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Association between serum C-reactive protein and DSM-IV generalized anxiety disorder in adolescence: Findings from the ALSPAC cohort

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ABSTRACT

Background: Animal studies suggest a role of inflammation in the pathophysiology of anxiety, but human studies of inflammatory markers and anxiety disorders are scarce. We report a study of serum C-reactive protein (CRP) and generalised anxiety disorder (GAD) from the general population-based ALSPAC birth cohort.

Methods: DSM-IV diagnosis of GAD was obtained from 5365 cohort members during face-to-face clinical assessment at age 16 years, of which 3392 also provided data on serum high sensitivity CRP levels. Logistic regression calculated odds ratio (OR) for GAD among individuals in top and middle thirds of CRP distribution compared with the bottom third. Effect of comorbid depression was assessed. Age, sex, body mass, ethnicity, social class, maternal education, maternal age at delivery, and family history of inflammatory conditions were included as potential confounders.

Results: Forty participants met DSM-IV criteria for GAD (0.74%). CRP levels were higher in GAD cases compared with the rest of the cohort ($P = 0.005$). After adjusting for potential confounders, participants in the top third of CRP values compared with the bottom third were more likely to have GAD; adjusted OR 5.06 (95% CI, 1.31–19.59). The association between CRP and GAD was consistent with a linear dose-response relationship. The pattern of association between CRP and GAD remained unchanged after excluding cases with co-morbid depression.

Conclusions: The findings are consistent with a role of inflammation in anxiety disorders. Longitudinal studies of inflammatory markers, subsequent anxiety taking into account current and past psychological stress are required to understand this association further.

1. Introduction

Emerging evidence indicates an important role of inflammation in the pathophysiology of mood and anxiety disorders where inflammatory cytokines are thought to play a key role (Khandaker et al., 2014; Dantzer et al., 2008; Hodes et al., 2014). In healthy volunteers, simulated bacterial infection with the injection of an immune activating agent, lipopolysaccharide (LPS, a bacterial cell wall endotoxin), has been reported to produce anxiety and low mood as well as increased serum levels of interleukin 6 (IL-6, an inflammatory cytokine) (Reichenberg et al., 2001). Similarly, in mice immune activation is associated with anxiety-like behaviour as well as increased proinflammatory cytokines both in peripheral circulation and the brain (Gibney et al., 2013; Rossi et al., 2012). Moreover, anxiety inducing effects of social stress could be blocked by intra-cerebroventricular (ICV) administration of IL-1β (a

Abbreviations: CRP, C-reactive protein; GAD, generalized anxiety disorder; ALSPAC, Avon Longitudinal Study of Parents and Children; DSM-IV, diagnostic and statistical manual of mental disorders, fourth edition; ICV, intra-cerebroventricular; CNS, central nervous system; DAWBA, Development and Well-being Assessment; BMI, body mass index; IQR, interquartile range; OR, odds ratio; CI, confidence internal.

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proinflammatory cytokine) receptor antagonist immediately after stress exposure (Rossi et al., 2012). The results indicate inflammatory cytokines are important mediators of the relationship between psychological stress and anxiety in the central nervous system (CNS). However, despite these intriguing findings from preclinical research human studies of inflammation and anxiety are scarce. Rarer still are general population-based studies that are less prone to bias arising from inappropriate control selection. Existing studies are limited in number and have mixed findings; some suggest an association between anxiety symptoms and circulating inflammatory markers, such as C-reactive protein (CRP) (Liukkonen et al., 2011; Pitsavos et al., 2006), while others do not (Copeland et al., 2012; Baune et al., 2012). Methodological issues, such as study design, physical comorbidity, methods for measuring CRP and anxiety, might account for some of the discrepancy in findings in existing studies (see discussion). A clearer understanding of the association between anxiety and inflammation would come from studies that are based on general population samples, include robust assessment of inflammation, anxiety disorder as well as relevant socio-demographic, physical and clinical parameters, thus reducing chances of bias and confounding.

Using data from the general population-based Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, we report a study of generalised anxiety disorder (GAD), diagnosed according Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) criteria, and serum CRP levels in adolescence at age 16 years. We have used a clinical diagnosis of GAD as the outcome rather than all anxiety disorders or anxiety symptoms, because anxiety disorders include a range of conditions with varied presentations that might have different pathophysiology and aetiology, whilst anxiety symptoms are non-specific and may accompany many physical and mental illnesses.

2. Methods and materials

2.1. Sample

The ALSPAC birth cohort comprises 14,062 live births from pregnant women resident in county Avon, a geographically defined region in southwest of England, with expected dates of delivery between April 1991 and December 1992 (http://www.bristol.ac.uk/alspac/) (Boyd et al., 2013; Fraser et al., 2013). Avon included both urban and rural areas, and the population was broadly representative of all children in the UK. The parents completed regular postal questionnaires about all aspects of their child's health and development since birth. Since the age of 7 years the entire cohort attended an annual assessment clinic during which they participated in a range of face-to-face interviews and physical tests.

The current study is based on 5365 cohort members who took part in psychiatric assessment at age 16 years, of which 3392 also provided data on serum CRP levels. Analysis of the association between CRP and GAD was based on this sample of 3392. We subsequently repeated the analysis after imputation of missing CRP data (N = 5365).

Ethical approval for the study was obtained from ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

2.2. Assessment of GAD

Diagnosis of GAD according to DSM-IV criteria was obtained using computerised child version of the Development and Well-being Assessment (DAWBA), which was administered on 5365 cohort members during face-to-face clinical assessment at age 16 years (Goodman et al., 2000; APA, 1995). DAWBA assessed symptoms of GAD occurring in the six-month period preceding the assessment. In addition, it included questions on symptom severity, duration, and their effect on life and development. All individuals with no psychiatric diagnosis or a diagnosis other than GAD were included in the comparison group.

2.3. Measurement of CRP

Blood samples were collected from participants who gave consent for venepuncture during clinical assessment at age 16 years. Participants fasted overnight before attending the clinic if seen in the morning, or at least for 6 h if seen in the afternoon. Blood samples were immediately spun, frozen and stored at −80 °C, which were analysed within 3–9 months of blood sampling with no freeze-thaw cycles in between. High sensitivity CRP was measured by automated particle-enhanced immunoturbidimetric assay (Roche UK, Welwyn Garden City, UK). A valid measure of serum CRP was obtained from 3490 participants in total, which ranged from 0.07 to 72.55 mg/L (60 subjects over 10 mg/L). No other inflammatory makers were measured.

The sample was divided into three groups based on the tertiles of CRP distribution in all 3490 subjects regardless of their case or non-case status at follow-up. Values for the 33rd and 66th percentiles of the CRP distribution were 0.26 mg/L and 0.64 mg/L respectively. Thus, CRP levels for participants in the bottom, middle and top thirds of the distribution were 0.07–0.25 mg/L, 0.26–0.63 mg/L, and 0.64–72.55 mg/L respectively.

2.4. Assessment of potential confounders

We included a number of socio-demographic, physical and clinical parameters that are relevant to CRP levels and psychiatric illnesses. The data were collected during face-to-face clinical assessments or by postal questionnaires completed by parents. We included age at the time of assessment of GAD (in weeks), sex, body mass index (BMI) at the time of assessment of CRP, ethnicity, father's social class, maternal age at delivery and educational level. As per the UK Office of National Statistics classification system, father's social class was recorded in six categories: I, II, III non-manual, III manual, IV, V (in descending order with professionals and higher managerial workers representing social class I). Maternal age at delivery was grouped into six categories (age in years: <20, 20–24, 25–29, 30–34, 35–40, >40). Maternal highest educational achievement was recorded in four groups (secondary school, vocational qualification, O level, A level, degree). In addition, we included family history of chronic inflammatory conditions: arthritis and rheumatism in mothers and maternal grandparents. Finally, we examined the effect of concurrent infection, and separately, depression (assessed by DAWBA) on the association between GAD and CRP (see below). We did not include the use of anti-inflammatory or psychotropic medications as potential confounders as the numbers of adolescent subjects taking these drugs are likely to be small.

2.5. Statistical analysis

2.5.1. Baseline comparison

Baseline characteristics between cases of GAD and the rest of the cohort were compared using the Chi-square test and independent sample t-test for categorical and continuous variables, respectively.

2.5.2. Comparison of serum CRP levels between GAD cases and non-cases

Values of serum CRP level were not normally distributed. Therefore, we used median and interquartile range (IQR) for descriptive results. Independent sample Kruskal–Wallis test was