Schizophrenia Research 229 (2021) 27–37

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Association of the kynurenine pathway metabolites with clinical, cognitive features and IL-1β levels in patients with schizophrenia spectrum disorder and their siblings

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ARTICLE INFO

Article history:
Received 19 August 2020
Received in revised form 11 January 2021
Accepted 19 January 2021
Available online xxxx

Keywords:
Cytokine
Inflammation
Kynurenine
Liquid chromatography
Schizophrenia
Tryptophan

ABSTRACT

Objective: There is evidence suggesting that tryptophan (TRP)-kynurenine (KYN) pathway dysregulation is involved in the pathophysiology of schizophrenia and is regulated by inflammatory cytokines. The study investigate for the first time whether this dysregulation occurs in advanced stages of the disease as a byproduct or emerges as one of the early and inherited manifestations of schizophrenia.
Method: Sera of 148 patients with schizophrenia spectrum disorders (SCZ), 139 unaffected siblings (SIB) and 210 controls were investigated. Serum interleukin (IL)-1β levels were measured by ELISA, and TRP, KYN and kynurenic acid (KYNA) levels were measured by a high-performance liquid chromatography system. Also, we collected clinical data by applying Comprehensive Assessment of Symptoms and History in SCZ, and SIS-R in SIB and control groups.
Results: Compared to controls, SCZ and SIB groups had lower TRP and higher KYNA levels. TRP levels showed significant differences only between SCZ and controls ($p < 0.01$). KYNA levels of both SCZ ($p \leq 0.001$) and SIB ($p < 0.05$) were higher than controls. No statistical significance was found for KYN levels across groups. SCZ and SIB groups had higher serum IL-1β levels than controls ($p \leq 0.001$).
Conclusions: Patients with SCZ and their siblings exhibited similar clinical features and TRP metabolite levels suggesting that TRP-KYN dysregulation may be an inherited component of the disease putatively conferring increased risk to schizophrenia. Elevation of IL-1β is one of the factors promoting overconsumption of the TRP-KYN pathway leading to increased production of neuroregulatory KYNA and presumably to neurodegeneration.
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1. Introduction

Schizophrenia is characterized by positive symptoms such as hallucinations and delusions, negative symptoms such as emotional withdrawal and apathy and findings of cognitive dysfunction such as attention, learning, and memory (Buchanan and Carpenter, 1994; Seeman, 1997). The lifetime prevalence of schizophrenia is about 0.5% (Saha et al., 2005) and genetic and environmental factors influence development of the disease (van Os et al., 2008).

Although dysregulated dopaminergic neurotransmission is known to be involved in the pathogenesis of schizophrenia, dopamine antagonists mainly treat positive symptoms without significantly affecting negative symptoms and cognitive dysfunction (Müller et al., 2013). Recent studies have suggested that glutamatergic dysfunction may lie beneath dopaminergic dysregulation (Swerdlow et al., 2009). Notably, glutamatergic system is regulated by the tryptophan (TRP)-kynurenine (KYN) pathway, which can be activated by inflammatory cytokines

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https://doi.org/10.1016/j.schres.2021.01.014
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(Müller and Schwarz, 2007). Several genetic, neuropathological and clinical studies suggest an involvement of the immune system in the pathogenesis of schizophrenia (Fillman et al., 2013; Müller et al., 2013). Moreover, increased levels of inflammation mediators have been reported in post-mortem brain and plasma samples of patients with schizophrenia (Fillman et al., 2016). Altogether, these findings indicate that the interaction between KYN pathway and cytokines may be centrally involved in the pathogenesis of schizophrenia.

In mammals, TRP is primarily metabolized by the KYN pathway resulting in several neuroactive downstream products such as the *N*-methyl-D-aspartate receptor (NMDAR) agonist quinolinic acid and the NMDAR and α7-nicotinic acetylcholine receptor (AChR) antagonist kynurenic acid (KYNA) (Schwarcz and Pellicciari, 2002). However, it is also reported that KYNA may regulate neuronal excitability and plasticity by its action on nicotinic receptors (nAChRs) in the CNS (Hilmas et al., 2001). In line with this, a recent review (Stone, 2020) has extensively discussed that there is some controversy as to whether KYNA acts to inhibit the α7-nicotinic receptor. The review (Stone, 2020) has remarked that KYNA activates the aryl hydrocarbon receptor (AHR), which is also significant regulators of neuronal development, differentiation, and synaptic function (Huang et al., 2004, 2011). Therefore, contrary to the other kynurenine pathway products, including quinolinic acid, the KYNA is considered as a neuroregulatory, not neurotoxic.

Dysregulation of KYN pathway has been associated with neurodegenerative disorders, depression, and schizophrenia (Schwarcz et al., 2012). Increased levels of KYN pathway products have been shown in brain tissue and cerebrospinal fluid (CSF) samples of patients with chronic schizophrenia (Linderholm et al., 2012; Sathyasaikumar et al., 2011; Wang and Miller, 2018), and in sera of first-episode neuroleptic-naive patients with schizophrenia (Condray et al., 2011).

Dysregulation of TRP-KYN pathway is also associated with microglial activation and subsequent release of inflammatory cytokines (Dantzer et al., 2011; Myint et al., 2012). Notably, patients with psychosis display enhanced serum levels of microglia-derived cytokines IL-6, IL-1β and tumor necrosis factor α (TNF-α) (Mohammadi et al., 2018) and cytokine elevation may be one of the underlying mechanisms of TRP-KYN pathway (Johansson et al., 2013). Also, elevated serum cytokine levels are associated with higher KYN and KYNA levels, reduced attention performance, executive functions and prefrontal cortex volume (Fillman et al., 2016; Kindler et al., 2020). Kynurenine metabolites and cytokine levels are also associated with psychiatric symptoms and treatment response in chronic and first-episode patients with schizophrenia (Chase et al., 2016; Condray et al., 2011; Myint et al., 2011).

First-degree relatives of patients with schizophrenia may often exhibit subthreshold psychiatric symptoms and signs of biological dysfunction without fulfilling the criteria for schizophrenia and thus, they putatively represent endophenotypes of the disease (Gottesman and Gould, 2003). Therefore, investigation of the interaction between TRP-KYN pathway, cytokines and psychiatric-cognitive features in first-degree relatives of patients with schizophrenia may provide valuable clues about the pathogenesis of the disease. To our knowledge, there is a single such study focused on twin pairs discordant for schizophrenia or bipolar disorder showing elevated CSF cytokine levels that correlate with TRP-KYN pathway metabolite levels. Moreover, in this study, CSF KYNA levels have been associated with psychotic symptoms, paranoid, schizoid and schizotypal personality disorders, indicating a genetic influence on cytokine-induced TRP-KYN pathway dysregulation (Kegel et al., 2017).

In this study by measuring serum TRP, KYN, KYNA, and IL-1β levels in patients with schizophrenia (SCZ), their unaffected siblings (SIB) and controls, we initially aimed to specify differences in the peripheral measurements of TRP-KYN pathway metabolites and IL-1β in patients together with their siblings, relative to controls. Also, our purpose was to investigate whether cytokine-associated TRP-KYN pathway dysregulation observed in schizophrenia is an intermediate phenotype. For this purpose, we analysed the presence of potential correlations between levels of these mediators and clinical features in three study samples. Furthermore, in line with our this aim and depending on relevant previous findings (Fillman et al., 2016; Kindler et al., 2020 e.g.), we examined whether differences in terms of the TRP-KYN pathway metabolites levels and also, clinical and cognitive features among cytokine subgroups of patients with schizophrenia and their siblings whose were classified on being to the lower and higher measurable levels of serum IL-1β. We hypothesized that TRP would activate the KYN pathway rather than the serotonin pathway in SCZ and SIB groups. Therefore, KYN and KYNA serum levels of two groups might be significantly higher compared to controls. Also, we expected that serum levels of IL-1β as an indicator of inflammation, would be higher in SCZ and SIB groups than controls. Finally, we assumed that levels of KYN pathway metabolites and IL-1β would be correlated with clinical symptoms in SCZ and SIB groups.

2. Material and methods

2.1. Participants

This study was conducted with 150 patients were diagnosed with schizophrenia spectrum disorders according to the DSM-IV-TR, their unaffected siblings (n = 140) and 214 controls was conducted, all of whom were selected from among the participants of a large gene-environment interaction study: European Network of National SCZ Networks studying Gene-Environment Interactions (EU-GEI), Work Package 6 (Vulnerability and Severity). Details of the EU-GEI project were provided elsewhere (European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EU-GEI) and van Os, 2014; Guloksuz et al., 2019). The diagnosis was later confirmed by the Operational Criteria Checklist for Psychotic and Affective Illness (McGuffin et al., 1991), based on the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) F20 criteria. Since this study is a part of a larger study which focused on possible interactions between genetics and life events in childhood and adolescence, only full siblings were included in the study. There were no twins in the study sample. The siblings with an age difference of >5 years with the patients were also excluded. By doing this, we aimed to study the siblings who shared the same environment in their childhood. The controls with no lifetime psychotic disorder were recruited from the same population as the cases. Exclusion criteria for all participants were diagnosis of psychotic disorder due to another medical condition, a history of head injury with loss of consciousness, and an intelligence quotient <70.

The projects of the EU-GEI were approved by the medical ethics committees of all participating sites and conducted in accordance with the Declaration of Helsinki. Also, the present study was approved by the Ethics Committee of Istanbul University, Faculty of Medicine for Clinical Research Ethics. All participants gave their written informed consent.

2.2. Clinical and cognitive assessments

Clinical and cognitive assessments were applied via face-to-face interviews at the research site for the sibling-pairs, and at home for the control group. All interviews were conducted by a research team who received specific training for the use of standardized assessments. Clinical assessment of the patients with schizophrenia, their unaffected siblings and the controls included Nottingham Onset Schedule (NOS)-Modified DUP version (Singh et al., 2005), CASH (Andreasen et al., 1992) used to rate the severity of psychotic symptoms only in the patients, the Structured Interview for Schizotypy-Revised (SIS-R) (Vollema and Ormel, 2000) applied to determine subclinical positive and negative symptoms in the siblings and controls. Also, psychosocial functioning was examined in all groups using the Global Assessment Functioning (GAF) Scale (Moos et al., 2000), as described in the DSM-IV. In addition to clinical assessment, sociodemographic information was obtained for all groups.

The brief version of the Weschler Adult Intelligence Scale-Third Edition (Brief WAIS-III R), including the subscales of Arithmetic, General Information, Digit-Symbol Coding, and Block Design was used of global IQ (Wechsler, 1997). As the reliability and validity of WAIS-III R in Turkey are in progress, age-adjusted *Z*-scores-based on the present sample- were used in statistical analyses, since normative data for WAIS-III R are not available from any other study. Also, the Beads Task was used to assess jumping to conclusion (Beck and Rector, 2005).

2.3. Measurement of serum TRP, KYN, KYNA and IL-1β levels

All sera (obtained from fasting blood samples in the morning) collected from patients, siblings and healthy controls were kept at −80 °C until use (Teunissen et al., 2014). In case of temporary clinical infection (e.g. upper respiratory infection or flu), sampling postponed until patient is fully recovered from infection.

Sample preparation procedure was carried out by mixing 150 μL serum with an equal volume of 0.6 mol/L $HClO_4$ in Eppendorf tubes, which were vortexed and centrifuged at 12000 rpm for 5 min in room temperature to precipitate protein. Volumes of 100 μL were injected onto the column for the calibration curves to calculate TRP, KYN and KYNA concentrations. The measurements of serum TRP, KYN and KYNA were performed using an HPLC system (Azura p2.1L system with ultraviolet detector, UV, Krauer, Germany, and LC-20A fluorescence detector, FLD, Shumadzu, Japan) including a C18 column (Agilent HC-18, 250× 4.6 mm i.d.; 5 m particle size). The mobile phase, freshly prepared prior to the study, was composed of 20 mmol/L NaAc, 3 mmol/L $ZnAc_2$ and 7% acetonitrile, filtered through a 0.45-μM membrane filter and degassed by an ultrasonic equipment for 20 min. UV condition was 365 nm wavelength for KYN analysis and FLD conditions were excitation 344 nm with detection at emission 398 nm for TRP and KYNA analysis for the simultaneous analysis of TRP, KYN and KYNA as previously reported (Zhao et al., 2010). Separation was achieved at an ambient temperature with a flow rate of 1 mL/min in 40 min. The concentrations were calculated from peak areas.

In the current study, a ratio of KYN/TRP was calculated in which levels of KYN divided by mmol expression of the TRP considering a confounder effect of the reducing in dietary intake of TRP on lower plasma TRP and KYN levels. Results are expressed as KYN(μM/L)/TRP(mmol/L) ratios (KTRμM/mmol).

Serum levels of IL-1β were measured with a commercial ELISA kit (Abcam, Cambridge, UK) as per manufacturer's recommendations. Optical density was measured at 450 nm, and concentrations were calculated by referring to a standard curve. Results are expressed as pg/mL.

Low and high cytokine subgroups in two study samples were identified as the based on the measurement sensitivity of ELISA in which was 1 pg/mL in measurable levels of IL-1β.

2.4. Statistical analysis

Statistical Package for the Social Sciences (SPSS) 21 software was used for all statistical analyses. To provide parametric distribution univariate outliers for continuous variables were detected by converting to *Z*-standard scores where Z value in excess of ±3.29 ($p \leq 0.001$, two-tailed) was considered as an univariate outlier (Tabachnick and Fidell, 2013). Thus, the data-sets of total 7 participants (4 controls, 2 patients, 1sibling) were excluded from our large data, and all statistical analyses were been conducted with number of 148 patients, 139 unaffected siblings, and 210 controls (Table 1).

The distribution of data was determined with a normality test of Kolmogorov-Smirnov method (for all, $p > 0.05$). Also, additional criteria were regarded for normality assumption depending on the assessment of skewness and kurtosis statistics for each variable and visual inspection of the data set was performed using histograms, boxplots, normal P—P plots and Q-Q plots.

For data-sets of the variables detected not to show an approximately normal distribution, A data transformation process including the functions of a logarithmic (log) at logarithm base 10 (for positively skewed data), and a reflecting log 10 (for negatively skewed data) was applied. Thus, our data-sets of IL-1β, KTR, GAF, number of beads scores and age variables were transformed. After the transforms, normal distributions were been approximately achieved for all continuous variables.

When a normality assumption statistically rejected by the Kolmogorov-Smirnov test, an approximately normal distribution were accepted in following ways: (1) Having the skewness statistic in a value less than ±1. (2) As a result of dividing the statistics of skewness/kurtosis by standard errors of those, finding a value less than at ±3.29 (Tabachnick and Fidell, 2007, 2013). (3) Confirmation of visual inspected to a relative bell-shaped curve. These criteria are been validated in both data-sets to raw and transformed.

The parametric statistical comparisons of groups were executed using a one-way or two-way variance/covariance analyses and independent samples *t*-test. Whereas, comparisons of the data-sets for ordinal variables (in here, all scores of clinical instruments providing a Likert scale), were done with non-parametric a Mann Whitney U or Kruskal-Wallis H tests, where in applicable. Categorical variables were analysed using a Pearson chi-square test.

Additional assumptions of a linearity and homogeneity of regression slopes among dependent variables or covariate, effects of interaction between fixed factor(s) and covariate, a multicollinearity of variables in proposed model were tested to covariance analyses. Thus, differences in TRP and KTR metabolites in which were been provided all assumptions of covariance analysis, between groups and sex were assessed using analyses of two-way covariance (ANCOVA) with covaried age, and following that all significant results, post-hoc test of Bonferroni adjusted for multiple comparisons, were used. However, alterations of other metabolites (KYN, KYNA) and IL-1β levels relative to group were tested by analyses of one-way variance (ANOVA) while sex differences were performed by the independent samples *t*-tests separately. Because all of the combined variance/covariance analysis assumptions could not be met by the data-sets of these variables. Additional multiple comparisons of one-way variance analyses were conducted with the Tukey HSD (Honestly Significant Difference) or Tamhane's T2 tests for homogeneous and non-homogeneous variances, respectively.

Relationships between the continuous variables of levels/ratios TRP-KYN pathway metabolites were investigated with a partial correlation analysis using a Pearson's correlation coefficients when controlling the effect of age; whereas were performed by a Spearman's rank correlation coefficients in ordered variables (e.g. Likert scale in clinical measurements) in all groups separately.

Before analysing of multiple correlations all necessary assumptions (the normality, linearity, multi-collinearity, homogeneity e.g.) have been checked. Thus, KYNA was not been used in analysing of multiple correlations due to be creating of a multi-collinearity (r value≥0.9). As well, correlations of IL-1β and KYN pathway metabolites tested by the non-parametric Spearman correlation analysis since, our IL-1β data-sets did not met further assumptions for parametric analyses of multiple correlations.

The threshold of statistical significance was set at $p < 0.05$, two-tailed. However, in analysing of multiple comparisons or correlations were applied a Bonferroni correction/adjusted *p* value, where was divided the determined significance level by the number of group or variables. Thus, in analyses of multiple comparisons, a two-tailed *p* of ≤0.02; in multiple correlations at $p \leq 0.01$

were considered to statistically significant, and are reported both as uncorrected and corrected. A clinical significance as an effect size (ES) was been determined using a partial eta squared as an indicator of amount of explained variance, on testing in comparation of groups based on *F or t* statistic test. In analysing of correlations, effect size is been used representing a coefficient of determination estimating by a r-squared, on which relation strength is used as additional relation size (RS) determination of clinical significance. The threshold of clinical significance was set at RS ≥ 0.10, and results are presented by together with *p* values by interpreting as regards Cohen's d (Cohen, 1988) thresholds specified in the notes of relevant Tables.

3. Results

3.1. Sociodemographic, clinical and cognitive characteristics of the participants

Sociodemographic and clinical characteristics of study groups were presented in Table 1.

There were more males in SCZs than both groups to SIB (χ^2 = 28.90, $p \leq 0.001$, corrected) and control (χ^2 = 65.93, $p \leq 0.001$, corrected), and the controls were significantly older than the other two groups (for all, $p \leq 0.001$, corrected). Moreover, all SIS-R subscores were significantly high in SIBs than controls (Table 1).

Table 1
Sociodemographic and clinical characteristics with cognitive performances and statistical comparison of the groups.

Sociodemographic characteristics	Group			χ^2/Z/F/RBF, *p*
	SCZs (n = 148)	SIBs (n = 139)	Cnts (n = 210)	
Sex, female/male, n (%)	37(25)/111(75)	78 (56.1)/61(43.9)	144(68.6)/66(31.4)	χ^2 = 67.28, **$p \leq 0.001$*****
Age/log age, mean (SD)	31.64(8.65)/1.49(0.11)	31.29(9.98)/1.47(0.13)	36.72(11.45)/1.54(0.14)	RBF = 14.9, **$p \leq 0.001$*****
Education (years), mean (SD)	11.24 (3.87)	11.54 (4.22)	11.16 (4.51)	RBF = 0.30, p = 0.74
Marital status, single/married or living with somebody, n (%)	121(88.3)/16 (11.7)	70(63.1)/41(36.9)	83(39.9)/125(60.1)	χ^2 = 81.28, **$p \leq 0.001$*****
Job status, unemployed*/employed/Student, n (%)	97(71.3)/26(19.1)/ 13 (9.6)	37(33.6)/60(54.5)/ 13(11.8)	67(32.8)/124(60.8)/13(6.4)	χ^2 = 65.94, **$p \leq 0.001$*****
DUP (weeks), mean (SD)	85.03 (138.02)	N/A	N/A	N/A
Duration of disease (months), mean (SD)	67.21 (63.06)	N/A	N/A	N/A
Type of AP medication, typical/atypical/both n (%)	8(6.6)/103(85.1)/10(8.3)	NIA	N/A	N/A
Smoking, yes/no, n (%)	69 (55.6)/55 (44.4)	59 (45.4)/71 (54.6)	91(43.8)/117(56.3)	χ^2 = 4.70, p= 0.09
BMI, mean (SD)	25.24 (5.63)	25.03 (3.53)	25.31 (4.25)	RBF = 0.04 p= 0.96
Clinical assessment scales, median (IQR)				
CASH, total score of delusions	14 (9–19)	N/A	N/A	N/A
CASH, total score of severity of delusions	4 (3–4)	N/A	N/A	N/A
CASH, total score of hallucinate.	5 (1–9)	N/A	N/A	N/A
CASH, total score of severity of hallucinations	3 (1–4)	N/A	N/A	N/A
CASH, total score of Schneiderian symptoms	5 (1.5–11)	N/A	N/A	N/A
CASH, total score of negative symptoms	11 (9–13)	N/A	N/A	N/A
GAF score, symptom/log GAF symptom, mean (SD)	44.22(14.39)/1.74(0.13)	76.31(13.16)/1.32(0.29)	87.97(4.12)/1.09 (0.15)	RBF =375.20, **$p \leq 0.001$*****
GAF score, functioning/log GAF functioning, mean (SD)	48.78(14.99)/1.69(0.16)	78.73(12.07)/1.27(0.31)	89.09(3.95)/1.05 (0.15)	RBF =314.02, **$p \leq 0.001$*****
SIS-R, social isolation(long)	N/A	0 (0–1)	0 (0–0)	Z = −4.71, **$p \leq 0.001$*****
SIS-R, introversion	N/A	1 (0–1)	0 (0–1)	Z = −4.62, **$p \leq 0.001$*****
SIS-R, hypersensitivity	N/A	1 (1–2)	1 (0–1)	Z = −7.22, **$p \leq 0.001$*****
SIS-R, referential thinking (being watched)	N/A	0 (0–1)	0 (0–0)	Z = −6.08, **$p \leq 0.001$*****
SIS-R, referential thinking (seeing meanings)	N/A	0 (0–1)	0 (0–0)	Z = −3.58, **$p \leq 0.001$*****
SIS-R, suspiciousness	N/A	1 (1–2)	1 (0–1)	Z = −5.86, **$p \leq 0.001$*****
SIS-R, restricted affect	N/A	0 (0–1)	0 (0–0)	Z = −4.99, **$p \leq 0.001$*****
SIS-R, magical ideation	N/A	1 (0–1)	0 (0–1)	Z = −3.48, **$p \leq 0.001$*****
SIS-R, illusions	N/A	0 (0–1)	0 (0–0)	Z = −2.66, **$p \leq 0.001$*****
SIS-R, psychotic symptoms	N/A	0 (0–0)	0 (0–0)	Z = −1.98, **$p \leq 0.001$*****
SIS-R, derealization/depersonalization	N/A	0 (0–0)	0 (0–0)	Z = −2.26, **$p \leq 0.001$*****
SIS-R, focus of attention (observation)	N/A	0 (0–1)	0 (0–0)	Z = −7.45, **$p \leq 0.001$*****
SIS-R, increased associativity (observation)	N/A	0 (0–0)	0 (0–0)	Z = −5.29, **$p \leq 0.001$*****
SIS-R, poverty of content of speech (observation)	N/A	0 (0–1)	0 (0–0)	Z = −6.23, **$p \leq 0.001$*****
SIS-R, oddness	N/A	0 (0–1)	0 (0–0)	Z = −6.73, **$p \leq 0.001$*****
Suicidal thoughts, yes/no, n (%)	74 (49.3)/76 (50.7)	N/A	N/A	N/A
Attempted suicide, yes/no, n (%)	27 (18.0)/123 (82.0)	N/A	N/A	N/A
Cognitive performance				
Age adjusted WAIS-III R total Z scores, mean (SD)	15.38 (6.97)	20.47 (5.23)	14.96 (6.54)	F = 5.38, **p = 0.006**** Partial η^2 (%): 0.07 (7%)
Beads score, blue/red n (%)	100 (78.7)/27 (21.3)	100 (76.9)/30 (23.1)	142 (69.6)/62 (30.4)	χ^2 = 4.12, p = 0.13
Beads score, number of beads/log beads mean (SD)	3.68 (4.12)/0.37 (0.39)	6.46 (5.25)/0.64 (0.42)	5.09 (3.93)/0.55 (0.40)	F = 15.04, **$p \leq 0.001$***** Partial η^2 (%): 0.06 (6%)

Notes. Mean (SD) are used represent mean and standard deviation, respectively. The variables of transformed to logarithm (10) functions are been presented by mean (SD) values both of the raw and transformed, respectively. Comparisons of groups in continuous variables of normally distributed were conducted by one-way variance (ANOVA) analysis *F* test statistic. The non-parametric a Pearson chi-square or Kruskal-Wallis H (χ^2) or a Mann Whitney U (Z) tests were used in group comparisons of variables in nominal and ordinal/Likert scale (in here for all the scores of clinical instruments) measurements. The all continuous variables are been shown with values of mean (SD); whereas, those of the ordinal levels of measurement are been presented with their values of median and interquartile range (IQR). η^2 = indicates eta-squared. Values of partial η^2 and its percentage (%) are used to estimate amount of the explained variance. Effects sizes are been determined in accordance with thresholds of Cohen's (1988) *d* statistics. Following that: d: 0.2 to partial η^2: 0.01 or 1% represents a small effect; d: 0.5 to partial η^2: 0.06 or 6% represents a moderate effect; d: 0.8 to partial η^2: 0.14 or 14% represents a large effect size. In here, only if the effects sizes have been estimated for the variables which based on *F* statistic test and found that a moderately effect size for all. Bold *p*-values indicate $^{**}p < 0.01$, $^{***}p \leq 0.001$ (two-tailed). Note that for three-groups comparisons all statistical analysis values belong to multiple group comparison tests. Following significant results of the variance analysis, for additional multiple-comparisons of groups please refer to the text. Abbreviations as follows: SCZs, Patients with Schizophrenia Spectrum Disorders; SIBs, Unaffected Siblings; Cnts, Controls; RBF, Robust Brown-Forsythe (for asymptotically F distributed); Log, The logarithm function for data transformation, DUP, Duration of Untreated Psychosis; AP, Antipsychotic; BMI, Body Mass Index; CASH, Comprehensive Assessment of Symptoms and History; GAF, Global Assessment of Functioning; SIS-R, Structured Interview for Schizotypy-Revised; N/A: Not applicable. * Unemployed or economically inactive (i.e. house person, physical illness/disability, career, retired).

As for the cognitive tests, SIBs showed better performance on WAIS-III R test than controls (20.47 vs. 14.96, $p = 0.004$, corrected) and SCZs (15.38 vs. 14.96, $p = 0.04$, uncorrected). There were no significant differences in total WAIS-III R test scores between SCZs and controls. Additional multiple comparisons of bead numbers yielded significant differences among SCZs and controls (3.68 vs. 5.09, $p \leq 0.001$, corrected) with SCZs and SIBs (3.68 vs. 6.46, $p \leq 0.001$, corrected).

3.2. Statistical comparisons of TRP, KYN, KYNA and IL-1β levels of groups

The groups of SCZ and SIB were found out to display lower TRP and higher KYNA, and IL-1β levels than controls. Moreover, a large percentage of variances are explained by group factor both for KYNA (46%) and IL-1β (17%) levels. The statistical significances in a trend level were found for KYN levels among groups (by both to three-groups at $p = 0.07$ and to multiple comparisons of SCZs and controls at $p = 0.06$). Our results of two-way ANCOVA test with group and sex as fixed factors, age as a covariate with TRP levels as dependent variable revealed that there was a statistically significant difference in terms of TRP levels among groups, when controlled the impact of age covariate ($F_{(2,326)} = 4.48$, $p < 0.01$, corrected, ES < 1), but only 3% of variance in TRP levels is explained by the group factor. Also, the covariate age did not have a significant effect on TRP levels of the groups ($p > 0.05$). However, our other two-way ANCOVA model with the KTR as dependent variable showed only a significant

Table 2
Descriptive statistics and statistical comparisons of levels/ratios of TRP-KYN pathway metabolites according to group and sex.

Dependent variables	Group	Group mean (SD)	Sex	Sex Mean (SD)	Test statistic *F*/RBF/*t*	*p* (2-tailed)	Partial η^2 (variance%)
TRP[a] (μM)	SCZ	46.26 (35.77)	Female	46.52 (36.47)			
			Male	46.15 (35.73)			
	SIB	52.76 (34.87)	Female	56.15 (35.23)			
			Male	48.07 (34.23)			
	Control	61.02 (32.90)	Female	62.71 (33.50)			
			Male	58.02 (31.95)			
	Main and interaction effects of factor(s)/covariate						
	Log age				$F = 0.46$	0.50	0.001 (0.1)
	Group				$F = 4.48$	**0.01****	0.03 (3)
	Sex				$F = 1.26$	0.26	0.004 (0.4)
	Group*Sex				$F = 0.31$	0.73	0.001 (0.1)
Log KTR/ KTR[a] (μM/mmol)	SCZ	1.89(0.45)/131.0(156.49)	Female	1.98 (0.5)/173.66 (220)			
			Male	1.86 (0.43)/114 (120.2)			
	SIB	1.94(0.51)/164.41(207.95)	Female	1.90(0.48)/143.88(184.11)			
			Male	1.98(0.57)/194.12(237.73)			
	Control	2.03(0.40)/162.85(176.17)	Female	2.04(0.39)/167.83(194.06)			
			Male	2.01(0.43)/153.56(138.75)			
	Main and interaction effects of factor(s)/covariate						
	Log age				$F = 5.36$	**0.02***	0.02 (2)
	Group				$F = 0.54$	0.58	0.004 (0.4)
	Sex				$F = 0.05$	0.83	0.000
	Group*Sex				$F = 0.93$	0.39	0.006 (0.6)
KYN[b] (μM)	SCZ	5.57 (4.28)	Female	5.44 (4.03)	$t = -0.14$	0.89	
			Male	5.57 (4.41)			
	SIB	5.97 (3.94)	Female	5.78 (3.96)	$t = -0.55$	0.59	
			Male	6.23 (3.93)			
	Control	6.75 (3.71)	Female	6.82 (3.46)	$t = -0.24$	0.81	
			Male	7.02 (4.27)			
	Main and interaction effects of factor(s)/covariate						
	Group*Log age				$F = 3.65$	**0.01****	0.03 (3)
	Group				RBF = 2.68	0.07	0.02 (2)
KYNA[b] (nM)	SCZ	0.06 (0.02)	Female	N/A			
			Male	N/A			
	SIB	0.06 (0.02)	Female	N/A			
			Male	N/A			
	Controls	0.04 (0.01)	Female	0.04 (0.005)	t = −1.16	0.25	
			Male	0.04 (0.006)			
	Main and interaction effects of factor(s)/covariate						
	Group*Logage				$F = 22.71$	**≤0.001*****	0.47 (47)
	Group				RBF = 13.83	**≤0.001*****	0.46 (46)
Log IL-1β/IL-1β[b] (pg/mL)	SCZ	1.47(0.27)/17.54(25.83)	Female	20.97(30.39)/1.49(0.3)	$t = 0.47$	0.64	
			Male	16.60(24.30)/1.46(0.26)			
	SIB	1.37(0.20)/8.17(17.92)	Female	7.34(17.27)/1.36(0.2)	t = −0.25	0.80	
			Male	8.19(17.94)/1.37(0.2)			
	Control	1.22 (0.22)/−0.69 (8.01)	Female	−0.18(8.68)/1.23(0.21)	$t = 0.71$	0.48	
			Male	−1.58(6.44)/1.20(0.23)			
	Main and interaction effects of factor(s)/covariate						
	Group*Logage				$F = 19.65$	**≤0.001*****	0.16 (16)
	Group				RBF = 33.87	**≤0.001*****	0.17 (17)

Notes. Mean (SD) are used represent mean and standard deviation values of group and sex factors, respectively. The variables of transformed to logarithm (10) functions are been presented by mean (SD) values together with those of raw data. a. Statistical comparison of two-way covariance analyses (ANCOVA) with group and sex as fixed factors, age as covariate with TRP and KTR as dependent variables, respectively. Results of the ANCOVA are presented with main effects of group and sex factors and their interactions indicated as group*sex. b. Comparisons in variables of KYN, KYNA, IL-1β relative to group by one-way variance analyses (ANOVA), differences sex with independent samples *t*-test are been shown. Values of partial η^2 indicates an effect size and its percentage (%) are used to estimate amount of explained variance. Effect sizes are been determined in accordance with thresholds of Cohen's (1988) d statistics as 0.01(1%) small, 0.06 (6%) moderate, 0.14 (14%) large. Bold *p*-values indicate *$p < 0.05$, **$p < 0.01$, *** $p \leq 0.001$ (two-tailed). Note that for three-groups comparisons all statistical analyses values. For additional multiple-comparisons of groups please refer to the text. Abbreviations as follows: SCZ, Patient with Schizophrenia Spectrum Disorders; SIB, Unaffected Sibling; RBF, Robust Brown-Forsythe (the test value for asymptotically F distributed); Log, Logarithm function for data transformation; TRP, Tryptophan; KTR, Kynurenine/Tryptophan Ratio; KYN, Kynurenine; KYNA, Kynurenic Acid; IL-1β, Interleukin-1β. N/A, Not applicable for statistical comparisons by sex in measurements.

difference for age covariate ($F_{(1,290)} = 5.36$, $p = 0.02$, ES < 1). But, a small percentage of variance (1.8%) is explained by age. No any significant effects of sex and/or interaction of factor variables were found in differentiates of TRP-KYN pathway metabolites and levels of IL-1β for all groups. Though, our general linear analysis model is suggested that age might be one of confounder by indicating of a significant interactions of group and age variables for KYN, KYNA and IL-1β levels (Table 2).

Additional multiple comparisons of TRP levels revealed existing in a significant difference for between only SCZs and controls ($p = 0.01$, corrected). In contrast, KYNA levels of both of SCZs ($p \leq 0.001$, corrected) and SIBs ($p = 0.04$, uncorrected) were higher than controls. There were no significant differences SCZs and SIBs in terms of KYNA levels. The IL-1β levels of SCZs were significantly higher than both controls ($p \leq 0.001$, corrected) and SIBs ($p = 0.01$, corrected). Furthermore, comparing to controls a significant elevation in IL-1β levels of SIBs were detected ($p \leq 0.001$, corrected).

The results with descriptive statistics relevant to alterations in levels of TRP-KYN among low cytokine (n = 69) and high cytokine (n = 34) subgroups of SCZs were shown in Fig. 1. When comparisons of clinical features on the basis of low-high cytokine subgroups of SCZs; delusion of thought insertion scores of whose had an increased IL-1β levels were significantly higher-ordered (mean rank, MR: 50.54 vs. 40.45) than those of low subgroup (Z = -2.09, $p = 0.04$). In addition, high cytokine SCZ subgroup was worse in regard to positive symptoms of Schneider (MR: 50.5 vs. 39.69) relative to low subgroup of those, in a trend level (Z = -2.87, $p = 0.06$).

According to classifying lower (n = 17) and higher (n = 82) levels of sera IL-1β between cytokine subgroups of SIBs, did not change the levels of TRP-KYN pathway metabolites (for all, $p > 0.05$). Only if, we observed a trend level difference for the scores of psychotic phenomena regarding low and high cytokine SIB subgroups ($Z = -1.92$, $p = 0.05$). In this subscale, SIBs with reduced levels of sera IL-1β had lower-ordered scores (MR: 47.4 vs. 54) as to high subgroup of those.

Additionally, cognitive test scores of low-high cytokine subgroups were similar in the groups of both SCZ and SIB (for all $p > 0.05$).

3.3. The correlations of TRP, KYN, KTR and IL-1β levels and their relations to cognitive and clinical features

Our results of the partial correlations on the Fig. 2, while findings of Spearman correlation analyses including significant relations of clinical test scores to IL-1β and TRP-KYN pathway metabolites in the Table 3 were presented. We did not found a statistically significant relationship of IL-1β and levels/ratios of TRP-KYN metabolites for all study samples. Only if, IL-1β levels showed a negative trend correlation with KYN levels in patients ($r = -0.21$, $p = 0.07$, RS < 0.30) and with TRP levels in controls ($r = -0.20$, $p = 0.08$, RS < 0.30), but not in SIB group. On the other hand, IL-1β levels showed a weak albeit significant (uncorrected levels of significance) positive correlation with the clinical symptoms in patients and their siblings (Table 3).

4. Discussion

Although there is substantial evidence supporting the existence of TRP-KYN pathway dysregulation in patients with schizophrenia, few studies have explored this issue from an endophenotype perspective. An important question is whether cytokine-mediated TRP-KYN dysregulation is an inborn attribute, which ultimately leads to full-scale schizophrenia upon intensification with other genetic and/or environmental triggers. We investigated this view through clinical assessment and measurements of IL-1β/TRP metabolite levels in patients with schizophrenia and their unaffected siblings. Siblings showed psychosocial dysfunction and subthreshold psychotic and negative symptoms in relevant scales. Moreover, schizophrenia and sibling groups displayed identical patterns of IL-1β and TRP metabolite production.

The TRP-KYN hypothesis is based on altered levels of TRP metabolites in central nervous system (CNS), CSF and serum samples of patients with schizophrenia, well-established neurotoxic action of TRP-KYN metabolites and elevated TRP metabolite/IL-1β expression of brain regions that are selectively impaired in patients with schizophrenia (Braff et al., 2001; Brébion et al., 2007; Fillman et al., 2016; Kindler et al., 2020). These supporting evidences by the study designs based

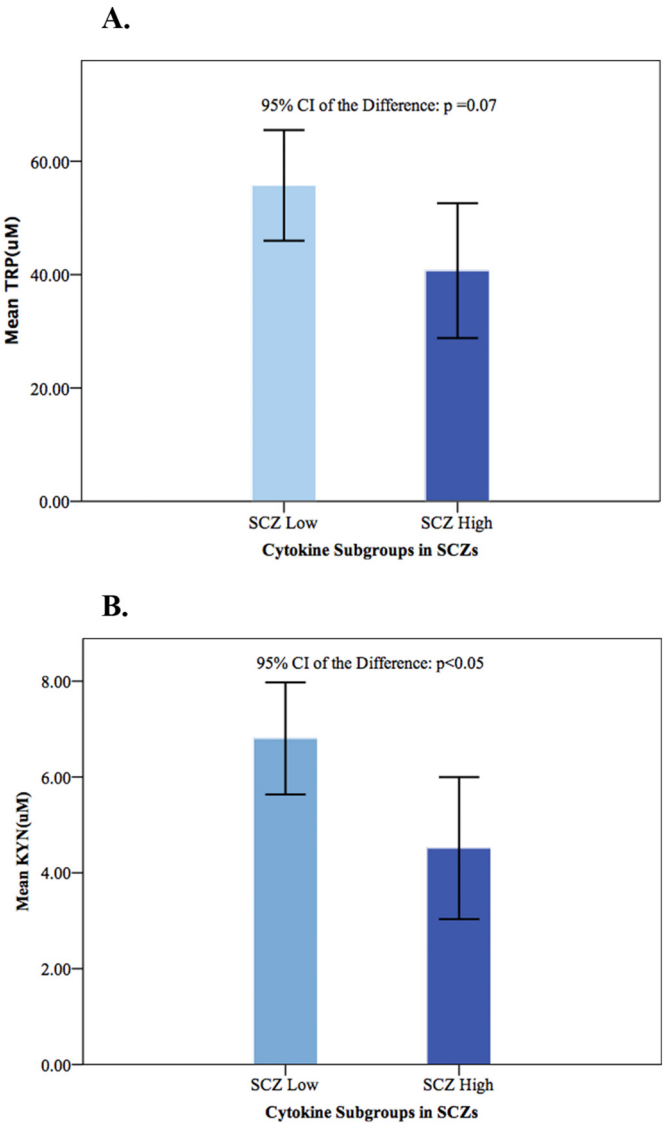


Fig. 1. Bar plots (mean) with standard deviation (SD) error bars for serum TRP and KYN levels for low cytokine (represented by light blue) and high (represented by navy blue) subgroups of the patients. Error bars represents at 95% confidence interval of the difference. Height of the bars indicates the mean. **A.** Serum TRP levels and its differences at SCZ cytokine (IL-1β) subgroups. There was a trend level difference among cytokine subgroups of the SCZs ($t_{(82)} = 1.87$, $p = 0.07$) ($\eta^2 = 0.04$, ES < 1). 4% variance is explained by IL-1β subgroup. TRP means of having elevated IL-1β levels of SCZ cytokine subgroup (navy blue) (mean: 40.70 ± 30.94) was lower than low cytokine subgroup (light blue) (mean: 55.75±36.61) of SCZs **B.** Serum KYN levels and its differences in the SCZ low-high cytokine subgroups. KYN means of low cytokine subgroup (light blue) (mean: 6.80 ± 4.27) was significantly increased than high subgroup of those (navy blue) (mean: 4.51 ± 3.64) ($t_{(76)} = 2.31$, $p = 0.03$) ($\eta^2 = 0.06$, ES < 1). 6% variance on KYN levels is explained by IL-1β subgroup. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

on measurements of both the brain activity and plasma levels of TRP-KYN pathway metabolites, are been shown as a proof that could be used KYN pathway metabolites as peripheral markers in investigations of brain dysfunctions in schizophrenia (Kindler et al., 2020).

In our study, both patients and their siblings showed increased KYNA and decreased TRP and KYN levels. In line with our results, KYNA has been shown to be increased in brain and CSF (Kindler et al., 2020; Linderholm et al., 2012; Müller et al., 2013; Wang and Miller, 2018) and TRP and KYN have been shown to be decreased in peripheral blood of patients with schizophrenia (Joaquim et al., 2018; Lee et al., 2011).

Reduction of TRP and KYN levels in parallel with an increase in KYNA levels might be explained by increased degradation of TRP into its breakdown products. In animal studies, elevation of KYNA levels has led to the impairment of spatial learning, working memory and sensorimotor gating, all of which are also observed in patients with schizophrenia (AhnAllen, 2012). Moreover, elevation of cerebral KYNA levels has been implicated to induce psychotic symptoms and cognitive dysfunction through NMDAR and AChR antagonism (Schwarcz and Pellicciari, 2002). Therefore, overall, our results are been seen in agreement with the TRP-KYN hypothesis. However, further studies in which analysing the genetic factors in the patients with schizophrenia including their first-degree relatives, are needed to reveal more clearly the link of the several environmental and biological factors.

On the other hand, in contrast with our findings, some studies have reported decreased plasma KYNA levels (Chiappelli et al., 2018; Myint et al., 2011). There are several elements that regulate the TRP-KYN pathway and thus may act as confounding factors in cohort studies focused on a heterogeneous group of patients with schizophrenia. For instance, a genetic variation resulting in decreased expression of sorting nexin 7 activator of IL-1β (Erhardt et al., 2017; Sellgren et al., 2016) and another variant leading to kynurenine-3-monooxygenase deficiency lead to altered TRP breakdown product levels (Oxenkrug et al., 2017). Estrogen level has a significant impact on the activity of KYNA producing enzymes and KYNA is lower in Caucasian women (Badawy and Dougherty, 2016; Jayawickrama et al., 2017). Finally, antipsychotic treatments increase KYNA levels and patients with treatment-resistant schizophrenia display lower KYNA levels compared to non-treatment resistant ones (Lee et al., 2011; Myint et al., 2011). Thus, ethnic-gender differences, treatment status and different combinations of genes regulating the TRP breakdown may plausibly lead to divergent results in different schizophrenia cohorts.

In all study groups, TRP-KYN levels were positively correlated. But, the patients showed a high level correlation of TRP-KYN levels compare as other groups. This is an anticipated finding between a substrate molecule (e.g. TRP) and its breakdown product (e.g. KYN). By contrast, negative correlation of TRP levels with ratio of KYN/TRP was only detected in siblings and controls. Also, all groups showed a positive correlation between of their KYN levels and ratio of KYN/TRP. The level of this positive relationship of KYN with KYN/TRP ratio was been found the strongest in patients and their siblings. Our these results have been implied that existing an association of a decreased TRP and elevated KYN levels with an increased ratio of KYN/TRP in the groups. The ratio of KYN/TRP is shown as an indicator of the TRP breakdown and IDO activity (Widner et al., 1997). Consistently with that, an elevated KYN/TRP plasma ratios and that's negative correlation with changes of brain glutamate metabolism and cognitive functions related the frontal regions have been shown in schizophrenia (Chiappelli et al., 2016; Kindler et al., 2020). Moreover, it was reported that existence of accompanied by immune activation markers to the elevated ratio of KYN/TRP in schizophrenia (Schwieler et al., 2015).

Basing on all these previous findings, the plasma ratio of KYN/TRP has been suggested that could be a peripheral marker for the brain dysfunctions in schizophrenia by Kindler et al. (2020). For that, Kindler et al. (2020) focused on the investigating of associations between of peripheral ratio of KYN/TRP and cognitive functions. Their study has been revealed that in high cytokine subgroup of patients with schizophrenia displayed an increased levels of plasma KYNA and KYN/TRP ratio and that's related with attention and dorsolateral prefrontal cortex volume. In contrast that, our results in which we used to similar method with them, we did not detect an important correlations and/or difference in cytokine subgroups of the patients for these metabolites and their relations with cognitive functions. Only, we found a moderately positive correlation between plasma KYN/TRP ratio and a cognitive sub-test performance in siblings. Also, in our study, high and low cytokine subgroups of the patients did not differ on cognitive performance. But, basing on the previous findings our results have been shown that further investigations in which examination KYN/TRP ratio and that's relationships with cognitive and clinical features due to supporting brain activity reflection on TRP-KYN pathway metabolites might be important in schizophrenia.

It is well known that TRP-KYN pathway dysregulation is closely associated with elevation of inflammatory cytokine levels (Erbağci et al., 2001; Wang and Miller, 2018). Inflammatory cytokines activate indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) enzymes, which catalyze degradation of TRP to KYN, leading to production of several neurotoxic metabolites and depletion of TRP and serotonin (Chiappelli et al., 2018; Garrison et al., 2018). A special emphasis was given to the inflammatory cytokine IL-1β in our study, since increased peripheral blood IL-1β expression has been linked to volume reduction in Broca's area and congruent cognitive dysfunction in patients with schizophrenia (Fillman et al., 2016). Furthermore, a positive correlation has been indicated between IL-18, which is a member of IL-1β family of pro-inflammatory cytokines previously known as IFN-γ (Dinarello and Fantuzzi, 2003), and visuospatial/constructional domain of cognitive impairment in first-episode schizophrenia patients (Zhang et al., 2013). Moreover, in chronic schizophrenia patients, a level of serum IL-18 had been found that is significantly higher than in both controls and first-episode patients, and this level was positively correlated with the general psychopathology sub-score of the Positive and Negative Syndrome Scale (PANSS) (Xiu et al., 2012). In line with this finding, Kovács et al. (2020) have reported that the elevated serum levels of IFN-γ which also contribute to IDO induction (Myint and Kim, 2014; Yasui et al., 1986), and neutrophil-to-lymphocyte ratio (NLR) in schizophrenia correlated with the clinical test scores. As a consequence of these, Kovács et al. (2020) considered that IFN-γ level might be an indicative marker for illness severity in schizophrenia. All of these are significant to show that IL-1β is associated with IL-18 which has a critical role in the pathophysiology of schizophrenia. In line with the previous reports, IL-1β levels of patients with schizophrenia and their siblings were significantly increased in our study. Moreover, the sibling group showed an intermediate IL-1β level between patients and controls indicating a hereditary propensity to an inflammatory phenotype in first-degree relatives of patients schizophrenia. Intriguingly, patients with schizophrenia with higher IL-1β levels were more likely to display lower KYN levels, suggesting that IL-1β is a driving force behind the increased degradation of TRP to KYNA. Also, a trend level correlation of IL-1β with KYN levels of the patients and lack and/or weak of correlation between IL-1β with clinical and cognitive parameters might be due to the fact that IL-1β is one of many factors inducing TRP-KYN pathway dysregulation and associated clinical symptoms and thus overall impact of this particular cytokine alone on clinical features is relatively small. Therefore, levels of additional inflammation factors need to be studied in unaffected siblings of the patients.

In a similar recent study (Kegel et al., 2017) CSF levels of KYNA were associated with psychotic symptom scores in unaffected twins of schizophrenia and bipolar disorder patients, thus lending further support to the participation of KYNA in development of psychosis. In the same study, authors showed that CSF levels of TRP and KYNA showed trends towards being correlated with levels of IL-8 and TNF-α (Kegel et al., 2017). These results further support the presence of an inherited

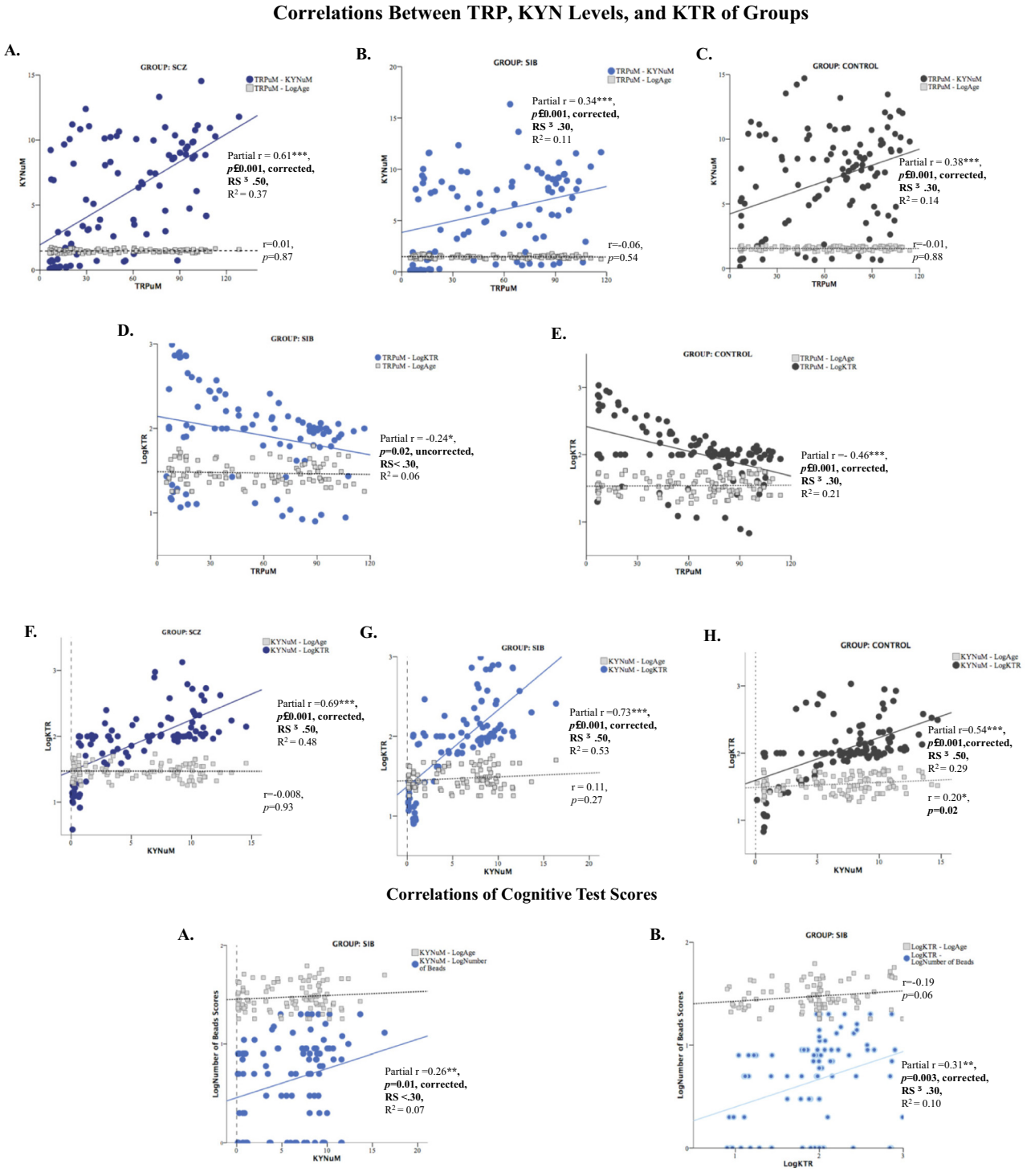


Fig. 2. Significant associations of partial correlation analyses as age controlled between the TRP, KYN levels and KTR, and their relationships of cognitive test scores of groups. When effect of age variable controlled, there was a positive correlation between TRP and KYN in all groups in high levels of significance both of statistically and clinical, and a moderate negative correlation between TRP and KTR in SIB and control groups. Also, all groups showed a positive correlation between of their KYN levels and KTR in a high level of significance for both clinical and statistically. Groups of SCZ and SIB showed the most strong relation among TRP-KYN and KYN-KTR. In SCZs, a variance of 37% was explained by the positive association of TRP-KYN. For two groups, the amount of explained variances by positive correlations between KYN-KTR were at 48% and 53% in SCZ and SIB groups, respectively. No statistically significant correlation between TRP and KTR in SCZ group ($p > 0.05$, uncorrected). The significant correlations of TRP-KYN pathway metabolites and cognitive test scores of groups, were been shown that in the second panel on the figure. It was observed that a positive significant correlation on a sub-score of the beads task and KYN levels and KTR of SIBs (all, $p \leq 0.01$, corrected, RS < 30 and RS ≥ 30 in a small-moderately relations). No other significant association of TRP-KYN pathway metabolites to cognitive test scores in groups, was found (all, $p > 0.05$).There was a weak significant correlation between age and KYN levels in controls ($r = 0.20$, $p < 0.05$, uncorrected). In SIBs, age correlated with the KTR in a negative trend level ($r = 0.19$, $p = 0.06$). No any significant correlation between age and other TRP-KYN pathway metabolites in the groups was detected (all, $p > 0.05$, uncorrected). When age statistically controlling, a slightly change on the correlation results of SIBs was observed as following that: The value of zero-ordered correlation coefficient of the TRP-KTR had been found at $r = -0.25$ for $p = 0.01$. Additionally, our all zero-ordered correlation results indicated that existed no any changes/or a noteworthy differences compare to controlled condition of the partial correlation. Herein, our correlation findings are been reported with partial correlation coefficients of Pearson's r values with their significance levels as both clinical and statistically. Relation size (RS) is been determined in accordance with thresholds of Cohen's (1988) for correlations as 0.10 ≤ RS < 0.30 small, 0.30 ≤ RS < 0.50 moderate, and 0.50 ≤ RS < 1.0 large. Bold values denote statistical significant at a two-tailed $^{*}p < 0.05$, uncorrected, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$, corrected levels. Abbreviations as follows: SCZ, Patient with Schizophrenia Spectrum Disorders; SIB, Unaffected Sibling; Log, Logarithm function for data transformation; RS, Relation size; TRP, Tryptophan; KTR, Ratio of Kynurenine/Tryptophan; KYN, Kynurenine.

Table 3
Results of Spearman's correlation analyses for all groups.

Spearman's rho	SCZs				SIBs				Controls			
	TRP	KYN	Log KTR	Log IL1β	TRP	KYN	KTR	Log IL-1β	TRP	KYN	Log KTR	Log IL-1β
Log IL-1β	-0.15	−0.21	−0.10	1	−0.16	−0.06	−0.03	1	−0.20	−0.05	0.06	1
RS	<0.30	<0.30	<0.30	N/A	<0.30	NS <0.10	NS <0.10	N/A	<0.30	NS <0.10	NS <0.10	N/A
Rho^2 (%)	0.02(2)	0.04(4)	0.01(1)	N/A	0.03(3)	N/A	N/A	N/A	0.04(4)	N/A	N/A	N/A
Thought of insertion	NS	NS	NS	**0.22***	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RS	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	0.04(4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Commenting voices	NS	NS	NS	**0.24***	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RS	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	0.06(6)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total Schneider symptoms	NS	NS	NS	**0.22***	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RS	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	0.04(4)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SIS-R, social isolation (long)	N/A	N/A	N/A	N/A	**0.21***	NS	NS	NS	**0.20***	NS	NS	NS
RS	N/A	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	<0.30	N/A	N/A	N/A
Rho^2, (%)	N/A	N/A	N/A	N/A	0.04(4)	N/A	N/A	N/A	0.04(4)	N/A	N/A	N/A
SIS-R, introversion	N/A	N/A	N/A	N/A	**0.25***	NS	NS	NS	NS	NS	NS	NS
RS	N/A	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	N/A	0.06(6)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SIS-R, restricted affect	N/A	N/A	N/A	N/A	**0.31****	**0.21***	NS	NS	**0.26****	NS	NS	NS
RS	N/A	N/A	N/A	N/A	≤0.30-< 50	<0.30	N/A	N/A	<0.30	N/A	N/A	N/A
Rho^2(%)	N/A	N/A	N/A	N/A	0.10(10)	0.04 (4)	N/A	N/A	0.07(7)	N/A	N/A	N/A
SIS-R, illusions	N/A	N/A	N/A	N/A	NS	**−0.22***	**−0.23***	NS	NS	NS	NS	NS
RS	N/A	N/A	N/A	N/A	NS	<0.30	<0.30	N/A	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	N/A	N/A	0.05 (5)	0.05 (5)	N/A	N/A	N/A	N/A	N/A
SIS-R, referential del.	N/A	N/A	N/A	N/A	NS	NS	NS	**0.24***	NS	NS	NS	NS
RS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.06(6)	N/A	N/A	N/A	N/A
SIS-R, suspiciousness	N/A	N/A	N/A	N/A	NS	NS	NS	**0.27****	NS	NS	NS	NS
RS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.07(7)	N/A	N/A	N/A	N/A
SIS-R, magical idea.	N/A	N/A	N/A	N/A	NS	NS	NS	**0.23***	NS	NS	NS	NS
RS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.30	N/A	N/A	N/A	N/A
Rho^2 (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.05(5)	N/A	N/A	N/A	N/A

Notes. Values of partial r^2 indicates an effect size and its percentage (%) are used to estimate amount of explained variance. Relation size (RS) is been determined in accordance with thresholds of Cohen's (1988) for correlations as 0.10 ≤ RS < 0.30 small, 0.30 ≤ RS < 0.50 moderate, and 0.50 ≤ RS < 1.0 large. No any statistical significance in the corrected level in the results of the correlations of levels/ratio TRP-KYN pathway metabolites and IL-1 beta and their relations to clinical features was found. Bold values denote statistical significant a two-tailed at *$p < 0.05$, uncorrected, **$p \leq 0.01$, corrected. Abbreviations as follows: SCZs, Patients with Schizophrenia Spectrum Disorders; SIBs, Unaffected Siblings; Log, Logarithm function for data transformation; RS, Relation size; TRP, Tryptophan; KTR, Ratio of Kynurenine/Tryptophan; KYN, Kynurenine; IL-1β, Interleukin-1β. N/A, Not applicable; NS: Non-significant.

tendency to inflammation induced TRP-KYN pathway dysregulation in patients with schizophrenia.

We found that IL-1 β levels were correlated to severity of psychotic symptoms not only in the patient group but also in siblings. This finding suggests that relationship between inflammation and psychosis is not specific to patients only. In addition, compared to low cytokine schizophrenia subgroup, high cytokine subgroup of them was worse in regard to severity in delusion of thought insertion significantly, and on Schneider symptoms in a trend level. As to, in the siblings, low cytokine subgroup displayed increasing for the severity of psychotic phenomena in a trend level.

We also found that kynurenine and its metabolite levels were positively correlated to a cognitive parameter which is an assessments of jumping to conclusion, in siblings. Also, our sibling group unexpectedly had showed a better performance on cognitive task than both patients and controls. It has been considered that an existing some confounding factors could be effect on these results (e.g., lack of more detail on cognitive baseline of the participants; the impact of task complexicity). For that, making an inference on our cognitive findings that contradicting with relevant previous findings is be difficult, and next studies which are designed considering possible confounding factors and that present an assessment of different cognitive process would be crucial.

The results of this study need to be interpreted in light of several limitations. An important limitation of our study was the absence of CSF measurements of TRP-KYN pathway components, which could have provided stronger correlation levels among clinical parameters, cytokines, and TRP metabolites. As we studied blood levels of TRP-KYN pathway components we do not know how accurately these metabolites reflect the situation in the brain. Furthermore, numerous previous studies present inconsistent findings on whether plasma TRP-KYN measurements can be a peripheral marker reflecting the brain activity might be thought. This limitation factor is valid for many relevant studies including our current study. However, a recent study (Kindler et al., 2020) presents a comprehensive examination to predict the association to or impact on TRP-KYN metabolites and the brain activity. Among the several inflammatory cytokines like IL-6, TNF-α, we only studied the IL-1 β. As we do not know the impact of these cytokines on TRP-KYN pathway in our study participants, our findings should be interpreted with caution. In addition that, our further analyses of cytokine subgroups in the study samples, did not allow to conduct an extensive investigations in the patients and their siblings comparing to controls since that distribution of unequal and relatively small sample size to the subgroups. The confounding effects in which could be caused by various environmental factor on changes of plasma TRP levels, might be shown as another restrictive factor of our study. As a matter of fact, ex-post facto research designs would be not provide a convenient way for experimental control. For instance, it is known that reduced dietary intake of TRP is responsible for decreased endogenous TRP, and serum KYN levels would be also decreased relevant to that (Widner et al., 1997). As a way to consider the possible confounding factor of dietary on our results related to low TRP and KYN levels, a plasma ratio of KYN/TRP of the participants was also been taken into account for all findings of the current study. Despite the

statistical differences in levels of TRP and KYNA, ratio of KYN/TRP did not changed between groups, in our study. Therefore, the effect of possible confounding environmental factors on our significant results should be considered. Finally, our study was basis on a cross-sectional investigation. Thus, to examine immune and metabolic changes as an indicators of clinical markers, and to analyse difference in magnitude in relation to the clinical features, study design of repeated measures in the similar study groups are been needed in the future.

5. Conclusion

In conclusion, both patients with schizophrenia and their relatives appear to show a liability to displaying TRP-KYN pathway dysregulation and increased cytokine expression, which may be causing premorbid abnormal personality traits. An additional triggering genetic and/or environmental factor such as further elevation of inflammatory cytokines might be shifting the balance towards characteristic findings of schizophrenia. We believe that further investigation of the full set of factors regulating the TRP-KYN pathway may result in characterization of biomarkers and effective treatment methods for this enigmatic disorder.

Acknowledgements

The study was supported by the European Community's Seventh Framework Program under grant agreement [grant number HEALTH-F2-2009-241909, Project EU-GEI] and the Scientific and Technological Research Council of Turkey [Project No: 115S596], and the Istanbul University Scientific Research Projects Unit of Turkey [Project No: 21615].

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