Socioeconomic Disadvantage Moderates the Association between Peripheral Biomarkers and Childhood Psychopathology

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Abstract

Background

Socioeconomic disadvantage (SED) has been consistently associated with early life mental health problems. SED has been shown to impact multiple biological systems, including the regulation of neurotrophic proteins, immune-inflammatory and oxidative stress markers, which, conversely, have been reported to be relevant to physiological and pathological neurodevelopment. This study investigated the relationship between SED, different domains of psychopathology, serum levels of interleukin-6 (IL6), thiobarbituric acid-reactive substance (TBARS) and brain-derived neurotrophic factor (BDNF). We hypothesized that a composite of socioeconomic risk would be associated with psychopathology and altered levels of peripheral biomarkers. In addition, we hypothesized that SED would moderate the associations between mental health problems, IL6, TBARS and BDNF.

Methods and Findings

Using a cross-sectional design, we measured the serum levels of IL6, TBARS and BDNF in 495 children aged 6 to 12. We also investigated socio-demographic characteristics and mental health problems using the Child Behaviour Checklist (CBCL) DSM-oriented scales. SED was evaluated using a cumulative risk model. Generalized linear models were used to assess associations between SED, biomarkers levels and psychopathology. SED was significantly associated with serum levels of IL6 (RR = 1.026, 95% CI 1.004; 1.049, p = 0.020) and TBARS (RR = 1.077, 95% CI 1.028; 1.127, p = 0.002). The association between SED...
Introduction

In children and adolescents, mental health problems are highly prevalent, debilitating and one of the main predictors of adult mental disorders [1–5]. It is well established that childhood psychopathology emerges in the context of an intricate relation between genetic and environmental risk factors [6–8]. Among these environmental risk factors, socioeconomic disadvantage (SED) has been described as one of the major contributors for the development and persistence of mental health problems [9–13]. Epidemiological and clinical evidence indicates that SED is associated with multiple dimensions of psychopathology, with more robust effects on externalizing problems, such as aggressive and delinquent behaviors, and a less robust, but still significant, association with internalizing symptoms, such as anxiety and depression [10–12, 14].

Several mechanisms have been proposed to explain the effects of SED on psychopathology. Low socioeconomic position is often associated with material deprivation, as well as with residence in neighborhoods where crime and substance abuse tend to be more prevalent; and educational/economic opportunities less available [15–17]. Exposure to chronic stress, frequently, but not exclusively, related to the experience of “social defeat”, or the experience of being excluded or isolated, has also been conceptualized as key factor [18, 19]. More recently, an association between SED and the neural substrates of psychopathology has been highlighted. Neuroimaging studies have reported and association between SED and alterations in brain structure and function, characterized, for example, by a decreased volume in the prefrontal cortex and its subdivisions (e.g. orbitofrontal cortex, anterior cingulate cortex), areas prominently involved in cognitive and emotional processing [13, 20–22]. Longitudinal studies have documented that SED was associated with divergent neurodevelopmental trajectories, with children from low socioeconomic background having slower gray matter growth during childhood [23, 24].

From a molecular perspective, neurotrophic proteins, immune-inflammatory and oxidative stress markers have been consistently reported to be associated with brain structure and function and to be relevant to physiological and pathological neurodevelopment [25, 26]. Associations between alterations in these systems have been reliably described in children and adults, across disparate mental disorders [27–32]. Moreover, convergent evidence indicates that early life SED is independently and strongly associated with inflammation in children [33], as well as prospectively in adults [34–37]. Conversely, serum levels of brain-derived neurotrophic factor (BDNF) and functional variations of the BDNF gene were also shown to be affected by SED [38, 39].

Conclusions

In children, SED is highly associated with mental health problems. Our findings suggest that this association may be moderated via effects on multiple interacting neurobiological systems.
We recently demonstrated that SED is associated with general psychopathology, independently from co-occurring risk factors (i.e. parental mental disorders, perinatal complications) (Mansur et al., unpublished data). Moreover, we also documented that exposure to environmental risk factors moderates the association between IL6 and general psychopathology [40]. However, the association between peripheral biomarkers and mental health problems is not completely understood, especially regarding the factors that mediate and/or moderate this relationship. Considering that SED could potentially impact the neural systems that underlie psychopathology, as well modulate systemic adaptations (e.g. immune and endocrine changes), it is possible that this factor could, at least partially, explain the association between SED and different domains of psychopathology. Herein we sought to extend results from these previous studies by evaluating the impact of SED on serum IL6, BDNF and the marker of lipid peroxidation thiobarbituric acid-reactive substance (TBARS). We also aimed to assess the impact of SED on the association between biomarkers and dimension of psychopathology. We hypothesized that (1) SED would be associated with altered levels of IL6, TBARS and BDNF levels; and (2) SED would moderate the association between biomarkers and psychopathology, wherein the correlation between biomarkers and dimensions of psychopathology would be stronger in children with exposure to high SED.

Methods

Participants

The sample herein is part of the High Risk Cohort Study for Psychiatric Disorders Study, which has been reported elsewhere [41]. From the total cohort of 2,512 subjects, 1,004 children were invited to participate in enriched imaging/biomarker cohort. A total of 741 subjects completed the imaging procedures and 495 children provided valid blood samples for the study herein. Primary reasons for missing blood samples were: caregiver refusal, children refusal and technical complications during blood processing procedures. Written informed consent was provided by all parents of participants, and verbal consent was obtained from all children. The study was approved by the Ethics Committee of the Universidade de São Paulo (IORG00048 84). All families were invited for an appointment with a trained psychologist and social worker in case they were interested in receiving the results of the study evaluation. All children identified as being under the need of care were referred for clinical evaluation. Situations involving serious risk of physical or psychological harm received special attention in accordance to competent authorities’ guidelines.

Measurements

Environmental risk factors. Questions about risk factors were determined after a critical review of the extant literature that has primarily reported on risk factors for mental disorders [41] and included inquiries about demographic and social factors (e.g. socio-economic status, parental education). We created a cumulative risk index, conceptualized as each individual’s cumulative exposure to a set of indicators of SED, according to previous studies [42–46]. Definitions and descriptive statistics of risk factors indicators are reported in Table 1. Each indicator was weighted equally and summed. For analyses of interaction we created a dichotomous variable for high exposure, defined as exposure to 2 or more indicators of SED.

Child Behavior Checklist (CBCL). Psychopathology was assessed dimensionally; using the CBCL, which is a parent-report questionnaire that assesses various behavioral and emotional problems. The CBCL is a widely used standardized measure of maladaptive behavior and emotional complications in individuals between ages 4 and 18 [47, 48]. For the study herein, we used the DSM-oriented scales (i.e. depressive problems, anxiety problems, somatic
problems, attention deficit/hyperactivity problems, oppositional defiant problems and conduct problems), which have good validity and clinical usefulness [49, 50].

**Blood samples collection and biomarkers assessment.** Whole blood samples were obtained from all children. All samples were obtained between 10:00am and 4:00pm. After collection, blood was allowed to clot by leaving it undisturbed at room temperature and then serum extracted after blood had been processed at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge. Serum was kept at −80°C until further analyzed. As the samples were labeled with numbers, without any group identification, the investigators were blinded for all procedures.

BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer’s instructions (Milipore, USA). For assessment of oxidative stress, serum levels of malondialdehyde (MDA), a product of lipid peroxidation, were measured by the TBARS (thiobarbituric acid reactive substances) method [51]. Serum IL6 levels were measured by flow cytometry using the Cytometric Bead Array (CBA) Flex Set Kit (BD Biosciences, San Jose, CA) (Cat. #558276). Acquisition was performed with a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA). The instrument has been checked for sensitivity and overall performance with Cytometer Setup and Tracking beads (BD Biosciences) prior to data acquisition. Quantitative results were generated using FCAP Array v1.0.1 software (Soft Flow Inc., Pecs, Hungary).

**Statistical analyses**

All statistical analyses were conducted using SPSS software for Windows (version 23.0). For the comparison of demographic and clinical data, the independent samples t-test was used for quantitative variables; the Chi-square test was used for categorical variables. Generalized linear models were used to assess associations between SED, biomarkers levels and psychopathology. We used linear, Poisson (for count data, e.g. CBCL scales) and gamma (for positively skewed distribution, e.g. serum TBARS and IL6 levels) distributions, as appropriate. Interactions between SED and biomarkers were assessed by adding the product term (i.e. SED x IL6) to the tested models. Due to the non-linearity of the models, the estimated β coefficients were transformed into rate ratio (RR) estimates. Post hoc correction to control for the false discovery rate was applied according to the Benjamini Hochberg procedure [52].

**Results**

**Sample characteristics**

The mean age was 10.06 years (SD 1.88) (males 9.94 years, SD 1.91; females 10.19 years, SD 1.85). 45.1% of the study population were female and 58% of the study population were

| Table 1. Definitions and descriptive statistics. |
|------------------------------------------------|
| **Socioeconomic Disadvantage Index**             |
| **Label**            | **Definition**                                     | **n, %**          |
|----------------------|---------------------------------------------------|-------------------|
| 1. Social Welfare    | Receiving governmental social assistance (e.g. Bolsa Família [Family Allowance]) | 113 (22.8%)       |
| 2. Low Income        | Income from both parents in the lower 25th percentile of the sample | 115 (23.2%)       |
| 3. Single Parent     | Living in a single-parent household               | 110 (22.2%)       |
| 4. Unemployment      | Having at least 1 parent who is currently unemployed | 48 (9.7%)         |
| 5. Low Educational Achievement | Both parents with incomplete primary education | 45 (9.1%)         |

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Caucasian. SED index range was 0–5 and the median was 2.0 (SD 1.05); 263 (53.1%) children were classified as high exposure (i.e. exposed to 2 or more social indicators). Twenty-eight children (5.7%) were not exposed to any SED factor, 204 children (41.2%) were exposed to 1 SED factor, 152 children (30.7%) were exposed to 2, 77 (15.6%) were exposed to 3, 27 (5.5%) were exposed to 4; and 7 (1.4%) were exposed to 5 SED factors. No significant correlation was found between the SED index and, respectively, age (r = 0.043, p = 0.339), gender (p = 0.826) or ethnicity (p = 0.644). There was also no association between the binary indicator of high SED and age (p = 0.532), gender (p = 0.925) and ethnicity (p = 0.650).

Means, SDs and ranges for the CBCL DSM-oriented scales were, respectively: depressive problems 4.43, 4.16, 0–22; anxiety problems 3.84, 2.63, 0–11; somatic problems 2.11, 2.08, 0–11; attention deficit/hyperactivity problems 5.86, 3.82, 0–14; oppositional defiant problems 3.98, 2.81, 0–10; and conduct problems 3.42, 2.81, 0–10. As for specific diagnosis, 76 children (15.4%) were diagnosed with an anxiety disorder, 25 (5.1%) with a mood disorder, 61 (12.3%) with attention deficit/hyperactivity disorder, 38 (7.7%) with oppositional/conduct disorders, 3 (0.6%) with tic disorders and 4 (0.8%) with eating disorders.

Socioeconomic disadvantage and peripheral biomarkers

Serum IL6 median was 2.58 pg/ml (interquartile range [IQR] 2.22–2.91), TBARS median was 14.04 pg/ml (IQR 9.63–21.72) and BDNF median was 26.04 pg/ml (IQR 20.12–33.54). There was no correlation between age, IL6 (r = 0.059, p = 0.190), TBARS (r = -0.016, p = 0.723) and BDNF levels (r = 0.065, p = 0.151). There was also no effect of ethnicity (p = 0.058, p = 0.708, p = 0.537, respectively); BDNF levels were higher in female participants (p = 0.002), but no effect of gender were observed on serum IL6 (p = 0.060) or TBARS (p = 0.695).

There was a positive correlation between the SED index, serum IL6 (r = 0.121, p = 0.007), TBARS (r = 0.145; p = 0.001) and BDNF levels (r = 0.098, p = 0.022). After adjustments for age, gender and ethnicity, the association between SED, IL6 (RR = 1.026, 95% CI 1.004; 1.049, p = 0.020) and TBARS remained significant (RR = 1.077, 95% CI 1.028; 1.127, p = 0.002), whereas there was a trend for BDNF (RR = 1.031, 95% CI 0.997; 1.066, p = 0.077).

Socioeconomic disadvantage, peripheral biomarkers and psychopathology

Table 2 shows that SED was positively associated with all CBCL scales when analyzed separately (Model 1) and together with the biomarkers (Model 2). The biomarkers had somewhat distinct patterns of associations with the different scales, with IL6 being more strongly associated with scores on the depressive and anxiety scales; and TBARS being associated with the anxiety and conduct scales. BDNF was only associated with the anxiety, attention and conduct scales when analyzed separately.

Interaction analyses indicated a significantly positive interaction between high SED and BDNF on depressive problems, indicating that a positive correlation between BDNF and depressive problems was only positive in the children from the high SED group (Fig 1). The moderating effect of SED on the association between BDNF and psychopathology was specific to depressive problems, as the results were non-significant for measures on other scales (Table 3). Moderating effects on IL6 and TBARS were, on the other hand, pleiotropic, as it was significant in almost all subscales, with the only exceptions being depressive and oppositional defiant problems, for IL6, and somatic problems, for TBARS (Table 3). All of the significant interactive effects with IL6 and TBARS were positive, indicating a stronger correlation between biomarkers and psychopathology in children from the high SED group, compared to children in the low SED group (Fig 1).
Discussion

Our results indicate that SED is robustly associated with multiple domains of psychopathology. The strongest association in our sample was with conduct problems; an association with anxiety symptomatology was also significant [11, 53]. Evidence indicates that familial context (i.e. parents educational level or occupational status) and residence in deprived neighborhoods, with more exposure to deviant peer behavior and lower social support, are more related to externalizing problems [54, 55]. Internalizing symptoms, on contrast, would be more, but not exclusively, associated with individual temperament and genetic vulnerabilities [12, 56].

Serum biomarkers also had differential associations with mental health problems, with the inflammatory cytokine IL6 being more strongly associated with the internalizing dimension (i.e. depressive, anxiety and somatic problems) and the oxidative stress marker TBARS with

| Table 2. Associations between socioeconomic disadvantage, IL6, TBARS and BDNF; considered separately (Model 1) and together (Model 2). All analyses included age, gender and ethnicity as covariates. |
|----------------------------------------|----------------------------------------|
| Depressive Problems                   |                                       |
| SED                                   | 1.118 (1.080; 1.158) \* < 0.001        |
| IL6                                   | 1.133 (1.075; 1.194) 0.005             |
| TBARS                                 | 1.003 (0.999; 1.008) 0.242             |
| BDNF                                  | 1.002 (0.998; 1.006) 0.392             |
| Anxiety Problems                      |                                       |
| SED                                   | 1.007 (1.033; 1.123) 0.002             |
| IL6                                   | 1.145 (1.081; 1.213) < 0.001           |
| TBARS                                 | 1.007 (1.002; 1.012) 0.012             |
| BDNF                                  | 1.005 (1.001; 1.011) 0.044             |
| Somatic Problems                      |                                       |
| SED                                   | 1.081 (1.022; 1.143) 0.016             |
| IL6                                   | 1.125 (1.038; 1.219) 0.012             |
| TBARS                                 | 1.008 (1.002; 1.015) 0.022             |
| BDNF                                  | 1.004 (0.998; 1.010) 0.282             |
| Attention Deficit/Hyperactivity Problems |                                   |
| SED                                   | 1.099 (1.062; 1.136) 0.004             |
| IL6                                   | 1.067 (1.017; 1.120) 0.019             |
| TBARS                                 | 1.005 (1.001; 1.009) 0.021             |
| BDNF                                  | 1.005 (1.001; 1.008) 0.032             |
| Oppositional Defiant Problems          |                                       |
| SED                                   | 1.094 (1.050; 1.140) < 0.001           |
| IL6                                   | 1.061 (1.000; 1.126) 0.080             |
| TBARS                                 | 1.006 (1.002; 1.011) 0.019             |
| BDNF                                  | 1.002 (0.998; 1.007) 0.392             |
| Conduct Problems                      |                                       |
| SED                                   | 1.289 (1.237; 1.343) < 0.001           |
| IL6                                   | 1.061 (0.995; 1.132) 0.108             |
| TBARS                                 | 1.011 (1.006; 1.016) < 0.001           |
| BDNF                                  | 1.006 (1.002; 1.011) 0.019             |

* Adj. p-value: Benjamini Hochberg post hoc corrected p-value.
RR: rate ratio; CI: confidence interval; SED: socioeconomic disadvantage, IL6: interleukin-6; TBARS: thiobarbituric acid-reactive substance; BDNF: brain-derived neurotrophic factor.

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externalizing symptoms (i.e. attentional, oppositional and conduct problems). The neurotrophin BDNF had, in comparison, weaker associations, especially in the models that analyzed all the variables together. Effect sizes were relatively small, although were largely consistent with previous mechanistic studies [29, 33]. Considering the complexity of mental illnesses etiology, which involves multiple interacting genetic, environmental and biological factors; large effect

Fig 1. Mental health problems and peripheral biomarkers within socioeconomic disadvantage exposure subgroups. Correlations between (A) somatic problems and serum IL6; (B) attention deficit/hyperactivity problems and serum TBARS and (C) depressive problems and serum BDNF; within socioeconomic disadvantage exposure subgroups. SED: socioeconomic disadvantage; IL6: interleukin-6; TBARS: thiobarbituric acid-reactive substance; BDNF: brain-derived neurotrophic factor.

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sizes are unlikely to be detected; therefore the magnitude of the associations described in this community-based study are noteworthy and possibly indicative of clinical relevance.

Evidence on putative specific effects of different pathophysiological pathways is scarce. There are reports of positive correlations between plasma markers of oxidative stress and attention deficit hyperactivity disorder, as well as aggressive behavior in adults [57, 58]. Oxidative stress induces damage to nucleic acids or lipids, which has the potential to impair basic cellular/neuronal functions [59]. Indeed, there is evidence that lipid peroxidation is associated with white matter damage, which could potentially affect the circuits that regulate aggressive/confrontational behavior [60, 61]. Consistent with our results, alterations in inflammatory markers, including an increase in serum IL6, have been observed in children and adolescents with major depressive disorder [32]. The relationship between inflammation and mood has been extensively studied, with potential mechanisms including, but not limited to, effects on monoamine levels through activation of indoleamine 2,3-dioxygenase (IDO), which degrades tryptophan, and pathologic microglial cell activation [62, 63].

Peripheral biomarkers were positively correlated with SED, with higher levels of IL6, TBARS and BDNF being found in children with low socioeconomic position. As hypothesized, SED moderated the association between IL6, TBARS, BDNF and domains of psychopathology. All significant interactions were in the same direction, with a stronger association between peripheral markers and mental health problems in children exposed to high SED. Interestingly, we observed an interaction between SED and BDNF on depressive problems, even though there was no significant association between BDNF and depressive symptoms, indicating that BDNF’s relationship with this domain of psychopathology may be fully dependent of socioeconomic position. Associations between IL6 and internalizing problems, as well as between TBARS and externalizing symptoms, were also modified by the presence of SED. These results indicate that the differential effects of SED on each domain of psychopathology may be subserved by differential activations of neurobiological pathways.

Conceptually, the accumulation of multiple adverse conditions (e.g. SED) may lead to several different types of emotional or behavioral outcomes, which has been termed multifinality [64]. This model has been empirically supported [53, 65, 66]; nonetheless, there is evidence that specific developmental trajectories are more likely than others. For example, some

Table 3. Effects of interactions between high socioeconomic disadvantage and peripheral biomarkers on CBCL scales. All analyses included age, gender and ethnicity as covariates.

| RR  | 95% CI   | Adj. p-value* | RR  | 95% CI   | Adj. p-value* |
|-----|----------|---------------|-----|----------|---------------|
|     | Depressive Problems |          |     | Attention Deficit/Hyperactivity Problems |          |
| IL6 | 1.124    | 1.010; 1.251  | 0.055 | 1.118    | 1.017; 1.230  | 0.038      |
| TBARS | 1.022 | 1.012; 1.031 | < 0.001 | 1.020    | 1.012; 1.028  | < 0.001   |
| BDNF | 1.013    | 1.005; 1.021  | 0.006 | 1.004    | 0.997; 1.011  | 0.373      |
|     | Anxiety Problems |          |     | Oppositional Defiant Problems |          |
| IL6 | 1.193    | 1.065; 1.336  | 0.006 | 1.092    | 0.973; 1.225  | 0.185      |
| TBARS | 1.014 | 1.004; 1.023 | 0.014 | 1.020    | 1.010; 1.029  | < 0.001   |
| BDNF | 1.005    | 0.996; 1.014  | 0.298 | 1.001    | 0.993; 1.010  | 0.771      |
|     | Somatic Problems |          |     | Conduct Problems |          |
| IL6 | 1.207    | 1.031; 1.413  | 0.036 | 1.198    | 1.048; 1.369  | 0.019      |
| TBARS | 1.003 | 0.991; 1.016 | 0.681 | 1.033    | 1.021; 1.044  | 0.003      |
| BDNF | 1.006    | 0.995; 1.018  | 0.355 | 1.003    | 0.993; 1.012  | 0.667      |

* Adj. p-value: Benjamini Hochberg post hoc corrected p-value.
RR: rate ratio; CI: confidence interval; IL6: interleukin-6; TBARS: thiobarbituric acid-reactive substance; BDNF: brain-derived neurotrophic factor.

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psychopathology constructs are more likely to predict themselves (i.e. homotypic continuity), whereas some domains are more likely to predict others (i.e. heterotypic continuity). Evidence indicates that conduct problems are mostly stable over time; oppositional/defiant problems, instead, are stronger predictors of affective and attentional problems [66, 67]. These developmental pathways are dynamically influenced by genetic and non-genetic factors [6, 68, 69]. Interestingly, this transition from oppositional problems toward mood/anxiety symptoms seems to be partially mediated by environmental risk factors [66, 70]. Our data suggest that SED’s relationship with different neurobiological substrates, likely determined by each individual genetic vulnerabilities and/or previous or co-occurring exposure to other environmental factors, accounts, at least partially, to its differential associations with disparate domains of psychopathology.

Nevertheless, the principle of equifinality, which refers to a diversity of pathways leading to same phenotype, may also apply (62). Low socioeconomic position is frequently correlated with other risk factors, including, but not limited to, parental mental disorders and exposure to perinatal complications [11, 71–73]. We recently reported, using data from this same sample, that SED is associated with parental mental disorders, but that its association with general psychopathology was independent and did not interact with familial mental illness (Mansur et al., unpublished data), a finding that is consistent with other studies [10]. However, we separately documented an interaction between SED and parental psychopathology on IL6 levels[40], indicating that the results described herein may not represent an isolated effect of SED. Our sample size and study design does not allow the disentangling of multiple risk factors’ effects, therefore, these findings need to be replicated and refined, with consideration for reciprocal and interacting associate factors, in larger, prospective samples.

This study has limitations that limit inferences and interpretations of the data. The cross-sectional design precludes conclusions about causality. It is not possible to determine, based on our data, whether exposure to SED or alterations in serum biomarkers precede the onset of psychopathology. We used a cumulative composite of SED, therefore other important determinants of environmental factors impact, such as extent and timing of exposure, were not directly assessed. Our SED index weighted equally all components. The studied factors are not interchangeable and may impact different etiological pathways; it is also possible that different combinations may have divergent effects [74, 75]. Moreover, as there are no longitudinal studies evaluating the markers assessed in this study, there are questions about their stability over time. We collected the samples in a relatively narrow period of the day; however, it is possible that the biomarker’s levels were affected by temporal and contextual factors. Nonetheless, our study also has a number of strengths. Our study population was derived from a large, community-based sample, enriched for the presence of psychopathology. We used a multi-informant clinical evaluation with validated instruments, thus obtaining data directly from parents and limiting rater and information biases. Finally, we simultaneously assessed a range of dimensional domains of psychopathology, which provides more insight on the diverse effects of SED.

In summary, SED was associated with disparate domains of psychopathology in children, as well as with increased serum levels of IL6, TBARS and BDNF. In addition, SED was also shown to moderate the association between IL6, TBARS and BDNF, and mental health problems, suggesting that SED’s different associations with psychopathology are, at least partially, related to its engagement of different neurobiological pathways. Prospective evaluation of this cohort may provide further information about the interaction between SED, serum biomarkers, psychopathology, and the onset of psychiatric disorders.

Supporting Information

S1 Dataset. Dataset.
(SAV)
Author Contributions
Conceived and designed the experiments: LAR ECM RAB EB.
Performed the experiments: RBM GRC EA AZG ACR SS PKM MLL AG PMP.
Analyzed the data: RBM GRC EA AZ EB.
Contributed reagents/materials/analysis tools: LS SIB MKS ALT.
Wrote the paper: RBM GRC EA AZ JJM LAR ECM RSM RGO RAB EB.

References
1. Hofstra MB, Van Der Ende J, Verhulst FC. Adolescents’ self-reported problems as predictors of psychopathology in adulthood: 10-year follow-up study. Br J Psychiatry. 2001; 179:203–9. PMID: 11532796.
2. Carter AS, Wagmiller RJ, Gray SA, McCarthy KJ, Horwitz SM, Briggs-Gowan MJ. Prevalence of DSM-IV disorder in a representative, healthy birth cohort at school entry: sociodemographic risks and social adaptation. J Am Acad Child Adolesc Psychiatry. 2010; 49(7):686–98. doi: 10.1016/j.jaac.2010.03.018 PMID: 20610138; PubMed Central PMCID: PMCPMC3166638.
3. Reef J, Diamantopoulou S, van Meurs I, Verhulst FC, van der Ende J. Developmental trajectories of child to adolescent externalizing behavior and adult DSM-IV disorder: results of a 24-year longitudinal study. Soc Psychiatry Psychiatr Epidemiol. 2011; 46(12):1233–41. doi: 10.1007/s00127-010-0297-9 PMID: 20936464; PubMed Central PMCID: PMCPMC3214259.
4. Reef J, van Meurs I, Verhulst FC, van der Ende J. Children’s problems predict adults’ DSM-IV disorders across 24 years. J Am Acad Child Adolesc Psychiatry. 2010; 49(11):1117–24. doi: 10.1016/j.jaac.2010.08.002 PMID: 20970699.
5. Petresco S, Anselmi L, Santos IS, Barros AJ, Fleitlich-Bilyk B, Barros FC, et al. Prevalence and comorbidity of psychiatric disorders among 8-year-old children: 2004 Pelotas Birth Cohort. Soc Psychiatry Psychiatr Epidemiol. 2014; 49(6):575–83. doi: 10.1007/s00127-014-0626-z PMID: 24486152; PubMed Central PMCID: PMCPMC4028510.
6. van Os J, Kenis G, Rutten BP. The environment and schizophrenia. Nature. 2010; 468(7321):203–12. doi: 10.1038/nature09565 PMID: 21068828.
7. Burt SA. Research review: the shared environment as a key source of variability in child and adolescent psychopathology. J Child Psychol Psychiatry. 2014; 55(4):304–12. doi: 10.1111/jcpp.12173 PMID: 24261560.
8. Gratten J, Wray NR, Keller MC, Visscher PM. Large-scale genomics unveils the genetic architecture of psychiatric disorders. Nat Neurosci. 2014; 17(6):782–90. doi: 10.1038/nn.3708 PMID: 24866044; PubMed Central PMCID: PMCPMC4112149.
9. Leventhal T, Brooks-Gunn J. The neighborhoods they live in: the effects of neighborhood residence on child and adolescent outcomes. Psychol Bull. 2000; 126(2):309–37. PMID: 10748654.
10. Amone-P’Olak K, Burger H, Huisman M, Oldehinkel AJ, Ormel J. Parental psychopathology and socioeconomic position predict adolescent offspring’s mental health independently and do not interact: the TRAILS study. J Epidemiol Community Health. 2011; 65(1):57–63. doi: 10.1136/jech.2009.092569 PMID: 19858541.
11. Amone-P’Olak K, Burger H, Ormel J, Huisman M, Verhulst FC, Oldehinkel AJ. Socioeconomic position and mental health problems in pre- and early-adolescents: the TRAILS study. Soc Psychiatry Psychiatr Epidemiol. 2009; 44(3):231–8. doi: 10.1007/s00127-008-0424-z PMID: 18714424.
12. Ashford J, Smit F, van Lier PA, Cuijpers P, Koot HM. Early risk indicators of internalizing problems in late childhood: a 9-year longitudinal study. J Child Psychol Psychiatry. 2008; 49(7):774–80. doi: 10.1111/j.1469-7610.2008.01889.x PMID: 18341546.
13. Holz NE, Laucht M, Meyer-Lindenberg A. Recent advances in understanding the neurobiology of childhood socioeconomic disadvantage. Curr Opin Psychiatry. 2015; 28(5):365–70. doi: 10.1097/YCO.0000000000000178 PMID: 26147616.
14. Kapi A, Veitsista A, Kavadias G, Lekea V, Bakoula C. Social determinants of self-reported emotional and behavioral problems in Greek adolescents. Soc Psychiatry Psychiatr Epidemiol. 2007; 42(7):594–6. doi: 10.1007/s00127-007-0201-4 PMID: 17520162.
15. Evans J, Middleton N, Gunnell D. Social fragmentation, severe mental illness and suicide. Soc Psychiatry Psychiatr Epidemiol. 2004; 39(3):165–70. doi: 10.1007/s00127-004-0733-9 PMID: 14999447.
16. Schneiders J, Drukker M, van der Ende J, Verhulst FC, van Os J, Nicolson NA. Neighbourhood socioeconomic disadvantage and behavioural problems from late childhood into early adolescence. J Epidemiol Community Health. 2003; 57(9):699–703. PMID: 12933776; PubMed Central PMCID: PMCPMC1732581.

17. Reijneveld SA, Brugman E, Verhulst FC, Verloove-Vanhorick SP. Area deprivation and child psychosocial problems—a national cross-sectional study among school-aged children. Soc Psychiatry Psychiatr Epidemiol. 2005; 40(1):18–23. doi: 10.1007/s00127-005-0850-0 PMID: 15624070.

18. Stansfeld S, Head J, Bartley M, Fonagy P. Social position, early deprivation and the development of attachment. Soc Psychiatry Psychiatr Epidemiol. 2008; 43(7):516–26. doi: 10.1007/s00127-008-0330-4 PMID: 18344050.

19. Selten JP, van der Ven E, Rutten BP, Cantor-Graae E. The social defeat hypothesis of schizophrenia: an update. Schizophr Bull. 2013; 39(6):1180–6. doi: 10.1093/schbul/sbt134 PMID: 24062592; PubMed Central PMCID: PMCPMC3796093.

20. Holz NE, Boecker R, Hohm E, Zohsel K, Buchmann AF, Blomeyer D, et al. The long-term impact of early life poverty on orbitofrontal cortex volume in adulthood: results from a prospective study over 25 years. Neuropsychopharmacology. 2015; 40(4):996–1004. doi: 10.1038/npp.2014.277 PMID: 25315195; PubMed Central PMCID: PMCPMC4330514.

21. Luby J, Belden A, Botteron K, Marrus N, Harms MP, Babb C, et al. The effects of poverty on childhood brain development: the mediating effect of caregiving and stressful life events. JAMA Pediatr. 2013; 167(12):1135–42. doi: 10.1001/jamapediatrics.2013.3139 PMID: 24165822; PubMed Central PMCID: PMCPMC4001721.

22. Haddad L, Schafer A, Streil F, Lederbogen F, Grimm O, Wust S, et al. Brain structure correlates of urban upbringing, an environmental risk factor for schizophrenia. Schizophr Bull. 2015; 41(1):115–22. doi: 10.1093/schbul/sbu072 PMID: 24894884; PubMed Central PMCID: PMCPMC4266290.

23. Hair NL, Hanson JL, Wolfe BL, Pollak SD. Association of Child Poverty, Brain Development, and Academic Achievement. JAMA Pediatr. 2015; 169(9):822–9. doi: 10.1001/jamapediatrics.2015.1475 PMID: 26192216; PubMed Central PMCID: PMCPMC4687953.

24. Hanson JL, Hair N, Shen DG, Shi F, Gilmore JH, Wolfe BL, et al. Family poverty affects the rate of human infant brain growth. PLoS One. 2013; 8(12):e80854. doi: 10.1371/journal.pone.0080854 PMID: 24349025; PubMed Central PMCID: PMCPMC3859472.

25. Garcia-Bueno B, Caso JR, Leza JC. Stress as a neuroinflammatory condition in brain: damaging and protective mechanisms. Neurosci Biobehav Rev. 2008; 32(6):1136–51. doi: 10.1016/j.neubiorev.2008.04.001 PMID: 18488869.

26. Pfaffenseller B, Fries GR, Wollenhaupt-Aguiar B, Colpo GD, Stertz L, Panizzutti B, et al. Neurotrophins, inflammation and oxidative stress as illness activity biomarkers in bipolar disorder. Expert Rev Neurother. 2013; 13(7):827–42. Epub 2013/08/01. doi: 10.1586/14737175.2013.811981 PMID: 23898853.

27. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biol Psychiatry. 2013; 74(1):15–25. doi: 10.1016/j.biopsych.2013.01.007 PMID: 23419545.

28. Upthegrove R, Manzanares-Teson N, Barnes NM. Cytokine function in medication-naïve first episode psychosis: a systematic review and meta-analysis. Schizophren Res. 2014; 155(1–3):101–8. doi: 10.1016/j.schres.2014.03.005 PMID: 24704219.

29. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB. Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. JAMA Psychiatry. 2014; 71(10):1121–8. doi: 10.1001/jamapsychiatry.2014.1332 PMID: 25133871; PubMed Central PMCID: PMCPMC4561502.

30. Fernandes BS, Berk M, Turk CW, Steiner J, Goncalves CA. Decreased peripheral brain-derived neurotrophic factor levels are a biomarker of disease activity in major psychiatric disorders: a comparative meta-analysis. Mol Psychiatry. 2014; 19(7):750–1. doi: 10.1038/mp.2013.172 PMID: 24342989.

31. Brown NC, Andreazza AC, Young LT. An updated meta-analysis of oxidative stress markers in bipolar disorder. Psychiatry Res. 2014; 218(1–2):61–8. doi: 10.1016/j.psychres.2014.04.005 PMID: 24794031.

32. Mitchell RH, Goldstein BI. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. J Am Acad Child Adolesc Psychiatry. 2014; 53(3):274–96. doi: 10.1016/j.jaac.2013.11.013 PMID: 24565356.

33. Miller GE, Cole SW. Clustering of depression and inflammation in adolescents previously exposed to childhood adversity. Biol Psychiatry. 2012; 72(1):34–40. doi: 10.1016/j.biopsych.2012.02.034 PMID: 22494534; PubMed Central PMCID: PMCPMC3493164.

34. Packard CJ, Bezyak V, McLean JS, Batty GD, Ford I, Burns H, et al. Early life socioeconomic adversity is associated in adult life with chronic inflammation, carotid atherosclerosis, poorer lung function and
decreased cognitive performance: a cross-sectional, population-based study. BMC Public Health. 2011; 11:42. doi: 10.1186/1471-2458-11-42 PMID: 21241479; PubMed Central PMCID: PMCPMC3032683.

35. Stringhini S, Batty GD, Bovet P, Shipley MJ, Marmot MG, Kumari M, et al. Association of life course socioeconomic status with chronic inflammation and type 2 diabetes risk: the Whitehall II prospective cohort study. PLoS Med. 2013; 10(7):e1001479. doi: 10.1371/journal.pmed.1001479 PMID: 23843750; PubMed Central PMCID: PMCPMC3699448.

36. Matthews KA, Chang Y, Bromberger JT, Karvonen-Gutierrez CA, Kravitz HM, Thurston RC, et al. Childhood Socioeconomic Circumstances, Inflammation, and Hemostasis Among Midlife Women: Study of Women's Health Across the Nation. Psychosom Med. 2015. doi: 10.1097/PSY.0000000000000283 PMID: 26716815.

37. John-Henderson NA, Marsland AL, Kamarck TW, Muldoon MF, Manuck SB. Childhood Socioeconomic Status and the Occurrence of Recent Negative Life Events as Predictors of Circulating and Stimulated Levels of Interleukin-6. Psychosom Med. 2013; 75(1):91–101. doi: 10.1097/PSY.0b013e318283e0d6 PMID: 23843750; PubMed Central PMCID: PMCPMC3699448.

38. Lasky-Su J, Faraone SV, Lange C, Tsuang MT, Doyle AE, Smoller JW, et al. A study of how socioeconomic status moderates the relationship between SNPs encompassing BDNF and ADHD symptom counts in ADHD families. Behav Genet. 2007; 37(3):487–97. doi: 10.1007/s10519-006-9136-x PMID: 17216343.

39. Bus BA, Molenijik ML, Penninx BJ, Buitelaar JK, Kenis G, Prickaerts J, et al. Determinants of serum brain-derived neurotrophic factor. Psychoneuroendocrinology. 2011; 36(2):228–39. doi: 10.1016/j.psyneuen.2010.07.013 PMID: 20702043.

40. Mansur RB, Cunha GR, Asevedo E, Zugman A, Rizzo LB, Grassi-Oliveira R, et al. Association of serum interleukin-6 with mental health problems in children exposed to perinatal complications and social disadvantage. Psychoneuroendocrinology. 2016; 71:94–101. doi: 10.1016/j.psyneuen.2016.05.015 PMID: 27258821.

41. Flouri E, Tzavidis N, Kallis C. Adverse life events, area socioeconomic disadvantage, and psychopathology and resilience in young children: the importance of risk factors' accumulation and protective factors' specificity. Eur Child Adolesc Psychiatry. 2010; 19(6):535–46. doi: 10.1007/s00787-009-0068-x PMID: 19820985.

42. Gustafsson PE, Hammarstrom A, San Sebastian M. Cumulative contextual and individual disadvantages over the life course and adult functional somatic symptoms in Sweden. Eur J Pub Health. 2015; 25(4):592–7. doi: 10.1093/eurpub/cku213 PMID: 25527526.

43. Shalev I, Caspi A, Ambler A, Belsky DW, Chapple S, Cohen HJ, et al. Perinatal complications and aging indicators by midlife. Pediatrics. 2014; 133(5):e1315–23. doi: 10.1542/peds.2014-1669 PMID: 25349321; PubMed Central PMCID: PMCPMC4210799.

44. Bordin IA, Rocha MM, Paula CS, Teixeira MC, Achenbach TM, Rescorla LA, et al. Child Behavior Checklist (CBCL), Youth Self-Report (YSR) and Teacher’s Report Form (TRF): an overview of the development of the original and Brazilian versions. Cad Saude Publica. 2013; 29(1):13–28. PMID: 23370021.

45. Ivanova MY, Dobrean A, Dofnner M, Erol N, Fonbonne E, Fonseca AC, et al. Testing the 8-syndrome structure of the child behavior checklist in 30 societies. J Clin Child Adolesc Psychol. 2007; 36(3):405–17. doi: 10.1080/15374410701443463 PMID: 17689894.
51. Wills ED, Wilkinson AE. Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. Biochem J. 1966; 99(3):657–66. PMID: 5964962; PubMed Central PMCID: PMCPMC1265055.

52. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing Journal of the Royal Statistical Society Series B (Methodological). 1995; 57(1):289–300.

53. Viisaint CL, Aiyer SM, Wilson MN, Shaw DS, Dishon TJ. The ecology of early childhood risk: a canonical correlation analysis of children’s adjustment, family, and community context in a high-risk sample. J Prim Prev. 2013; 34(4):261–77. doi: 10.1007/s10935-013-0305-4 PMID: 23700232; PubMed Central PMCID: PMCPMC4749130.

54. Dodge KA, Pettit GS, Bates JE. Socialization mediators of the relation between socioeconomic status and child conduct problems. Child Dev. 1994; 65(2 Spec No):649–65. PMID: 8013245.

55. Atzaba-Poria N, Pike A, Deater-Deckard K. Do risk factors for problem behaviour act in a cumulative manner? An examination of ethnic minority and majority children through an ecological perspective. J Child Psychol Psychiatry. 2004; 45(4):707–18. doi: 10.1111/j.1469-7610.2004.00265.x PMID: 15056303.

56. Oldehinkel AJ, Hartman CA, De Winter AF, Veenstra R, Ormel J. Temperament profiles associated with internalizing and externalizing problems in preadolescence. Dev Psychopathol. 2004; 16(2):421–40. PMID: 15467604.

57. Coccaro EF, Lee R, Gozal D. Elevated Plasma Oxidative Stress Markers in Individuals With Intermittent Explosive Disorder and Correlation With Aggression in Humans. Biol Psychiatry. 2016; 79(2):127–35. doi: 10.1016/j.biopsych.2014.01.014 PMID: 24582164.

58. Guney E, Cetin FH, Alisik M, Tunca H, Tas Torun Y, Iseri E, et al. Attention Deficit Hyperactivity Disorder and oxidative stress: A short term follow up study. Psychiatry Res. 2015; 229(1–2):310–7. doi: 10.1016/j.psychres.2015.07.003 PMID: 26188640.

59. Gemma C, Vila J, Bachstetter A, Bickford PC. Oxidative Stress and the Aging Brain: From Theory to Prevention. In: Riddle DR, editor. Brain Aging: Models, Methods, and Mechanisms. Frontiers in Neuroscience. Boca Raton (FL) 2007.

60. Versace A, Andreazza AC, Young LT, Fournier JC, Almeida JR, Stiffler RS, et al. Elevated serum measures of lipid peroxidation and abnormal prefrontal white matter in euthymic bipolar adults: toward peripheral biomarkers of bipolar disorder. Mol Psychiatry. 2014; 19(2):200–8. doi: 10.1038/mp.2012.186 PMID: 23358158; PubMed Central PMCID: PMCPMC3640681.

61. Lin WM, Chen MH, Wang HC, Lu CH, Chen PC, Chen HL, et al. Association between peripheral oxidative stress and white matter damage in acute traumatic brain injury. Biomed Res Int. 2014; 2014:340936. doi: 10.1155/2014/340936 PMID: 24804213; PubMed Central PMCID: PMCPMC3996315.

62. Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequel and concomitants play a role in the pathophysiology of unipolar depression. Neurosci Biobehav Rev. 2011; 35(2):764–82. doi: 10.1016/j.neubiorev.2011.12.005 PMID: 22197082.

63. Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: a review of the interactions between inflammation and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2014; 53:23–34. doi: 10.1016/j.pnpbp.2014.01.013 PMID: 24468642.

64. Cicchetti D, Rogosch FA. Psychopathology as risk for adolescent substance use disorders: a developmental psychopathology perspective. J Clin Child Psychol. 1999; 28(3):355–65. doi: 10.1207/S15374442jccp280308 PMID: 10446685.

65. Marsh P, McFarland FC, Allen JP, McElhaney KB, Land D. Attachment, autonomy, and multifinality in adolescent internalizing and risky behavioral symptoms. Dev Psychopathol. 2003; 15(2):451–67. PMID: 12931837; PubMed Central PMCID: PMCPMC1774591.

66. Nobile M, Colombo P, Bellina M, Molteni M, Simone D, Nardocci F, et al. Psychopathology and adversities from early- to late-adolescence: a general population follow-up study with the CBCL DSM-Oriented Scales. Epidemiol Psychiatr Sci. 2013; 22(1):63–73. doi: 10.1017/S2045760612000145 PMID: 22794669.

67. Copeland WE, Shanahan L, Costello EJ, Angold A. Childhood and adolescent psychiatric disorders as predictors of young adult disorders. Arch Gen Psychiatry. 2009; 66(7):764–72. doi: 10.1001/archgenpsychiatry.2009.85 PMID: 19581568; PubMed Central PMCID: PMCPMC2891142.

68. Howes OD, Murray RM. Schizophrenia: an integrated sociodevelopmental-cognitive model. Lancet. 2014; 383(9929):1677–87. doi: 10.1016/S0140-6736(13)62036-X PMID: 24315522; PubMed Central PMCID: PMCPMC4127444.
69. Kofink D, Boks MP, Timmers HT, Kas MJ. Epigenetic dynamics in psychiatric disorders: environmental programming of neurodevelopmental processes. Neurosci Biobehav Rev. 2013; 37(5):831–45. doi: 10.1016/j.neubiorev.2013.03.020 PMID: 23567520.

70. Rowe R, Maughan B, Eley TC. Links between antisocial behavior and depressed mood: the role of life events and attributional style. J Abnorm Child Psychol. 2006; 34(3):293–302. doi: 10.1007/s10802-006-9032-0 PMID: 16718539.

71. Vendlinski MK, Lemery-Chalfant K, Essex MJ, Goldsmith HH. Genetic risk by experience interaction for childhood internalizing problems: converging evidence across multiple methods. J Child Psychol Psychiatry. 2011; 52(5):607–18. doi: 10.1111/j.1469-7610.2010.02343.x PMID: 21198591; PubMed Central PMCID: PMCPMC3079020.

72. Vos AA, Posthumus AG, Bonsel GJ, Steegers EA, Denktas S. Deprived neighborhoods and adverse perinatal outcome: a systematic review and meta-analysis. Acta Obstet Gynecol Scand. 2014; 93(8):727–40. doi: 10.1111/aogs.12430 PMID: 24834960.

73. Snelgrove JW, Murphy KE. Preterm birth and social inequality: assessing the effects of material and psychosocial disadvantage in a UK birth cohort. Acta Obstet Gynecol Scand. 2015; 94(7):766–75. doi: 10.1111/aogs.12648 PMID: 25846179.

74. Lahelma E, Laaksonen M, Martikainen P, Rahkonen O, Sarlio-Lahteenkorva S. Multiple measures of socioeconomic circumstances and common mental disorders. Soc Sci Med. 2006; 63(5):1383–99. doi: 10.1016/j.socscimed.2006.03.027 PMID: 16690186.

75. Shavers VL. Measurement of socioeconomic status in health disparities research. J Natl Med Assoc. 2007; 99(9):1013–23. PMID: 17913111; PubMed Central PMCID: PMCPMC2575866.