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Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood maltreatment

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Abstract

Background. Inflammation is a possible biological mechanism underlying the association between childhood maltreatment and psychosis. Previous investigations on this regard were mainly conducted on chronic schizophrenia and lacked control for confounders. We aim to investigate the role of familial liability, childhood maltreatment and recent stress in determining cytokine abnormalities at the onset of psychosis.

Methods. We recruited 114 first-episode psychosis (FEP) patients, 57 unaffected biological siblings of FEP patients, and 251 community-based controls. Plasma cytokines (IL-1β, IL-6, TNF-α, IFN-γ, IL-4, IL-10 and TGF-β) were measured and differences across the groups analysed after adjusting for potential confounders.

Results. FEP had a higher pro- and anti-inflammatory cytokine profile (IL-1β, IL-6, TNF-α, IL-10 and TGF-β), which was not observed in unaffected siblings. Siblings presented decreased IL-1β when compared with patients and controls. Childhood maltreatment was associated with higher levels of TGF-β in both patients and siblings when compared with controls. Physical childhood abuse was associated with increased levels of TGF-β in FEP patients but with decreased levels in controls. Other childhood maltreatment subtypes or recent stressors did not affect cytokine levels in any of the groups.

Conclusions. Normal or reduced cytokines in siblings represent possibly a protective factor and suggest that the identified inflammatory profile in FEP can be a real pathophysiological component of psychosis. Experience of childhood maltreatment may contribute as long-term immune priming for the TGF-β pathway, and increased levels of this cytokine in both patients and siblings exposed to childhood maltreatment point to a possible biological candidate of familial risk for psychosis.

Introduction

Increasing body of evidence suggests a role of the immune system in psychosis (Goldsmith *et al.*, 2016). To date, it remains unclear whether the immune activation present at the onset of psychosis is mainly to be ascribed to genetic predisposition or to the exposure to environmental factors, such as childhood maltreatment, which is well-known to be associated with both onset of psychosis and inflammatory abnormalities (Baumeister *et al.*, 2015; McGrath *et al.*, 2017).

Increased inflammation in psychosis has been supported by a number of meta-analyses demonstrating enhanced levels of cytokines and cytokines receptors in chronic schizophrenia, as well as in drug-naïve patients in their first episode of psychosis (FEP) (Goldsmith *et al.*, 2016; Pillinger *et al.*, 2018). Supporting the contribution of genetic predisposition, genome-wide association studies (GWAS) suggest an association between schizophrenia and the major histocompatibility complex on chromosome 6p22.1, which is strictly linked to the immune system (Ripke *et al.*, 2014).

However, genetic predisposition may not be the only factor playing a role in immune dysregulation. There is now robust cross-national epidemiological evidence that childhood maltreatment increases the risk of psychotic experiences across the life span in more than two-fold and in a dose-response fashion, with the highest association attributed for sexual

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and physical abuse (McGrath *et al.*, 2017). Furthermore, patients with psychosis are almost three times more likely to have been exposed to childhood maltreatment, and the prevention of traumatic experiences could reduce the incidence of psychosis by 33% (Varese *et al.*, 2012). Interestingly, one of the possible biological mechanisms underlying the association between childhood maltreatment and psychosis is the trigger of an immune dysfunction characterised by abnormal production of inflammatory cytokines (in particular IL-6 and TNF-α) (Coelho *et al.*, 2014; Baumeister *et al.*, 2015). Studies suggest that not all the types of trauma may affect the immune system in the same way and that physical and sexual abuse is associated with the strongest effects (Baumeister *et al.*, 2015).

Few studies have attempted to investigate whether augmented inflammation in psychosis could be attributed to childhood maltreatment, and the current evidence is limited and conflicting. Earlier studies reporting increased IL-6 (Dennison *et al.*, 2012) and TNF-α (Dennison *et al.*, 2012; Di Nicola *et al.*, 2013) in patients with psychosis exposed to childhood maltreatment were limited by sample sizes ($n < 50$) and lacked control for confounders.

Subsequent studies conducted in larger samples of patients with diagnoses of chronic schizophrenia/bipolar disorder reported associations between C-reactive protein and childhood abuse severity (combined as effects of sexual, emotional and physical abuse) (Aas *et al.*, 2017) or sexual abuse only (Quidé *et al.*, 2018), but the significant associations disappeared when controlling for body mass index (BMI) (Aas *et al.*, 2017). An important limitation of these studies is the sample, composed by patients suffering from chronic schizophrenia, in whom the inflammatory markers could have been largely affected by the effects of chronic antipsychotic treatment on metabolic parameters or BMI (Baumeister *et al.*, 2016; Calevro *et al.*, 2018).

In the present study, we investigated the association between childhood maltreatment and inflammation in psychosis in an epidemiological sample of FEP patients recruited in Brazil, where the estimates of childhood maltreatment can reach figures over 40% in the southeast part of the country (Rates *et al.*, 2015), and government's budget for childhood maltreatment prevention has been considered inadequate (ISPCAN, 2014).

We used a number of strategies to overcome the methodological limitations identified previously. First, we studied FEP patients in order to reduce possible confounding related to illness duration, weight gain and long exposure to pharmacological treatment. Second, we included community-based controls to reduce risk of selection bias. Third, we investigated the different subtypes of childhood maltreatment, which were before considered as one phenomenon. Fourth, we controlled for a broad range of confounders (age, gender, BMI, tobacco smoking, psychoactive substance use, pharmacological treatment, years of study and relationship status) that were not taken into account in previous investigations. Fifth, we included unaffected siblings of FEP patients in order to investigate the possible role of familial liability to immune activation. The inclusion of high-risk groups provides the advantage of controlling for possible effects of shared environmental and genetic risks in the context of inflammatory dysregulation that has not been explored elsewhere. Sixth, we considered a comprehensive innate and adaptive immune-related profile, representing activation of the inflammatory response system (monocyte (M) type-1: IL-1β, TNF-α, IL-6; T helper 1: IFN-γ) and the compensatory system (M-2: IL-10; T helper 2: IL-4; and T regulatory: IL-10, TGF-β).

We aimed to: (i) investigate plasma cytokine levels among patients, siblings, and community-based controls, controlling for a set of confounders; and (ii) investigate the role of childhood maltreatment and recent stress in determining the differences in cytokine levels shown among the above groups, controlling for confounders. We inferred that: (i) FEP patients would have increased levels of inflammatory cytokines when compared with controls, and unaffected siblings would represent an intermediate group; (ii) reports of traumatic events would be associated with increased levels of inflammatory markers in all groups; and (iii) the subtypes of childhood maltreatment would impact differently on the levels of the inflammatory markers.

A better understanding of the low-grade inflammatory profile and its association with environmental stressors can improve risk stratification in groups with illness as well as in high- and average-vulnerability groups, in order to improve the effectiveness of interventions.

Methods

This case-sibling-control study is part of the epidemiological investigation named STREAM (Schizophrenia and Other Psychoses Translational Research: Environment and Molecular Biology) conducted in Ribeirão Preto catchment area (comprised of 26 counties with around 1.3 million inhabitants, located in the São Paulo state, Brazil) between April 2012 and March 2015 (Del-Ben *et. al.*, accepted). The STREAM study is part of the international multicentre consortium EU-GEI (European Network of National Schizophrenia Networks Studying Gene-Environment Interactions; http://www.eu-gei.eu/), an incidence and case-sibling-control study investigating biological and environmental interactions in psychosis (Jongsma *et al.*, 2018).

Participants

As previously described (Loureiro *et al.*, 2018), we recruited patients in their first contact with mental health services due to psychotic symptoms during the study period. Any patients with psychotic symptoms originated from other medical condition or substance intoxication/withdrawal were excluded.

Siblings were invited to participate in the study considering patients' agreement and the absence of lifetime history of psychotic symptoms.

Community-based controls were recruited considering the demographic characteristics of the Ribeirão Preto catchment area stratified by age and gender, according to the Brazilian Official Census Bureau 2010 (Instituto Brasileiro de Geografia e Estatística, IBGE, www.ibge.gov.br). Controls with lifetime history of psychotic symptoms were not included.

All the participants aged between 16–64 years old and were living in the Ribeirão Preto catchment area. This study was approved by the local research ethics committee.

Initially, we recruited 507 participants with blood collection (166 FEP, 76 siblings, 265 community-based controls). From these, we excluded 17 participants (eight patients, one sibling and eight controls) presenting with any of the following: nephropathy, urinary tract infection, dengue fever, rheumatic fever, human immunodeficiency virus, syphilis, Crohn's disease, throat infection, pregnancy, corticosteroid treatment, multiple sclerosis, pneumonia, and hidradenitis suppurative. BMI data were missing in 42 patients, 18 siblings, and six controls, and two patients did

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not answer the childhood trauma questionnaire completely, and therefore were also excluded from the study.

Clinical assessment

Clinical assessment was conducted by trained researchers (psychologists and nurses with experience in mental health) and during all the study period weekly meetings were held with the senior staff (one psychiatric nurse, two psychologists and three senior psychiatrists).

Diagnosis was obtained for all participants using the Structured Clinical Interview for DSM-IV, clinical version (SCID-CV) (First *et al.*, 1997; Del-Ben *et al.*, 2001). We used the Brief Psychiatric Rating Scale (BPRS) for the clinical assessment of symptom severity at the moment of blood collection (Overall and Gorham, 1962; Crippa *et al.*, 2001), and the Nottingham Onset Schedule (Singh *et al.*, 2005) to register psychosis onset date and the pharmacological treatment starting date. History of lifetime and/or current psychoactive substance use (cannabis, alcohol, cocaine/crack, inhalants, sedatives, amphetamine and hallucinogens) was assessed by The Cannabis Experience Questionnaire-Modified Version (CEQmv) (Di Forti *et al.*, 2009).

Stress measurements

We used the Childhood Trauma Questionnaire (CTQ) (Bernstein *et al.*, 2003; Grassi-Oliveira *et al.*, 2006) to assess the history of childhood maltreatment. The CTQ short form is a self-report questionnaire consisting of 25 items rated on a 5-point Likert scale (1 = never true; 5 = very often true) ranging from 5 to 25 points in order to assess the exposure to emotional, physical and sexual abuse, and physical and emotional neglect. The sum of values of the five scales generates the CTQ total score, which ranges from 25 to 125 points. In addition, four cut-off scores are provided for each scale: none to low; low to moderate; moderate to severe and severe to extreme. We used a pre-defined cut-off score based on the moderate to severe threshold ($\geqslant$13 for emotional abuse; $\geqslant$10 for physical abuse; $\geqslant$8 for sexual abuse; $\geqslant$15 for emotional neglect; and $\geqslant$10 for physical neglect) to classify participants in the maltreated (moderate to severe; severe to extreme) or non-maltreated (none to low; low to moderate) groups (Bernstein *et al.*, 2003).

The occurrence of adverse life events over the past 12 months was assessed by a questionnaire proposed by the EU-GEI, which was based on the List of Threatening Experiences (Brugha *et al.*, 1985). The translation and adaptation of this questionnaire to Portuguese was performed by the STREAM research team, and the final version was submitted for backtranslation by a bilingual researcher associated with the EU-GEI. The proposed questionnaire comprises 21 questions assessing the occurrence of the following adverse events: personal problems; relational problems; work related and financial problems; severe events in family or friends; problems with law. Participants reporting any of these were classified as having experienced recent stress.

Cytokines measurement

Peripheral blood was collected after the diagnosis evaluation and the samples were processed as previously described (Loureiro *et al.*, 2018). Cytokines (IL-1β, IL-6, TNF-α, IFN-γ, IL-4, IL-10 and TGF-β) were quantified in plasma (25 μL) using the Milliplex MAP Human Cytokine/Chemokine magnetic bead panel (#HCYTOMAG-60K; #HTH17MAG-14K; #TGFBMAG-64K-01 EDM Millipore, Billerica, MA, USA; https://www.emdmillipore.com/US/en). The assay was performed in 96-well plates according to the manufacturer's instructions and the results were expressed in pg/mL. Briefly, each assay plate layout consisted of seven standards, two positive controls, two blank wells, all run in duplicate, and up to 76 samples. Results were analysed on a Luminex-200 System (Luminex, Austin, TX, USA) and reported on xPOTENT software version 3.1. Cytokines concentrations were calculated through the five-parameter logistic curve-fitting method using the median fluorescence intensity (MFI). All data were corrected using Milliplex Analyst software.

Statistical analysis

Data were analysed using SPSS version 24.0 (IBM Corp: Armonk, NY, USA). Demographic and clinical data were analysed using descriptive statistics. Statistical associations between categorical variables were analysed by Pearson's χ^2 tests with column proportions compared by the z-test (adjusted p values with Bonferroni method), and for continuous variables by analysis of variance with Bonferroni correction.

Plasma cytokines were logarithmically transformed for the statistical analyses, while the raw values are provided (adjusted means ± S.E.M.). To test the overall difference among the groups on the cytokine levels, we used general linear model (GLM) with Bonferroni corrections, adjusted for age, gender, BMI, tobacco smoking, years of study, relationship status and psychoactive substance use. The effects of childhood maltreatment or recent stress were also analysed by GLM to test for the between-group interaction and the within-group differences, controlling for the same confounders. When results were significant, recent stress or childhood maltreatment were also entered as possible confounders. In the patient group, we also explored whether associations could have been influenced by pharmacological treatment, and significant associations between cytokines and childhood maltreatment were also further explored by taking into account the trauma severity. Statistical significance was set at $\alpha < 0.05$ (two-tailed).

Results

Sample characteristics

The final sample was comprised of 114 FEP patients, 57 siblings and 251 controls ($n = 422$) (Table 1). The groups did not significantly differ for age ($p > 0.05$), but differed for gender; in particular, siblings had a higher proportion of females when compared with FEP or controls ($p < 0.001$). Patients had lower BMI than controls ($p = 0.026$) but presented the highest frequency of cannabis (49.1%), tobacco (36.8%) and other psychoactive substances use (50.0%), whereas siblings presented the lowest frequency, with significant differences among the groups ($p < 0.001$).

Clinical characteristics of the patients are presented in Table 2. Half of the patients were under pharmacological treatment for less than 12.5 weeks.

Stress measurements

The groups differed in the proportion of experience of childhood maltreatment ($p < 0.001$), with patients having the highest

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Table 1. Socio-demographic characteristics of the sample ($n = 422$)

	First episode psychosis ($n = 114$)	Siblings ($n = 57$)	Controls ($n = 251$)	Test and significance		
				χ^2; F	df	p
Male, *n* (%)	73 (64.0)	18 (31.6)	129 (51.4)	16.176	2	<0.001[a,b]
Mean age (s.d.)	30.8 (12.5)	30.7 (10.5)	31.3 (11.0)	0.122	2,421	0.885
⩾9 years of study, *n* (%)	49 (43.0)	42 (73.7)	193 (76.9)	42.182	2	<0.001[a,c]
Stable union, *n* (%)	32 (28.1)	34 (59.6)	136 (54.2)	25.087	2	<0.001[a,c]
Body mass index (kg/m^2), mean (s.d.)	24.8 (5.1)	24.9 (4.9)	26.2 (5.3)	3.699	2,421	0.026[c]
Waist circumference (cm), valid/missing	111/3	56/1	243/8			
Waist circumference (cm), mean (s.d.)	86.4 (13.3)	82.9 (14.6)	87.4 (14.9)	2.223	2,409	0.110
Cannabis						
Ever used (yes), *n* (%)	56 (49.1)	4 (7.0)	50 (19.9)	47.098	2	<0.001[a,c]
Current use (yes), *n* (%)	11 (9.6)	–	7 (2.8)	11.971	2	<0.003[a,c]
Other psychoactive substance, yes *n* (%)[d]	57 (50.0)	1 (1.8)	39 (15.5)	69.376	2	<0.001[a,b,c]
Tobacco (yes), *n* (%)	42 (36.8)	10 (17.5)	42 (16.7)	19.162	2	<0.001[a,c]
Childhood Childhood maltreatment						
Total, *n* (%)	50 (43.9)	20 (35.1)	57 (22.7)	17.451	2	<0.001[c]
Emotional abuse, *n* (%)	27 (23.7)	13 (22.8)	27 (10.8)	12.179	2	0.002[b,c]
Physical abuse, *n* (%)	21 (18.4)	4 (7.0)	19 (7.6)	10.704	2	0.005[c]
Sexual abuse, *n* (%)	7 (6.1)	5 (8.8)	8 (3.2)	3.888	2	0.143
Emotional neglect, *n* (%)	27 (23.7)	11 (19.3)	23 (9.2)	14.618	2	<0.001[c]
Physical neglect, *n* (%)	19 (16.7)	8 (14.0)	20 (8.0)	6.553	2	<0.038[c]
Recent stress, *n* (%)	66 (57.9)	43 (75.4)	186 (74.1)	10.749	2	0.005[c]

Post-hoc analysis significance is reported as follows:
[a]First-episode psychosis *v.* siblings.
[b]Siblings *v.* controls.
[c]First-episode psychosis *v.* controls.
[d]Other psychoactive substance including the following: alcohol, cocaine/crack, inhalants, sedatives, amphetamine, and hallucinogens.

proportion (43.9%), followed by their unaffected siblings (35.1%), and controls (22.7%). This pattern was observed for all the subtypes of childhood maltreatment, except for sexual abuse (Table 1).

Controls had a significantly higher frequency of recent stress than patients ($p = 0.005$) (Table 1).

Cytokine levels in FEP patients, siblings and community-based controls

Table 3 shows the results of the plasma cytokines among the groups controlling for age, gender, BMI, tobacco smoking, psychoactive substance use, years of study and relationship status. We found differences among the groups in plasma concentrations of IL-1β, IL-6, TNF-α, IL-10 ($p < 0.001$) and TGF-β ($p = 0.004$), but not IFN-γ ($p = 0.942$) or IL-4 ($p = 0.915$). FEP had significantly higher levels of IL-6, TNF-α, IL-10 ($p < 0.001$) and TGF-β ($p = 0.003$) when compared with controls. Patients also had higher levels of IL-1β ($p = 0.011$) and IL-10 ($p = 0.014$) when compared with their siblings. Siblings ($p = 0.006$) and patients ($p < 0.001$) had higher levels of TNF-α when compared with controls but siblings presented decreased IL-1β when compared with controls ($p < 0.001$).

Cytokines and history of childhood maltreatment

Cytokines found to be different among the groups (IL-1β, IL-6, TNF-α, IL-10, TGF-β) were further tested to investigate the between-group interaction with history of childhood maltreatment (global and subtypes) and the within-group differences, adjusted for age, gender, BMI, tobacco smoking, psychoactive substance use, years of study and relationship status. Significant results were found for TGF-β only (Figs 1 and 2).

Our between-group comparison showed a significant interaction between groups and childhood maltreatment for TGF-β ($F_{2,421} = 3.969$; $p = 0.020$), even after adjusting for recent stress ($F_{2,421} = 4.257$; $p = 0.015$). In *post-hoc* analysis ($F_{2,126} = 6.250$; $p = 0.003$), both patients and their siblings had higher levels of TGF-β when compared with controls ($p = 0.020$ and $p = 0.023$, respectively). When looking at childhood maltreatment subtypes, only physical abuse yielded significant results ($F_{2,421} = 4.411$; $p = 0.013$), remaining significant after adjusting for recent stress ($F_{2,421} = 4.571$; $p = 0.011$). *Post-hoc* analysis revealed higher levels of TGF-β in patients when compared with controls ($F_{2,43} = 4.237$; $p = 0.030$). Within each group, FEP patients with physical abuse had higher levels of TGF-β when compared with FEP without physical abuse ($F_{1,113} = 6.119$; $p = 0.015$), even after adjusting for recent stress ($F_{1,113} = 6.630$; $p = 0.011$). There was longer duration of pharmacological treatment, in weeks, in those with physical

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Table 2. Clinical characteristics of the FEP sample ($n = 114$)

Variables	First episode psychosis
Psychosis onset age	
Mean (s.d.)	30.0 (12.5)
Median	26.0
DUP (in weeks)	
Mean (s.d.)	52.8 (151.3)
Median	11.0
BPRS (Total score)	
Mean (s.d.)	8.6 (6.4)
Median	7.0
Duration of psychosis (in weeks)	
Mean (s.d.)	81.1 (154.7)
Median	35.5
Pharmacological treatment (in weeks)	
Mean (s.d.)	29.7 (43.3)
Median	12.5
Current treatment	
Antipsychotics (AP): *n* (%)	49 (43.0)
Antidepressants (AD): *n* (%)	1 (1.0)
Mood stabilisers (MS): *n* (%)	2 (1.8)
AP + AD: *n* (%)	23 (20.2)
AP + MS: *n* (%)	25 (22.0)
AP + AD + MS: *n* (%)	7 (6.0)
None: *n* (%)	7 (6.0)

FEP, first-episode psychosis; DUP, Duration of untreated psychosis; BPRS, Brief Psychiatric Rating Scale.

abuse (58.7 ± 69.2 *v.* 23.1 ± 32.1; $F_{1,113} = 12.739$; $p = 0.001$). The results remained significant after including the duration of treatment in the model ($F_{1,113} = 6.548$; $p = 0.012$). Furthermore, when we stratified physical abuse by different dimensions of severity [none to minimum ($n = 80$), low to moderate ($n = 13$), moderate to severe ($n = 13$) or severe to extreme ($n = 8$)], we found that the higher cut-offs of severity were associated with higher levels of TGF-β ($F_{3,113} = 3.510$; $p = 0.018$). Patients reporting the two highest cut-offs of severity had higher levels of TGF-β than patients reporting the low to moderate cut-off (moderate to severe: $p = 0.040$; severe to extreme: $p = 0.039$ *v.* low to moderate, respectively).

Control subjects with experience of childhood maltreatment had lower TGF-β levels than controls without childhood maltreatment ($F_{1,250} = 5.916$; $p = 0.016$), even after adjusting for recent stress ($F_{1,250} = 6.220$; $p = 0.013$). When focusing on subtypes of childhood maltreatment, controls with physical abuse showed lower TGF-β levels ($F_{1,250} = 3.965$; $p = 0.048$) when compared with controls without physical abuse, remaining significant after adjusting for recent stress ($F_{1,250} = 4.010$; $p = 0.046$).

There was no association between reports of childhood maltreatment and inflammatory cytokines in the sibling group.

The remaining cytokines IL-6, IL-1β, TNF-α, IL-10 were not associated with reports of childhood maltreatment in any of the groups ($p > 0.05$ for all).

Cytokines and history of recent stressors

Cytokine levels were not significantly different whether subjects experienced or not recent stress in none of the groups ($p > 0.05$).

Discussion

The present study showed that FEP patients have a high pro- and anti-inflammatory cytokine profile (IL-1β, TNF-α, IL-6, IL-10 and TGF-β), which was not observed in unaffected siblings. Nevertheless, in those exposed to childhood maltreatment, both patients and unaffected siblings showed increased levels of TGF-β when compared with controls. Physical childhood abuse was associated with increased levels of TGF-β in FEP patients but with decreased levels in controls.

Low-grade inflammatory profile of FEP patients

Our findings suggest an inflammatory profile characterised by activation of the inflammatory response system (M1: IL-1β, TNF-α, IL-6) and the compensatory system (M2 and T-regulatory cells: IL-10 and TGF-β) in psychosis. The inflammatory profile reported in our study adds important information to meta-analysis of peripheral cytokines in psychosis. For instance, whereas increased IL-6 and TNF-α are the most replicated findings (Goldsmith *et al.*, 2016), the participation of well-known anti-inflammatory cytokines are still vastly ignored. In this sense, we demonstrate the co-existence of an up-regulation of anti-inflammatory cytokines in FEP. The existence of an anti-inflammatory profile complements previous findings of enhanced levels of two cytokine receptors (sIL-2R and IL1-Ra) facilitating anti-inflammatory actions (Goldsmith *et al.*, 2016), and is also consistent with data showing higher percentages of both pro- and anti-inflammatory monocytes/T cells in recent-onset schizophrenia, including activation of $CD4^{+}CD25^{high}FoxP3^{+}$ T-regulatory cells, which produce both IL-10 and TGF-β (Drexhage *et al.*, 2011). In this sense, the concomitant up-regulation of pro- and anti-inflammatory cytokines may provide a compensatory response and may be favourable in preventing the detrimental effects of chronic inflammation.

Familial liability and low-grade inflammation

Overall, increased pro- and anti-inflammatory cytokines were not observed in unaffected siblings, which could indicate that familial liability does not play a major role in determining the inflammatory profile found in FEP. However, our results could have been largely affected by the relatively reduced sample size of siblings who took part in our study. Alternatively, immune dysregulation in siblings may exist during vulnerable periods for the development of psychosis (e.g. adolescence) but when the increased risk does not lead to full-blown psychosis, cytokine levels normalise to the level of those without familial risk. Longitudinal studies are needed to test this hypothesis. Given that siblings not only did not show increased inflammatory profile, but they instead showed lower levels of IL-1β – one of the main pro-inflammatory cytokines – when compared with both patients and controls, we could speculate that the relative lack of immune activation in siblings may represent a protective factor. Considering the higher proportion of females in our sibling sample and that higher vulnerability to psychosis is observed among males (Jongsma *et al.*,

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Table 3. Cytokines plasma levels in FEP, unaffected siblings and community-based controls

	FEP ($n = 114$)	Siblings ($n = 57$)	Controls ($n = 251$)	Test and significance		
				F	df	*p*
IL-1β (pg/mL)	3.0 (0.2)	2.4 (0.3)	3.3 (0.2)	8.028	2,421	<0.001[a,b]
IL-6 (pg/mL)	2.3 (0.3)	1.9 (0.4)	1.8 (0.2)	12.655	2,421	<0.001[c]
TNF-α (pg/mL)	6.1 (0.3)	5.4 (0.3)	4.5 (0.2)	20.708	2,421	<0.001[c,b]
IFN-γ (pg/mL)	21.8 (2.2)	22.8 (3.0)	21.8 (1.4)	0.060	2,421	0.942
IL-4 (pg/mL)	0.2 (0.02)	0.2 (0.03)	0.2 (0.01)	0.089	2,421	0.915
IL-10 (pg/mL)	6.9 (0.5)	4.4 (0.7)	3.9 (0.3)	18.166	2,421	<0.001[a,c]
TGF-β (pg/mL)	784.5 (60.7)	507.0 (80.2)	544.5 (38.1)	5.483	2,421	0.004[c]

FEP, First-episode psychosis.
GLM analysis with Bonferroni corrections, adjusted for age, gender, BMI, tobacco smoking, years of study, relationship status, and psychoactive substance use. Cytokines levels are presented as raw values (adjusted mean ± S.E.M.) with statistics performed on the logarithmically transformed values. Post-hoc analysis significance is reported as follows:
aFirst-episode psychosis *v.* siblings.
bSiblings *v.* controls.
cFirst-episode psychosis *v.* controls.

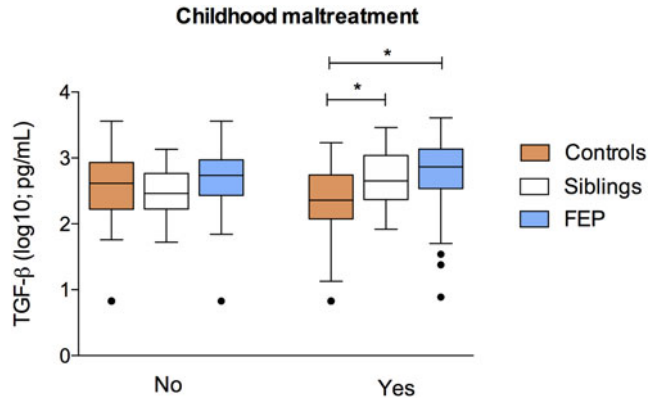

Fig. 1. TGF-β plasma levels in first-episode psychosis patients ($n = 114$), siblings ($n = 57$) and community-based controls ($n = 251$) with and without childhood maltreatment. Plasma cytokines were logarithmically transformed and data are expressed as mean ± S.E.M. and given as pg/mL. *$p < 0.05$ represents results for the GLM between-group interaction analysis adjusted for age, gender, BMI, tobacco smoking, years of study, relationship status, psychoactive substance use and recent stress. FEP, first-episode psychosis.

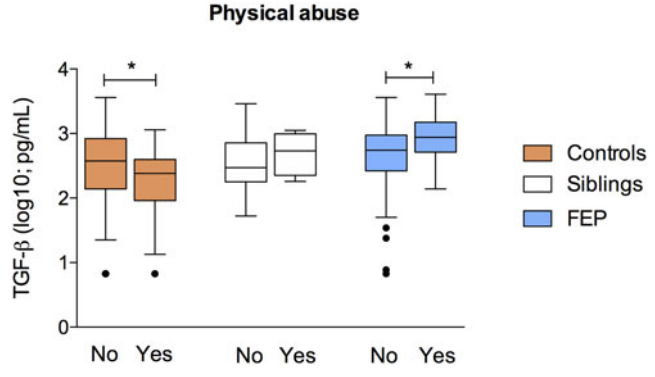

Fig. 2. TGF-β plasma levels in first-episode psychosis patients ($n = 114$), siblings ($n = 57$) and community-based controls ($n = 251$) with and without physical childhood abuse. Plasma cytokines were logarithmically transformed and data are expressed as mean ± S.E.M. and given as pg/mL. *$p < 0.05$ represents results for the GLM within-group interaction analysis adjusted for age, gender, BMI, tobacco smoking, years of study, relationship status, psychoactive substance use and recent stress. FEP, first-episode psychosis.

2018), future studies with a balanced sex distribution and larger sample size could help to clarify sex differences related to IL-1β.

One possible argument regarding the increased immune activation in our patients compared to both siblings and controls could be the exposure of our FEP patients to antipsychotic treatment. The immunomodulatory effects of antipsychotic treatment are not always consistent across studies and large part of these effects has been recently suggested to be partly consequence of their metabolic side effects (Baumeister *et al.*, 2016; Calevro *et al.*, 2018). In order to reduce the confounding effect of antipsychotics on the inflammatory markers, we focussed on the study of FEP patients, who had limited exposure to antipsychotic treatment, and we controlled all our analyses for important metabolic confounding factors, which have been discussed to contribute to cytokine abnormalities in the general population, specially the effects of age, gender, BMI and smoking (Goldsmith *et al.*, 2016). Hence, the identified inflammatory profile points towards a pathophysiological component of psychosis.

Childhood maltreatment and low-grade inflammation

Early-life stress has been argued as an important factor in the immune activation of psychosis (Baumeister *et al.*, 2016), but our results do not support previous studies reporting enhanced TNF-α (Dennison *et al.*, 2012; Di Nicola *et al.*, 2013) and IL-6 (Dennison *et al.*, 2012) in reduced sample sizes of psychotic patients exposed to childhood maltreatment. In accordance, larger studies in patients with chronic schizophrenia/bipolar disorder also failed to report associations of childhood maltreatment with TNF-α, IL-6 (Quidé *et al.*, 2018), the soluble TNF-1 receptor or gp130 (the IL-6 signal-transducing component) (Aas *et al.*, 2017). Inconsistent findings could possibly relate to methodological limitations observed (sample size, lack of control for confounders) or geographical and economic factors (high *v.* low-middle income countries). In our sample, the non-significant associations could be due to the non-specific effects of childhood maltreatment, as it is likely that the observed inflammatory profile may arise from cumulative risk factors, which should be the focus of future investigations.

Interestingly, we found that both FEP patients and their unaffected siblings exposed to childhood maltreatment exhibited

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higher levels of TGF-β when compared with controls exposed to childhood maltreatment, implying TGF-β as a possible biological candidate of familial risk for psychosis, which may result from the interplay between shared genetic and environmental factors. We also found that exposure to physical childhood abuse was associated with increased levels of TGF-β in FEP patients but with decreased levels in controls, and that the severity of childhood physical abuse was positively associated with the levels of TGF-β in patients. With regards to siblings, although we did not find significant differences in TGF-β between those with and without physical abuse, the pattern of TGF-β in the exposed group was similar to their FEP peers, further supporting TGF-β as a possible biological candidate of familial risk for psychosis. The lack of statistical significance could be explained by the relatively low number of siblings reporting physical abuse ($n = 4$).

The specificity for physical abuse, but not other subtypes, in augmenting TGF-β is intriguing is several ways, and may suggest exposure-specific mechanisms. Indeed, physical abuse is the most prevalent subtype of childhood maltreatment in subjects reporting psychotic experiences, contributing to the highest odds ratio for a subsequent psychotic episode in males (McGrath *et al.*, 2017). This is also consistent with previous findings of a stronger association of physical and sexual childhood abuse, rather than other childhood maltreatment, with increased levels of inflammatory markers in adulthood (Baumeister *et al.*, 2016).

We found an opposite effect in community-based controls, with low levels of TGF-β in those who reported general childhood maltreatment but also in the subtype physical abuse. A possible explanation behind this would further support the role of the immune system in mediating the association between childhood maltreatment and psychosis, with controls showing opposite immune activation as a possible protective factor.

GWAS implicate TGF-β in schizophrenia (Jia *et al.*, 2010; Sanders *et al.*, 2017), and increased TGF-β protein (Kim *et al.*, 2004; Borovcanin *et al.*, 2013), gene expression (Amoli *et al.*, 2019) and lymphocyte receptor (Numata *et al.*, 2008) were described before in medication-free and FEP patients, although previous findings were not corrected for confounders as in our study. TGF-β is a pleiotropic cytokine and a master regulator of the immune system, orchestrating the balance between innate and adaptive immune components (Th1, Th2, Th17, monocytes), and mediating the differentiation of naïve $CD4^+$ T-cells towards T_{reg}-cells producing anti-inflammatory cytokines (IL-10, TGF-β) (Kim *et al.*, 2004; Borovcanin *et al.*, 2013). However, TGF-β can also contribute to pro-inflammatory reactions and to a heightened risk of inflammation (Chen *et al.*, 2003). In the brain, TGF-β controls the excitatory/inhibitory transmission balance, facilitating neuronal hyperexcitability (Sun *et al.*, 2010). Genetic liability for increased TGF-β, would therefore, facilitate immune and neuronal dysregulation and consequent increased risk for psychosis, with childhood maltreatment acting as a possible trigger. Thus, screening for childhood maltreatment and subtyping the immune profile among low-and high-liability groups may provide insights for personalised interventions for better clinical outcomes.

Recent stress and low-grade inflammation

Lastly, our study does not support that recent stress (past 12 months) may have an effect on cytokines. It has been reported that acute psychological stress may induce short-term systemic inflammations, whereas chronic stress may be associated with more permanent inflammatory changes (Rohleder, 2014). A longitudinal study showed that childhood maltreatment but not recent stress accounted for the association between increased inflammation and depression in patients with cancer (Archer *et al.*, 2012). We are also in accordance with a study reporting association between cytokines and childhood maltreatment but not recent stress in depression (Grosse *et al.*, 2016). If that is true, these findings may inform about sensitive periods in stress-induced immune changes.

Strengths and limitations

In this study, we attempted to overcome several methodological limitations identified previously. First, this is the largest sample yet to test the association between childhood and recent stress in FEP patients, siblings, and community-based controls adjusting for a broad range of confounders. Second, participants' recruitment in this study followed the National Census of Brazil for a representative sample, which is distinctive from the previous investigations including convenience samples sensitive to selection bias. Third, we tested for different subtypes of childhood maltreatment, which were before considered as one phenomenon. Fourth, our cytokine profile was much broader, allowing us to test both pro- and anti-inflammatory cytokines. Fifth, we included siblings in the study in order to test more specific confounding effects of shared environment and genetic risk than the classic case-control design. The results presented herein provide strong additional support for an association between childhood maltreatment and cytokine abnormalities in adulthood across the different diagnostic groups.

Our study has some limitations. First, we relied on retrospective self-reports, and therefore recall bias can be an issue. Despite that, studies have shown the validity of retrospective self-reports in both patients and controls (Grassi-Oliveira *et al.*, 2006), and siblings' reports contributed towards validation of patients' reports in this study. Still, our results should be interpreted in the context of a retrospective measurement. Second, the majority of patients in this study were not drug-naïve. However, cytokine abnormalities have been reported in drug-naïve FEP (Pillinger *et al.*, 2018), and longitudinal studies have demonstrated association between inflammatory markers during childhood/adolescence and future risk for psychosis in young adulthood (Khandaker *et al.*, 2014; Metcalf *et al.*, 2017); therefore, it is unlikely that the cytokine changes found in our study may be solely from medication effects. Moreover, we controlled for duration of antipsychotic treatment in the within-group analyses for the patients when looking at the effects of childhood maltreatment and we controlled for metabolic factors (contributing to the effects of antipsychotic on inflammatory markers) in all our analyses.

Conclusion

We found increased pro- and anti-inflammatory cytokines in FEP patients but not in unaffected siblings who, similarly to patients, were exposed to higher childhood maltreatment than community-based controls, suggesting that the identified inflammatory profile can be a real pathophysiological component of psychosis and that normal or reduced immune activation may be protective in siblings.

Remarkably, our study suggests that individuals at high genetic liability for psychosis may be susceptible to immune dysregulation when exposed to childhood maltreatment, implicating increased

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TGF-β as a possible biological candidate of shared familial risk. Whether the opposite effects of childhood maltreatment on TGF-β reflect important biological mechanisms increasing risk or resilience to psychosis would need to be further explored in future studies.

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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