Brain, Behavior, and Immunity 58 (2016) 57–62

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Short Communication

Associations between cytokines, endocrine stress response, and gastrointestinal symptoms in autism spectrum disorder

Bradley J. Ferguson [a,e], Sarah Marler [b], Lily L. Altstein [c], Evon Batey Lee [d], Micah O. Mazurek [e,g], Aaron McLaughlin [f], Eric A. Macklin [c,h], Erin McDonnell [c], Daniel J. Davis [n], Anthony M. Belenchia [o], Catherine H. Gillespie [n], Catherine A. Peterson [o], Margaret L. Bauman [i], Kara Gross Margolis [j], Jeremy Veenstra-VanderWeele [k,l], David Q. Beversdorf [a,e,m,*]

[a] Interdisciplinary Neuroscience Program, University of Missouri, United States
[b] Department of Child and Adolescent Psychiatry, Vanderbilt University Medical Center, United States
[c] Massachusetts General Hospital Biostatistics Center, United States
[d] Departments of Pediatrics, Psychology, and Psychiatry, Vanderbilt University, United States
[e] The Thompson Center for Autism and Neurodevelopmental Disorders, University of Missouri, United States
[f] University of Missouri School of Medicine, United States
[g] Department of Health Psychology, University of Missouri, United States
[h] Harvard Medical School, United States
[i] Boston University School of Medicine, United States
[j] Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Columbia University, United States
[k] Department of Psychiatry and Sackler Institute for Developmental Psychobiology, Columbia University, United States
[l] New York State Psychiatric Institute, New York Presbyterian Hospital Center for Autism and the Developing Brain, United States
[m] William and Nancy Thompson Chair in Radiology, Departments of Radiology, Neurology, and Psychological Sciences, University of Missouri, United States
[n] Department of Veterinary Pathobiology, University of Missouri, United States
[o] Department of Nutrition and Exercise Physiology, University of Missouri, United States

ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 9 May 2016
Accepted 12 May 2016
Available online 12 May 2016

Keywords:
Autism spectrum disorder
Cortisol
Cytokines
Stress
Gastrointestinal disorders

ABSTRACT

Many children and adolescents with autism spectrum disorder (ASD) have significant gastrointestinal (GI) symptoms, but the etiology is currently unknown. Some individuals with ASD show altered reactivity to stress and altered immune markers relative to typically-developing individuals, particularly stress-responsive cytokines including tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). Acute and chronic stress is associated with the onset and exacerbation of GI symptoms in those without ASD. The present study examined whether GI symptoms in ASD were associated with increases in cortisol, a stress-associated endocrine marker, and TNF-α and IL-6 in response to stress. As hypothesized, a greater amount of lower GI tract symptoms were significantly associated with post-stress cortisol concentration. The relationship between cortisol response to stress and GI functioning was greater for children who had a history of regressive autism. Exploratory analyses revealed significant correlations between cortisol response, intelligence, and inappropriate speech. In contrast, symptoms of the lower GI tract were not associated with levels of TNF-α or IL-6. Significant correlations were found, however, between TNF-α and IL-6 and irritability, socialization, and intelligence. These findings suggest that individuals with ASD and symptoms of the lower GI tract may have an increased response to stress, but this effect is not associated with concomitant changes in TNF-α and IL-6. The relationship between cortisol stress response and lower GI tract symptoms in children with regressive autism, as well as the relationships between cortisol, IL-6, and intelligence in ASD, warrant further investigation.

1. Introduction

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and restricted, repetitive patterns of behavior that occur early in development (American Psychiatric Association, 2000). Many studies suggest an increased prevalence of gastrointestinal (GI) problems in individuals with ASD relative to typically-developing individuals (McElhanon et al., 2014;

* Corresponding author at: DC 069.10, One Hospital Drive, University of Missouri, Department of Radiology, Columbia, MO 65212, United States.
E-mail address: beversdorfd@health.mssouri.edu (D.Q. Beversdorf).

http://dx.doi.org/10.1016/j.bbi.2016.05.009
0889-1591/

Chaidez et al., 2014), especially for constipation, but the cause of this relationship is not currently known. Both acute and chronic stress are associated with the onset of GI disease as well as exacerbation of GI symptoms (Dinan et al., 2006). Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, which results in a cascade of neuroendocrine factors that modulate stress reactivity, digestion, and immunological functioning. Activation of the HPA axis in individuals with irritable bowel syndrome (IBS) without ASD is associated with an augmented adrenocorticotropic hormone (ACTH) and cortisol response, as well as increased sympathetic activity and low vagal tone (Aggarwal et al., 1994; Mayer, 2000). Furthermore, a generalized increase in the stress response is also characteristic of those with ASD relative to typically-developing individuals (Ming et al., 2005; Mazurek et al., 2013; Spratt et al., 2012). However, the relationship between the stress response and GI symptoms in ASD is poorly understood.

In addition to core ASD symptoms, many children with ASD have associated co-occurring symptoms, including anxiety, agitation, irritability, and aggression. Among individuals with ASD, GI symptoms predict increased behavioral symptoms in some domains, including heightened stress and anxiety, increased rigid-compulsive behavior, and irritability/agitation (Coury et al., 2012; Bishop-Fitzpatrick et al., 2015; Nikolov et al., 2009; Peters et al., 2014). Exposure to a social situation is associated with an enhanced cortisol response in ASD relative to typically-developing peers (Corbett et al., 2010), and there is a positive relationship between cortisol and self-reported social stress and anxiety during social situations in ASD (Lopata et al., 2008). Furthermore, a heightened cortisol response is linked to decreased intelligence as well as receptive and expressive language (Kidd et al., 2012), and some have proposed that the effects of stress on the HPA axis may contribute to these outcomes (Maldonado et al., 2008). In the mouse brain, elevated interleukin-6 (IL-6) levels are associated with increased autism-like features such as impairments in cognition, learning, anxiety, and social interaction, suggesting a potential cytokine of interest in ASD (Wei et al., 2012). Additionally, levels of IL-6 have been shown to be increased in individuals with regressive autism, (i.e. losing previously acquired skills such as language or social skills) (Ashwood et al., 2011). Determining biomarkers associated with GI symptoms in ASD has the potential to assist in the treatment of this and perhaps other medical and psychological complications in this population.

Activation of the HPA axis also leads to cascading immune system responses through the release of cytokines. Several studies have shown that individuals with ASD have an atypical immune response, including alterations in IL-12, IFN-γ, IL-2, IL-6, IL-10, and TNF-α (Lyte et al., 2011; Goines and Ashwood, 2013). Levels of the proinflammatory cytokines IL-6 and tumor necrosis factor-alpha (TNF-α), which lead to activation of the HPA axis (Dunn, 2006), have been shown to be increased in those with ASD (Ashwood et al., 2011; Emanuele et al., 2010; von Känel et al., 2005). In the general population, GI symptoms themselves have been reported to also be associated with alterations in TNF-α and IL-6, suggesting a potential interaction between stress and immune functioning in those with GI dysfunction (Lyte et al., 2011; von Känel et al., 2006). Therefore, these specific stress, ASD, and GI-associated cytokines were the focus for our study of the interaction with stress reactivity.

As a result, the goal of the present study was to examine the relationships between GI symptoms and cortisol response to stress, as well as the stress-responsive cytokines IL-6 and TNF-α. We hypothesized that GI symptoms would be positively correlated with change in cortisol concentrations after stress, and also with levels of IL-6 and TNF-α. We also examined the relationship between psychophysiological markers of autonomic nervous system functioning and GI symptomatology, which are known to be interrelated with lower GI symptomatology in ASD (Ferguson et al., in press). Furthermore, relationships between these measures and intelligence, ASD-associated behaviors, and adaptive functioning were explored, to determine how these findings relate to other co-occurring conditions associated with GI symptomatology.

2. Materials and methods

2.1. Participants

Participants were recruited through the Autism Speaks – Autism Treatment Network and through clinics at the University of Missouri Thompson Center for Autism and Neurodevelopmental Disorders in Columbia, Missouri, and the Vanderbilt Kennedy Center and Monroe Carrell Jr. Children's Hospital at Vanderbilt University in Nashville, Tennessee. A total of 120 individuals (mean age = 11.8, SD = 3.8, range = 6–18, mean Full Scale Intelligence Quotient = 84, SD = 22.6, Range = 36–130, 111 Caucasian, 108 males) with a diagnosis of ASD participated in the study. ASD diagnosis was based on the Diagnostic and Statistical Manual for Mental Disorders IV-TR criteria (American Psychiatric Association, 2000), and diagnoses were verified by administration of the Autism Diagnostic Observation Schedule (ADOS) Lord et al., 1989. Individuals with a known mitochondrial disorder, genetic disorder such as Fragile X syndrome or tuberous sclerosis, or a bleeding disorder were excluded.

Potential participants' medical records were screened, and the parents of those who were deemed eligible to participate were contacted. An effort was made to recruit a similar number of individuals with and without GI disorders at each study site. A total of 107 and 105 individuals provided pre-stress blood samples that were suitable for analysis for IL-6 and TNF-α, respectively. A total of 81 pre-stress and 79 post-stress salivary cortisol samples were suitable for analysis.

2.2. Assessment of gastrointestinal symptoms

The Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III (QPGS-RIII) Walker et al., 2006 was used to assess GI symptomatology. The QPGS-RIII is a 71-item parent report measure that assesses the frequency, severity, and duration of functional (i.e. no associated pathology observed in endoscopy, imaging, or blood) GI symptoms in children and adolescents. The QPGS-RIII has been used to assess GI dysfunction in ASD with clinician-parent agreement at 92.1% for presence of any QPGS-RIII disorder, and fair agreement for functional constipation (Gorrindo et al., 2012). Consistent with previous work from our team, continuous variables were created for upper and lower GI tract symptoms (Ferguson et al., in press). Briefly, the multiple choice responses to the questions pertaining to the ten functional pediatric GI disorders assessed by the QPGS-RIII were assigned ratings, and a quantitative score was created by summing the ratings (scored on scales of 1–3, 0–4, 1–5, or 0–5, in accordance with the QPGS-RIII scoring criteria for each designated item; Yes/No responses were assigned 1 point each). Separate scores were summed for upper and lower GI tract disorders that included the following GI disorders for each GI group: Upper GI – functional abdominal pain, abdominal migraine, aerophagia, upper abdominal pain associated with bowel symptoms; Lower GI – functional constipation, irritable bowel syndrome, non-retentive fecal incontinence, lower abdominal pain associated with bowel symptoms. Parent-report forms were used for all participants except for four individuals over the age of 16, as the parent indicated that the