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Chapter 4

Inflammatory cytokines and growth factors were not associated with psychosis liability or childhood trauma

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Abstract

Psychosis is a multifactorial condition arising from an interaction between genetic liability and exposure to environmental risk factors, in particular childhood trauma. Furthermore, accumulating evidence supports a role for the immune system in the aetiology of psychosis. Increased peripheral levels of pro-inflammatory cytokines and reduced neurotrophic factors are found in patients with psychosis. Childhood trauma is highly prevalent in psychosis patients and is also associated with increased pro-inflammatory cytokines and reduced neurotrophic factors. Recent studies suggest the increase in pro-inflammatory cytokines and decrease in neurotrophic factors seen in psychosis may be attributable to the effects of child maltreatment. The aim of this study was to improve understanding of the relation between childhood trauma, inflammation and psychosis. We examined separate and interaction effects of psychosis liability and childhood trauma on serum levels of BDNF, CCL-2, CRP, IFN-γ, IGFBP2, IL-6, PDGF, SCF and TNF-α in 40 patients with recent onset psychosis, 13 patients at Ultra-High Risk (UHR) for psychosis, 31 unaffected siblings of psychosis patients and 41 healthy controls. Childhood trauma was assessed retrospectively with the Childhood Trauma Questionnaire (CTQ). No statistically significant effects of psychosis liability or childhood trauma on concentrations of cytokines or growth factors in peripheral blood were found, nor were there any statistically significant interaction effects of psychosis liability with childhood trauma on serum levels of cytokines and growth factors.

Introduction

Psychosis is a multifactorial condition arising from an interaction between genetic liability and exposure to environmental risk factors[1]. The traditional biological explanatory model includes neurodevelopmental abnormalities and dopamine dysregulation. In recent decades, this view has been expanded by a hypothesized role for the immune system in the aetiology of psychosis. Counts and function of immune cells, including monocytes and T cells and serum levels of cytokines were shown to be altered in the peripheral blood of psychosis patients [2]. While interleukin (IL)-12, interferon (IFN)-γ, tumor necrosis factor (TNF)-α and soluble IL-2 receptor (sIL-2r) are elevated in both recent onset as well as chronic and medicated psychosis patients, pro-inflammatory cytokines IL-1β, IL-6 and transforming growth factor (TGF)-β are elevated only in patients with recent onset psychosis and acute relapse and normalized after antipsychotic treatment [3]. A recent study on a large series of 180 antipsychotic-naïve schizophrenia patients from four different sites [4] confirmed abnormal levels of both pro- and anti-inflammatory cytokines, which were also dependent on the duration and treatment of the disease. Immune dysregulation can affect brain function via several pathways [5,6]. For example, some cytokines can pass the blood brain barrier and are neurotoxic or induce neuroinflammation [7]. Also, the immune system has a beneficial role in neurodevelopment and plasticity which can be disturbed by both deficient and exaggerated immune activation [8]. Furthermore, the immune system is connected to other regulatory systems, e.g. the neuroendocrine stress response, glutamate transmission and secretion of neurotrophic factors. Inflammation has been linked to decreased brain-derived neurotrophic factor (BDNF) levels [9]. BDNF is a growth factor essential for neurodevelopment and plasticity. Reduced BDNF levels have been reported in schizophrenia [10]. It is still largely unknown whether altered levels of cytokines and (neuro)trophic factors in psychosis are a cause or consequence of psychotic diseases or an intermediate factor linking other risk factors to psychosis. 

Childhood trauma was consistently shown to be associated with psychotic disorders in a dose-response manner, across varying designs including large prospective studies [11]. Psychosocial stress activates the hypothalamic-pituitary-adrenal axis, resulting in release of glucocorticoids. Glucocorticoid receptors are ubiquitously expressed in immune cells. The acute effects of glucocorticoids are largely immunosuppressive [12,13]. However, prolonged stress especially during critical time frames may affect the immune system differentially and result in low-grade inflammation [14]. Childhood maltreatment during the first decade of life was associated with markers of inflammation in adults [15]. A dose-response relation was found between severity of maltreatment and increased levels of high sensitive C-reactive protein (CRP), fibrinogen
and white blood cell count. In a population of medicated and chronic patients with schizophrenia or schizoaffective disorder, IL-6 and TNF-α were significantly raised in the subset of 24 out of 40 patients that reported a history of childhood trauma compared to healthy and non-traumatized controls [16]. In the group of patients that did not report childhood trauma, levels of pro-inflammatory cytokines were comparable to healthy controls. In patients with recent onset psychosis, hsCRP levels were raised only in the subgroup of patients who had experienced sexual abuse [17].

These studies suggest the increase in pro-inflammatory cytokines and decrease in neurotrophic factors seen in psychosis may be attributable to the effects of child maltreatment. Another possibility is that individuals at (genetic) risk for psychosis are more vulnerable to effects of child maltreatment on the immune system. It is still largely unknown how psychosis liability and childhood trauma interact. Furthermore, only healthy controls and psychosis patients, but not other psychosis liability groups have been examined.

The aim of this study was to improve understanding of the association between childhood trauma, inflammation and psychosis. It concerns a secondary analysis of a previously described study sample [18]. We examined serum levels of cytokines and growth factors, namely BDNF, chemokine (C-C motif) ligand (CCL)-2, CRP, IFN-γ, insulin-like growth factor binding protein (IGFBP2), IL-6, platelet-derived growth factor (PDGF), stem cell factor (SCF) and TNF-α in groups with different liability for psychosis, with and without the experiences of childhood trauma.

Psychosis can be seen as the expression of a psychosis phenotype along a continuum [19,20], ranging from individuals with increased risk to psychotic disorder, to individuals with mild or nonspecific symptoms, to individuals with moderate, subthreshold symptoms and functional decline, to people who have a single psychotic episode and finally severe and unremitting psychotic illness [19]. In this study, four groups along this psychosis continuum were selected: patients with recent-onset psychotic disorder, patients with moderate, subthreshold psychotic symptoms (so-called ultra-high risk for psychosis), unaffected siblings of patients with psychotic disorder and healthy controls.

Furthermore, (interaction) effects of lower vs. higher psychosis liability and childhood trauma on serum levels of cytokines and growth factors were examined.

Methods

Participants

This study was a secondary analysis in a subset of a previously described study sample [18]. Individuals aged 18-35 with different phenotypic liability to psychosis were included: [1] 40 patients with a first diagnosis of any psychotic disorder - except for substance-induced psychotic disorder and psychotic disorder due to a medical condition - established within the last five years and [2] 13 Patients at Ultra-High Risk (UHR) for psychosis [3] 31 unaffected siblings of patients diagnosed with a psychotic disorder and [4] 41 healthy controls. Patients and siblings were recruited from psychiatric institutions in The Hague, Rotterdam, Delft and Castricum in the Netherlands. UHR patients were recruited from the patient population of the Early Detection and Intervention Team (EDIT) implemented in these institutions. Details of the screening methodology of EDIT are described elsewhere [21]. Briefly, all referrals to secondary mental healthcare facilities were pre-screened using the self-report Prodromal Questionnaire [22]. Those scoring above the cut-off score for subclinical positive psychosis symptoms were further assessed in a semi-structured clinical interview using the Comprehensive Assessment of At-Risk Mental States (CAARMS) [23] to determine presence, severity, frequency and distress of UHR symptoms. Criteria for UHR are based on the positive symptoms subscale (including unusual thought content, non-bizarre ideas, perceptual abnormalities and disorganized speech). The UHR patients included in this study all had both subclinical psychotic symptoms as determined by the CAARMS as well as a decline in social functioning as determined by the Social and Occupational Functioning Assessment Scale (SOFAS) [24].

Controls were recruited from the same communities through flyers in public facilities, including schools for vocational education and dentistry practices. Exclusion criteria were poor command of the Dutch language, an IQ lower than 75 and a history of epilepsy or autoimmune disorder. Psychosis patients and UHR patients were classified as high psychosis liability and siblings and healthy controls were classified as low psychosis liability, based on [1] phenotype of (subsyndromal) psychotic symptoms which was present in UHR and psychosis patients and absent in siblings and healthy controls and [2] life time risk for psychosis, which is 100 % in psychosis patients, 36% in UHR patients [25], 10% in siblings and 3% in controls from the general population [26]. The study was approved by the medical ethical committee of Leiden University Medical Centre. Participants received verbal and written information about the study and were given the opportunity to ask questions to the study or an independent researcher. At the start of experiments researchers repeated their explanation and made sure the participants understood all procedures, before written informed consent was obtained from all participants.
Questionnaires

Electronic self-report questionnaires were administrated to obtain information about medical history, length, weight, use of psychotropic and other (including over-the-counter) medication, substance use (smoking, alcohol, cannabis/THC and illicit drugs) and sociodemographic characteristics including sex, age, ethnicity and education level. Childhood trauma was assessed retrospectively with the Childhood Trauma Questionnaire Short Form (CTQ-SF), a well validated 25-item self-report questionnaire including five subscales: emotional abuse, emotional neglect, physical abuse, physical neglect and sexual abuse [27]. Childhood trauma was defined as present if any subscale scores was classified as moderate or severe according to published norm scores [28].

Serum measures

Blood samples were taken before questionnaires and collected in clotting tubes (5 ml) for serum preparation. Serum samples were stored in liquid nitrogen to enable testing patient and control immune cells in the same experiment. IFN-γ and TNF-α were measured using high sensitive ELISA kits (eBioscience) according to the manufacturer’s instructions. Briefly, stock sera were defrosted at 4 ºC. Pre-manufactured microwells absorbed with IFN-γ coating antibody or TNF-α coating antibody were incubated with 50 μl of sample (diluted 2-fold) and 50 μl Biotin-conjugated anti-human TNF-α antibody or anti-human IFN-γ antibody at room temperature on a microplate shaker. Microwells were washed and subsequently incubated with Streptavidin-HRP. After washing, amplification agent I (Biotinyl-Tyramide) was added and washed away after 15 minutes. Amplification reagent II (streptavidin-HRP) was added and washed away after 30 minutes. Finally, the wells were incubated with substrate solution and the reaction was terminated by addition of 1 M Posphoric acid. Absorbance was measured at 450 nm as the primary wave length and 620 nm as reference wave length. Each plate contained one sample from participants from all liability groups and 6 standard dilutions performed in duplicate.

A premixed multi analyte Luminex kit (LXSAHM, R&D) was used to measure BDNF, CCL-2, PDGF-BB and SCF. In short, 50 μl of sample (diluted 2-fold) or standard was incubated with microparticle cocktail (color-coded magnetic microparticles pre-coated with analyte specific antibodies) at room temperature on a microplate shaker. Any unbound substances were washed away using a magnetic plate washer (BioPlex, Bio-Rad). Microwells were subsequently incubated with a biotinylated antibody cocktail specific to the analytes, washed and incubated with streptavidin-phycoerythrin conjugate. After a final wash, the microparticles were resuspended in buffer and read on a Luminex MAGPIX Analyzer (Bio-Rad). Each plate contained one sample from participants from all liability groups and 6 standard dilutions performed in duplicate.

IGFBP-2 was measured using an ELISA-quantikine (R&D) according to the manufacturer’s instructions. Sample and standard dilutions were assayed in duplicate. Participants with CRP levels above 10 µg/ml and were excluded from analysis as this may be indicative of acute infections. This was the case for eight participants (2 healthy controls, 2 siblings, 2 UHR patients and 2 psychosis patients).

Range and sensitivity of assays is shown in Table 4.1. The lowest point of each calibration curve was considered the lower limit of quantification (LLOQ) and the highest point of each calibration curve the upper limit of quantification (ULOQ). Number of values outside limits of quantification are shown in Table 4.1. Where extrapolated values were available, these were used. Values below the lower limit of detection were imputed with values representing 0.5 times the lowest (extrapolated) value. Values above the upper limit of detection were imputed with value 1.5x the highest (extrapolated) value.

For three samples (1 healthy control and 2 psychosis patients), there was insufficient material to perform all assays.

Statistics

All analyses were conducted with IBM SPSS version 23. Significance was assumed at α < 0.05 (two-tailed). Continuous variables were inspected for normal distribution and log transformed (ln) to achieve a normal distribution if necessary. Sociodemographic characteristics and serum factor levels of cytokines and growth factors were compared between the four groups using one-way analysis of variance (ANOVA) with three predetermined contrasts (high vs. low liability, psychosis vs. UHR and siblings vs. controls) and explorative post-hoc Dunnett’s t-tests for continuous variables and χ² tests for categorical variables.

We used linear or logistic regression models to examine (interaction) effects of psychosis liability and childhood trauma. We explored association of covariates (age, sex, ln(BMI), smoking, cannabis/THC use, ethnicity, education and use of contraceptive or other relevant medication) with outcome measures by entering them separately in MANOVAs. Covariates with associations with outcome measures at significant or trend level (p < 0.1) were selected for correction of regression models. The basic model
Table 4.1. Assay sensitivity.

| Technique | BDNF | CCL2 | CRP | IFN-γ | IGFBP-2 | IL-6 | PDGF | SCF | TNF-α |
|-----------|------|------|-----|-------|---------|------|------|-----|-------|
| Range of quantification | Luminex | Luminex | hELISA | hELISA | hELISA | Luminex | Luminex | hELISA | hELISA |
| N | 116 | 115 | 117 | 114 | 117 | 115 | 115 | 117 | 117 |
| Below LLOD (%) | 0 | 0 | 2 | 0 | 0 | 4 | 0 | 0 | 0 |
| Above LLOD (%) | 0 | 0 | n.a. | 5 | 2 | 4 | 0 | 0 | 0 |
| Within limits of quantification N (%) | 108 (93.1) | 115 (100) | 98 (80.9) | 56 (47.8) | 114 (100) | 54 (46.2) | 36 (31.3) | 29 (24.8) |
| Below LLOQ (%) | 0 | 0 | 25 | 0 | 0 | 54 | 0 | 79 | 54 |
| Above ULOQ (%) | 8 | 0 | n.a. | 6 | 5 | 25 | 0 | 0 | 0 |

LLOD = lower limit of detection; ULOD = upper limit of detection; LLOQ = lower limit of quantification; ULOQ = upper limit of quantification. Participants with CRP levels above 10 μg/ml were excluded from analysis.

Results

Sociodemographic characteristics

After exclusion of participants with CRP levels above 10 μg/ml, data was available for 39 healthy controls, 29 siblings, 11 ultra-high risk (UHR) and 38 psychosis patients. Sociodemographic characteristics are shown in Table 4.2. Groups differed significantly on gender, smoking, education level and use of psychotropic medication. Psychosis patients were significantly more likely to be male (χ²(1) = 12.24, p < 0.001) than healthy controls. UHR patients were significantly more likely to smoke than healthy controls (χ²(1) = 11.07, p = 0.001) and there was a trend for psychosis patients to smoke more often than healthy controls (χ²(1) = 3.64, p = 0.056). Both UHR and psychosis patients had on average finished lower levels of education than healthy controls (τB = -2.77, p = 0.048 and τB = -0.286, p = 0.004 for UHR and psychosis patients, respectively). For none of the UHR patients this included an antipsychotic. The sample included 12 psychosis patients that did not use psychotropic medication.

Serum measures

Serum levels of BDNF, CCL2, CRP, IFN-γ, IGFBP-2, IL-6, PDGF, SCF, TNF-α in controls, UHR and psychosis patients with and without childhood trauma are depicted in Figure 4.1. Differences in serum levels between controls, siblings, UHR and psychosis patients were explored with uncorrected one-way analysis of variance (ANOVA). No significant group differences were found in serum levels of BDNF, CCL2, CRP, IFN-γ, IGFBP-2, IL-6, PDGF, SCF, TNF-α (Table 4.3). Results of predetermined contrasts (high vs. low liability, psychosis vs. UHR and siblings vs. controls) and explorative post-hoc Dunnett’s tests are available in supporting information.
Table 4.2. Sociodemographic characteristics.

|                     | Low psychosis liability | High psychosis liability | Controls | Siblings | UHR | Psychosis | N | p         |
|---------------------|-------------------------|--------------------------|----------|----------|-----|-----------|---|-----------|
| Age                 | 24.0                    | 25.5                     | 24.0     | 25.5     |     |           |   | 0.438     |
| (21.0-26.0)         | (21.3-30.0)             | (20.0-29.0)              | (22.0-30.0) |          |     |           |   |           |
| BMI                 | 22.8                    | 23.6                     | 23.1     | 23.0     |     |           |   | 0.979     |
| (20.2-24.6)         | (21.0-24.8)             | (18.5-26.2)              | (20.4-24.3) |          |     |           |   |           |
| Native Dutch        | 29 (74.4)               | 19 (67.9)                | 8 (72.7) | 21 (55.3) |     |           |   | 0.328     |
| Education           |                         |                          |          |          |     |           |   |           |
| No/primary          | 0 (0)                   | 0 (0)                    | 0 (0)    | 3 (7.9)  |     |           |   | 0.002     |
| Vocational          | 10 (25.6)               | 9 (32.1)                 | 7 (63.6) | 17 (44.7) |     |           |   |           |
| Secondary           | 8 (20.5)                | 2 (7.1)                  | 1 (9.1)  | 7 (18.4)  |     |           |   |           |
| Higher              | 21 (53.8)               | 17 (60.7)                | 3 (27.3) | 11 (28.9) |     |           |   |           |
| Smoking             | 6 (21.1)                | 7 (29.2)                 | 9 (81.8) | 16 (47.1) |     |           |   | 0.005     |
| Cannabis use        | 8 (20.5)                | 2 (7.1)                  | 4 (36.4) | 10 (26.3) |     |           |   | 0.138     |
| Medication          |                         |                          |          |          |     |           |   |           |
| Psychotropic        | 0 (0)                   | 1 (3.6)                  | 7 (63.6) | 26 (68.4) |     |           |   | <0.001    |
| Contraceptivea      | 12 (31.7)               | 3 (27.3)                 | 4 (66.7) | 1 (16.7)  |     |           |   |           |
| Other               | 3 (7.7)                 | 1 (3.6)                  | 1 (9.1)  | 5 (13.2)  |     |           |   | 0.584     |

Values displayed are median (interquartile range) or N (%). p-values of ANOVA (for continuous variables), X² tests (for dichotomous variables) or Kendall’s tau-B (for education) are given. BMI = body mass index. Smoking during last 24 hours. Cannabis use during last month. aPercentage of all females within group.

Table 4.3. ANOVA test statistics.

|                      | F    | df | p   | η²  |
|----------------------|------|----|-----|-----|
| Ln(BDNF)             | 1.296| 3, 112| 0.279 | 0.014 |
| Ln(CCL-2)            | 0.652| 3, 111| 0.583 | 0.017 |
| Ln(CRP)              | 0.673| 3, 113| 0.570 | 0.018 |
| Ln(IFN-γ)            | 0.318| 3, 113| 0.813 | 0.008 |
| Ln(IGFBP-2)          | 1.435| 3, 110| 0.237 | 0.038 |
| Ln(IgG)              | 0.290| 3, 113| 0.832 | 0.008 |
| Ln(PDG)              | 0.264| 3, 111| 0.851 | 0.007 |
| Ln(SCF)              | 0.690| 3, 111| 0.560 | 0.018 |
| Ln(TNF-α)            | 0.924| 3, 113| 0.432 | 0.024 |

Four psychosis liability groups (healthy controls, unaffected siblings, UHR patients and psychosis patients) were tested using one-way analysis of variance. η² = partial eta squared effect size (0.01-0.06: small effect, 0.06-0.14: moderate effect > 0.14: large effect).
Childhood trauma was reported by 17.9% of controls, 13.8% of siblings, 81.8% of UHR and 52.6% of psychosis patients. Both UHR and psychosis patients reported childhood trauma significantly more often than healthy controls ($\chi^2 (1) = 16.09$, $p < 0.001$ and $\chi^2 (1) = 10.17$, $p = 0.001$ for UHR and psychosis patients, respectively). Type and number of traumas are depicted in Figure 4.2. All types of trauma were more likely to be reported by high liability group (UHR and psychosis patients) compared to the low liability group (healthy controls and siblings). These differences were significant for emotional abuse ($\chi^2 (1) = 13.94$, $p < 0.001$), physical abuse ($\chi^2 (1) = 8.70$, $p = 0.003$), sexual abuse ($\chi^2 (1) = 5.77$, $p = 0.016$), emotional neglect ($\chi^2 (1) = 19.97$, $p < 0.001$) and at trend level for physical neglect ($\chi^2 (1) = 3.11$, $p = 0.078$).

Separate and interaction effects of psychosis liability and childhood trauma on serum levels were examined using linear regression models. Sex, age, BMI, smoking, cannabis use, education and oral contraceptive use were selected as covariates, as they were associated with outcome measures in MANOVA significantly or at trend level ($p < 0.10$). Regression coefficients of corrected models are shown in Table 4.4. Regression coefficients of uncorrected models and models with additional correction for psychotropic use are available in supporting information. Addition of group, childhood trauma or the interaction term did not significantly improve the model for any of the cytokines and growth factors. There were no significant or trend level main or interaction effects of psychosis liability or trauma on growth factors and cytokines.

Figure 4.2. Childhood trauma as reported by groups with different psychosis liability. The Childhood Trauma Questionnaire (CTQ) was used to assess different types of abuse and neglect retrospectively. Percentages of participants within each group that reported moderate or severe levels of emotional abuse, physical abuse, sexual abuse, and neglect are shown. 

Table 4.4. Regression coefficients. Regression coefficients of linear regression models are given. Models included psychosis liability (high vs. low), childhood trauma (yes/no), and psychosis liability x childhood trauma as predictors and were corrected for sex, age, BMI, smoking, cannabis use, education and oral contraceptive use.
Discussion

We examined separate and interaction effects of psychosis liability and childhood trauma on serum levels of cytokines and growth factors. No statistically significant effects of psychosis liability or childhood trauma on concentrations of cytokines or growth factors in peripheral blood were found, nor were there any statistically significant interaction effects of psychosis liability with childhood trauma on serum levels of cytokines and growth factors.

It is possible that the sample size in our study was too small to reach statistically significance. Furthermore, selection bias cannot be ruled out and could have resulted in a relatively healthy patient population. The disease trajectories of the recent onset psychosis patients in this sample are still unknown, some will completely recover and not experience any other psychotic episodes, whereas others will develop a more chronic debilitating pattern of disease. A large proportion was taking antipsychotic medication and many may have been (partly) in remission. Similarly, a large portion of the UHR sample will develop a full-blown psychotic episode, whereas in others, the psychotic-like symptoms will lessen and/or functioning will improve. Thus, homeostasis may still be maintained or already restored in our sample.

Confounding factors may also explain differences between our negative results and previous findings. BMI, sex and age are known to affect cytokine levels in peripheral blood [29–31] and were indeed associated with outcome measures in our sample. Especially obesity is considered a likely confounder in psychosis studies, as it is linked with inflammatory markers, more common among psychosis patients and further increased by the use of psychotropic medication and may have been (partly) in remission. Similarly, a large portion of the UHR sample will develop a full-blown psychotic episode, whereas in others, the psychotic-like symptoms will lessen and/or functioning will improve. Thus, homeostasis may still be maintained or already restored in our sample.

Patients with psychotic disorders, including this study sample, form a heterogenous group and previous work has shown the course of immune dysregulation in psychosis patients to be highly dynamic, involving both pro- and anti-inflammatory forces. Cytokines and growth factors in peripheral blood are the end-product of diverse processes and players, including non-immune cells, e.g. adipocytes and endothelial cells. Immune dysregulation may occur without affecting serum cytokine levels, especially as pro-inflammatory mechanisms may be - temporarily - compensated by an increased anti-inflammatory response.

Limitations

Childhood trauma was measured retrospectively, which has the potential for recall bias. We used the Childhood Trauma Questionnaire, which is considered a well-validated and reliable instrument [27,35]. Furthermore, retrospective report of childhood trauma by psychosis patients was shown to be reliable [36]. We previously reported on the increased prevalence of childhood trauma in patients with high psychosis liability in this sample and found that patients with a history of childhood trauma reported more psychotic and affective symptoms in daily life and more paranoid ideation and stress after exposure to social stress in a virtual reality environment [37]. Our findings are in line with previous research, finding a high prevalence of childhood trauma in UHR [38] and psychosis patients [11] and increased prevalence of childhood trauma in psychosis patients compared to unaffected siblings [39]. In this secondary analysis, we confirmed increased prevalence of childhood trauma in UHR and psychosis patients in this subset and show that all subtypes of traumas had an increased prevalence, with a trend level effect for physical abuse and statistically significant effects for all other subtypes.

We selected four groups along the psychosis liability spectrum. While psychosis liability is a continuous concept, we dichotomized it into lower and higher liability to analyse (interaction) effects of childhood trauma. The sample size was too small to analyse groups separately, especially as childhood trauma was extremely common IL-23 compared to over-weight controls [34]. Interestingly, there was no difference between over- and normal-weight controls, whereas overweight patients had increased concentrations of inflammatory markers compared to normal-weight patients. While no formal interaction test was performed in this study, these findings are suggestive of an interaction effect between obesity and psychosis liability. Such an interaction effect would explain the negative results of our study where psychosis liability was present, but obesity relatively rare. Thus, obesity may be an essential player in the complex relation between psychosis, metabolic syndrome and immune dysregulation rather than a simple confounding factor.
among UHR, limiting the number of UHR patients without childhood trauma. We considered classifying siblings and UHR patients as an intermediate risk group, but as siblings were more comparable to controls both in terms of life time psychosis risk and phenotype, we considered it more valid to classify controls and siblings as lower and UHR and psychosis patients as higher psychosis liability groups. The life time risk of psychotic disorders is approximately 3% for controls (general population), 10% for siblings [26], 36% for UHR [25] and, by definition, 100% for patients with recent onset psychotic disorder. UHR and psychosis patients all reported (subsyndromal) psychotic symptoms, which were uncommon among siblings and controls from the general population.

We used high sensitive assays. Still, some values were outside the limit of detection, either being undetectably low or out of range high. Furthermore, a larger proportion of values were extrapolated by software as they were outside the calibration curve. The latter proportions were especially high for IFN-γ, IL-6, SCF and TNF-α (Table 4.1). The reliability of these measurements may be decreased by increased measurement error. However, they are not invalid measurements and still confer meaning and we therefore included them in our analysis. We aimed to be detailed and transparent regarding assay sensitivity and data reliability, but were unable to compare the sensitivity of our assays to other studies as the information reported was very limited.

Conclusions
We did not find evidence for independent or interaction effect of psychosis liability or childhood trauma on peripheral levels of cytokines and growth factors in this sample. These negative results should be interpreted within a framework of meta-analytic work showing deregulation in psychosis - which is known to be highly dynamic, the heterogeneity of patients with psychotic disorders and signs for a complex interaction with obesity.

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## Supporting information

### Table S4.1. Results of predetermined contrasts (high vs. low liability, psychosis vs. UHR, and siblings vs. controls) and explorative post-hoc Dunnett’s t-tests of one-way analysis of variance of serum levels in four psychosis liability groups: healthy controls, siblings, ultra-high risk (UHR) patients, and psychosis patients.

| Post-hoc contrast | Dunnett’s t-test (vs. controls) |
|-------------------|---------------------------------|
| High vs. low liability | Siblings vs. controls | UHR vs. psychosis |
|                  | B                 | CRI       | IFN-γ   | IGFBP-2 | IL-6    | PDGF   | SCF     | TNF-a   |
|                  | p                 | CI        | p       | CI       | p       | CI       | CI       | p       |
| Ln(BDNF)         | 1.372             | 0.173     | 1.093   | 0.277    | 0.011   | 0.991    | 0.586    | 0.491   | 0.171   |
| Ln(CCL-2)        | -0.114            | 0.909     | 0.065   | 0.948    | 1.298   | 0.197    | 1.000    | 0.843   | 0.722   |
| Ln(CRP)          | 0.968             | 0.335     | -1.023  | 0.308    | -0.788  | 0.432    | 0.635    | 0.881   | 0.991   |
| Ln(IFN-γ)        | -0.654            | 0.527     | 0.748   | 0.456    | 0.026   | 0.979    | 0.815    | 0.998   | 0.997   |
| Ln(IGFBP-2)      | 1.885             | 0.280     | 1.613   | 0.110    | -0.098  | 0.922    | 0.471    | 0.203   |         |
| Ln(IL-6)         | 0.453             | 0.651     | 0.360   | 0.719    | 0.417   | 0.678    | 0.973    | 0.995   | 0.702   |
| Ln(PDGF)         | 0.476             | 0.635     | 0.646   | 0.519    | -0.511  | 0.610    | 0.870    | 0.794   | 0.964   |
| Ln(SCF)          | -0.086            | 0.932     | 0.063   | 0.950    | 1.324   | 0.188    | 1.000    | 0.845   | 0.692   |
| Ln(TNF-a)        | 1.218             | 0.228     | 0.557   | 0.580    | -1.786  | 0.088    | 0.906    | 0.271   | 0.994   |

Note that *p*-values for Dunnett’s *t*-tests are corrected for multiple testing whereas *p*-values for predetermined contrast *t*-tests are not.

### Table S4.2. Uncorrected models.

|             | Psychosis liability | Childhood trauma | Psychosis liability x childhood trauma |
|-------------|---------------------|------------------|---------------------------------------|
|             | B                   | CI               | *p*        | B             | CI               | *p*        | B           | CI               | *p*       |
| BDNF        | 0.03                | [-0.13; 0.19]    | 0.690      | -0.08         | [-0.28; 0.12]   | 0.433      | 0.16        | [-0.10; 0.43]   | 0.227     |
| CCL-2       | 0.04                | [-0.37; 0.25]    | 0.702      | -0.18         | [-0.44; 0.08]   | 0.179      | 0.13        | [-0.22; 0.48]   | 0.474     |
| CRP         | -0.12               | [-0.72; 0.48]    | 0.693      | -0.21         | [-0.96; 0.55]   | 0.590      | 0.57        | [-0.44; 1.58]   | 0.267     |
| IFN-γ       | 0.20                | [-0.10; 0.49]    | 0.185      | 0.05          | [-0.30; 0.41]   | 0.733      | -0.14       | [-0.62; 0.34]   | 0.563     |
| IGFBP-2     | 0.25                | [-0.39; 0.80]    | 0.438      | -0.59         | [-1.40; 0.23]   | 0.156      | 0.30        | [-0.79; 1.19]   | 0.585     |
| IL-6        | -0.39               | [-1.66; 0.88]    | 0.545      | -1.42         | [-3.03; 0.20]   | 0.084      | 1.19        | [-0.96; 3.34]   | 0.275     |
| PDGF        | 0.14                | [-0.11; 0.39]    | 0.259      | -0.06         | [-0.37; 0.25]   | 0.698      | -0.14       | [-0.56; 0.27]   | 0.501     |
| SCF         | 0.04                | [-0.16; 0.24]    | 0.706      | -0.08         | [-0.33; 0.17]   | 0.527      | 0.06        | [-0.28; 0.40]   | 0.717     |
| TNF-a       | 0.22                | [-0.49; 0.93]    | 0.540      | -0.48         | [-1.17; 0.42]   | 0.297      | 0.21        | [-0.99; 1.41]   | 0.731     |

Regression coefficients of uncorrected linear regression models are given. Models included psychosis liability (high vs. low), childhood trauma (yes/no) and psychosis liability x childhood trauma as predictors.

### Table S4.3. Correction for psychotropic medication use.

|             | Psychosis liability | Childhood trauma | Psychosis liability x childhood trauma |
|-------------|---------------------|------------------|---------------------------------------|
|             | B                   | CI               | *p*        | B             | CI               | *p*        | B           | CI               | *p*       |
| BDNF        | 0.06                | [-0.13; 0.26]    | 0.52       | -0.02         | [-0.23; 0.19]   | 0.87       | 0.01        | [-0.26; 0.27]   | 0.96      |
| CCL-2       | -0.28               | [-0.57; 0.02]    | 0.06       | 0.01          | [-0.30; 0.31]   | 0.97       | 0.10        | [-0.30; 0.50]   | 0.61      |
| CRP         | -0.10               | [-0.88; 0.67]    | 0.79       | 0.02          | [-0.81; 0.65]   | 0.96       | 0.15        | [-0.91; 1.20]   | 0.78      |
| IFN-γ       | 0.08                | [-0.21; 0.38]    | 0.57       | 0.20          | [-0.11; 0.52]   | 0.19       | -0.07       | [-0.67; 0.13]   | 0.18      |
| IGFBP-2     | 0.49                | [-0.34; 1.33]    | 0.25       | -0.23         | [-1.13; 0.66]   | 0.61       | -0.39       | [-1.59; 0.75]   | 0.50      |
| IL-6        | -0.12               | [-1.86; 1.62]    | 0.89       | -1.14         | [-3.00; 0.72]   | 0.23       | 1.19        | [-1.18; 3.56]   | 0.32      |
| PDGF        | 0.26                | [-0.04; 0.55]    | 0.08       | 0.20          | [-0.11; 0.51]   | 0.21       | -0.41       | [-0.81; -0.01]  | 0.04      |
| SCF         | -0.01               | [-0.28; 0.26]    | 0.93       | 0.09          | [-0.20; 0.37]   | 0.54       | -0.03       | [-0.40; 0.33]   | 0.86      |
| TNF-a       | 0.42                | [-0.60; 1.45]    | 0.41       | -0.75         | [-1.85; 0.36]   | 0.18       | 0.72        | [-0.68; 2.13]   | 0.31      |

Regression coefficients of linear regression models are given. Models included psychosis liability (high vs. low), childhood trauma (yes/no) and psychosis liability x childhood trauma as predictors and were corrected for sex, age, BMI, smoking, cannabis use, education level, oral contraceptive use, and psychotropic medication use.