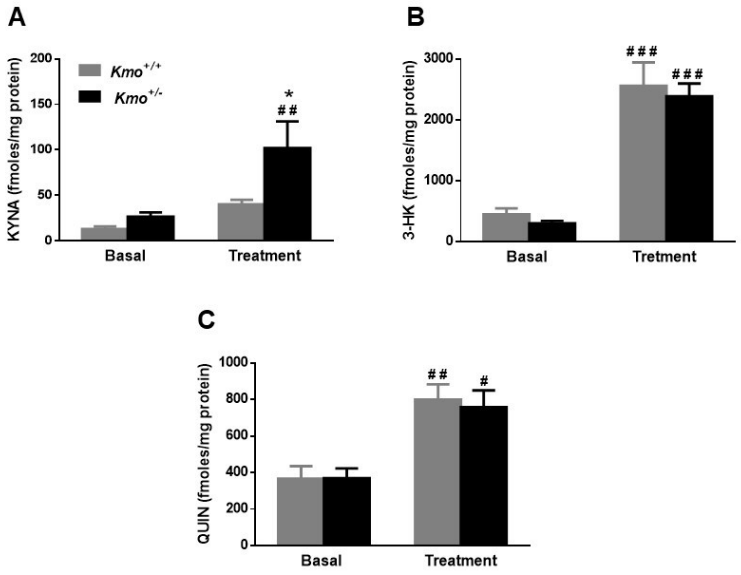


Figure 3

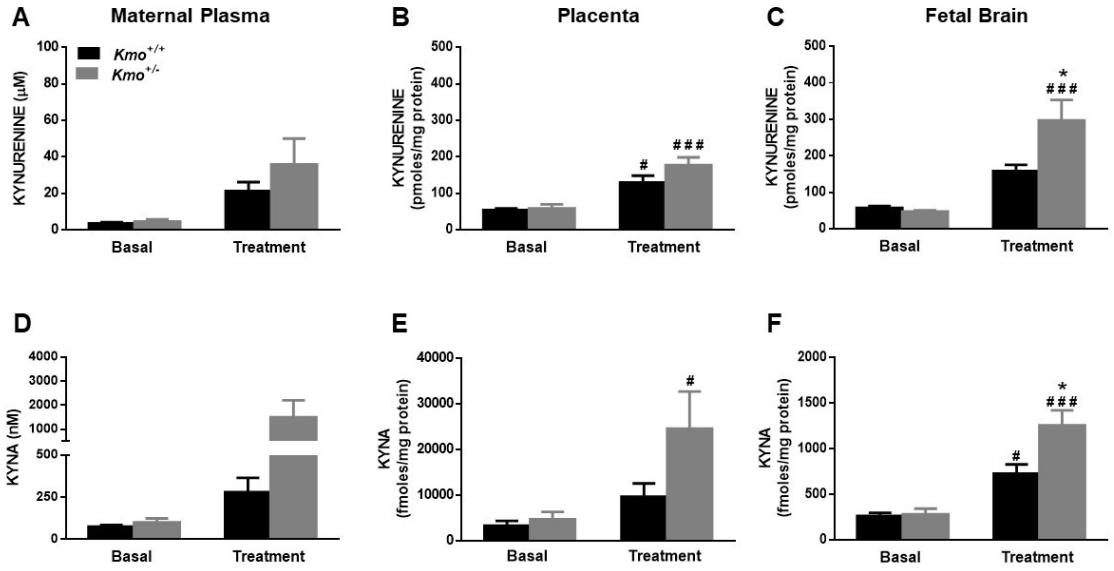


Figure 4

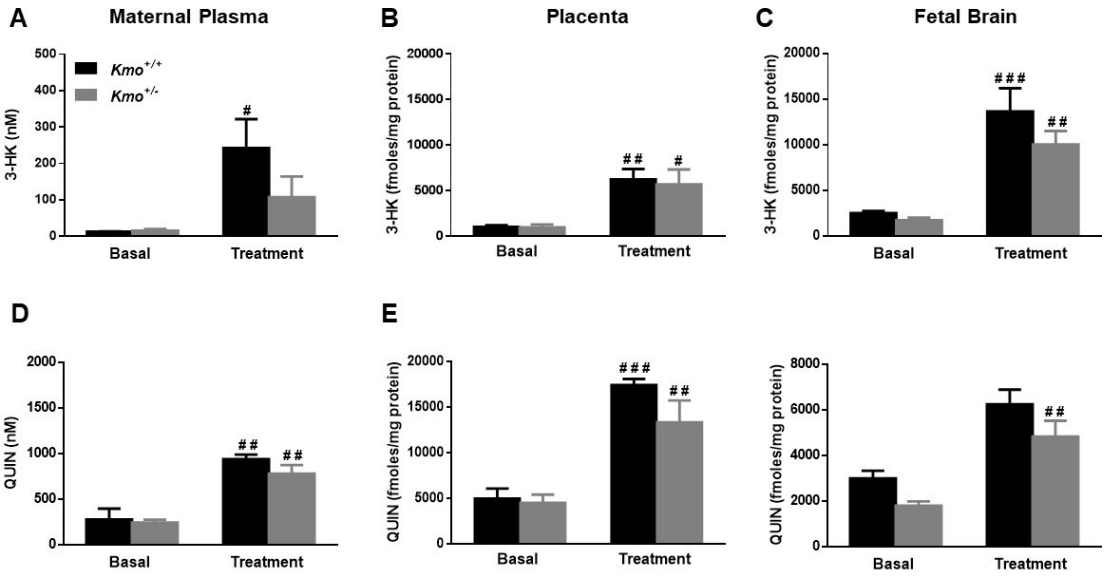


Figure 5