

Systemic Inflammation during the First Postnatal Month and the Risk of Attention Deficit Hyperactivity Disorder Characteristics among 10 year-old Children Born Extremely Preterm

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Abstract Although multiple sources link inflammation with attention difficulties, the only human study that evaluated the relationship between systemic inflammation and attention problems assessed attention at age 2 years. Parent and/or teacher completion of the Childhood Symptom Inventory-4 (CSI-4) provided information about characteristics that screen for attention deficit hyperactive disorder (ADHD) among 793 10-year-old children born before the 28th week of gestation who had an IQ ≥ 70 . The concentrations of 27 proteins in blood spots obtained during the first postnatal month were measured. 151 children with ADHD behaviors were identified by parent report, while 128 children were identified by teacher report. Top-

quartile concentrations of IL-6R, TNF- α , IL-8, VEGF, VEGFR1, and VEGFR2 on multiple days were associated with increased risk of ADHD symptoms as assessed by a teacher. Some of this increased risk was modulated by top-quartile concentrations of IL-6R, RANTES, EPO, NT-4, BDNF, bFGF, IGF-1, PIGF, Ang-1, and Ang-2. Systemic inflammation during the first postnatal month among children born extremely preterm appears to increase the risk of teacher-identified ADHD characteristics, and high concentrations of proteins with neurotrophic properties appear capable of modulating this increased risk.

Keywords Infant, premature/blood · Attention deficit hyperactivity disorder · Inflammation · Neuroprotection

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Introduction

The lower the gestational age at birth, the higher the risk of an attention deficit hyperactivity disorder (ADHD) (Sucksdorff et al. 2015). The reasons for this heightened risk with greater immaturity remain obscure.

One hypothesis, invokes inflammation. Assisted (i.e., mechanical) ventilation and bacteremia are likely the two most common contributors to sustained or recurrent inflammation in the extremely preterm newborn (Dammann and Leviton 2014). The reasons extremely preterm newborns are at heightened risk for bacteremia are complex, but probably include exposure to the mother's organisms, fragile skin, indwelling arterial and venous tubes, need for assisted ventilation, deficient expression of complement, as well as a deficiency of antimicrobial proteins and peptides (Dammann and Leviton

2014). Once the inflammation is initiated, it appears to be sustained (Dammann et al. 2016).

Support for an inflammation-ADHD relationship comes from human studies of early postnatal infection (Rand et al. 2016), early postnatal inflammation (O'Shea et al. 2014), and later postnatal inflammation (Ceylan et al. 2012; Shyu et al. 2012; Holtmann et al. 2013; Mitchell and Goldstein 2014), as well as genetic propensities to inflammation (Zayats et al. 2015).

Another hypothesis attributes some of this to a separation of the fetus from the source of growth factors provided by mother and/or placenta before the newborn can synthesize adequate amounts (Reuss et al. 1994; Dammann and Leviton 1999). Growth factors with neurotrophic and/or angiogenic properties have the potential to minimize the occurrence of brain damage (Larphaveesarp et al. 2015; Chew and DeBoy 2016), which has been associated with ADHD (van Ewijk et al. 2012).

We know of no study of the relationship between early postnatal inflammation and the risk of an attention disorder assessed at school age, nor do we know of any cohort study of the relationship between early blood concentrations of growth factors and the risk of an attention disorder assessed at school age. The ELGAN (Extremely Low Gestation Age Newborn) Study not only enrolled children born before the 28th week of gestation and followed them to age 10 years, but also measured concentrations of inflammation-related proteins in multiple blood specimens collected during the first postnatal month, as well as the concentrations of selected proteins that have neurotrophic and/or angiogenic properties. The ELGAN Study, therefore, provided an opportunity to begin to fill this void.

Methods

Participants

The ELGAN study is a multi-center prospective, observational study of the risk of structural and functional neurologic disorders in extremely preterm infants (O'Shea et al. 2009). A total of 1506 infants born before the 28th week of gestation were enrolled during the years 2002–2004.

At age 10 years, 966 of these children were recruited for an age-appropriate assessment of cognition, executive function, behaviors, and achievement. Of these, 889 (92%) returned for follow up and 874 were administered the neurocognitive tests. Enrollment and consent procedures for this follow up study were approved by the institutional review boards of all participating institutions.

General Cognitive Ability

General cognitive ability (or IQ) was assessed with the School-Age Differential Ability Scales–II (DAS-II) Verbal and Nonverbal Reasoning scales (Elliott 2007). Intellectual

disability (ID) was defined as a score below 70 on both the verbal and nonverbal reasoning scales.

ADHD Assessment

While the child was tested, the parent or caregiver completed questionnaires regarding the child's medical and neurological status and behavior, including the Child Symptom Inventory-4 Parent Checklist (CSI-4) (Sprafkin et al. 2002; Gadow and Sprafkin 2002). The child's current teacher was also asked to complete the Child Symptom Inventory-4 Teacher Checklist. Teachers and parents did not make any ADD DSM-IV diagnosis. Rather, the CSI-4 program identified children as screening positive for these diagnoses based on the parents' or teachers' acknowledging selected characteristics of the child that aligned with DSM-IV symptoms.

Both the parent checklist and the teacher version include the same 18 items specific for ADHD symptoms (9 for the inattentive domain and 9 for the hyperactive/impulsive domain) that are each rated on a scale from 0 (never) to 3 (very often). For this study, a child was classified as screening positive for each ADHD type if s/he was reported to have 6 or more of the 9 characteristics/symptoms either, sometimes, or very often. Parents were the source about the physician's diagnosis, which we recognize is often based, in part, on parent and teacher assessments (Power et al. 2008).

Blood Spot Collection and Measurement

Drops of blood were collected on filter paper on the first postnatal day (range: 1–3 days), the 7th postnatal day (range: 5–8 days), the 14th postnatal day (range: 12–15 days), the 21st postnatal day (range: 19–23 days), and the 28th postnatal day (range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at -70°C in sealed bags with a desiccant until processed. Details about the elution of proteins from the blood spots are provided elsewhere (Fichorova et al. 2015). The total protein concentration in each eluted sample was determined by BCA assay (Thermo Scientific, Rockford, IL) using a multi-label Victor 2 counter (Perkin Elmer, Boston, MA) and the measurements of each analyte normalized to mg total protein (Fichorova et al. 2015).

Proteins Measured

The Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston Massachusetts eluted all blood spots as previously described and measured all proteins reported here. The laboratory used the Meso Scale Discovery to measure: C-Reactive Protein (CRP), serum amyloid A (SAA), Myeloperoxidase (MPO) Interleukin-1 β (IL-1 β), IL-6, IL-6 Receptor (IL-6R), Tumor Necrosis Factor- α (TNF- α), TNF Receptor-1 (TNFR-1), TNFR-2, IL-8 (CXCL8),

Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES; CCL5), Intercellular Adhesion Molecule-1 (ICAM-1; CD54), Vascular Cell Adhesion Molecule-1 (VCAM-1; CD106), Vascular Endothelial Growth Factor (VEGF), VEGF Receptor-1 (VEGFR-1, also known as sFLT-1), VEGFR-2 (KDR), Insulin-Like Growth Factor-1 (IGF) Binding Protein-1 (IGFBP-1), thyroid stimulating hormone (TSH), Metalloproteinase (MMP)-9, and erythropoietin (EPO).

A multiplex immunobead assay manufactured by R&D Systems (Minneapolis, MN) and a MAGPIX Luminex reader (R&D Systems) were used to measure angiopoietin-1 (Ang-1), Ang-2, placenta growth factor (PIGF), Neurotrophin-4 (NT-4), Brain Derived Neurotrophic Factor (BDNF), and basic Fibroblastic Growth Factor (bFGF). ELISA (R&D Systems) was used to measure IGF-1.

Because the concentrations of inflammation-related proteins in the ELGAN Study varied with gestational age, and with the postnatal day of collection (Leviton et al. 2011b, 2012), we divided our sample into 15 groups defined by gestational age category (23–24, 25–26, 27 weeks), and postnatal day of blood collection (1, 7, 14, 21 and 28). Because we were interested in the contribution of both high and low concentrations, and the concentrations of most proteins did not follow a normal distribution, the distribution of each protein's concentration was divided into quartiles among children in each of the 15 groups (3 gestational age groups, 5 collection days).

Data Analyses

In the sample of all children who had protein measurements and a CSI-4 form completed by a parent or teacher, 65 children had an IQ of less than 70. These children have been excluded because we did not have a way of determining if the ADHD symptoms were “inconsistent with developmental level” as recommended by Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV).

We evaluated five null hypotheses linking blood concentrations during the first postnatal month to ADHD symptoms 10 years later. First, compared to children who had a concentration in the lower three quartiles one day, those who had a top quartile concentration that day were no more likely of satisfying CSI-4 criteria for ADHD at age 10 years (Appendix Tables 1–5, summarized in Table 2). Second, compared to their peers who had lower concentrations of a protein on two days of the early epoch (when specimens were obtained on days 1, 7, and 14), those who had top quartile concentrations of that protein on two days of the early epoch were no more likely to have ADHD symptoms at age 10 (Appendix Table 6, summarized in Table 2). Third, compared to their peers who had lower concentrations of a protein on both days 21 and 28 (of the late epoch), those who had top quartile concentrations of that protein on those days were no more likely to have ADHD symptoms at age 10 (Appendix Table 7, summarized in Table 2). Fourth,

compared to their peers who had concentrations of two proteins in the lower three quartiles on two days during the early epoch, those who had higher concentrations of one or both proteins were not at higher risk of ADHD symptoms at age 10 (Table 3 for teacher-identified ADHD and Table 5 for parent-and-teacher-identified ADHD). Fifth, compared to their peers who had concentrations of two proteins in the lower three quartiles on both days of the late epoch, those who had higher concentrations of one or both proteins were not at higher risk of teacher-identified ADHD symptoms at 10 (Table 4).

We created logistic regression models comparing the risk of ADHD symptoms among children who had a concentration in the top quartile to the risk among children who had a concentration below the 75th centile. This allowed us to calculate odds ratios and 95% confidence intervals. In the two-protein sets of analyses (Tables 3, 4, and 5), children who did not have high (i.e., top quartile) concentrations of either protein were the referent group, and their odds ratios of ADHD are set at 1.0.

In this sample, children who had top quartile concentrations of inflammation-related proteins were no more likely than their peers who had lower concentrations to have a mother who had limited educational achievement, a low score on the Kaufman Brief Intelligence Test, 2nd Edition, or was eligible for government-provided medical care insurance (Medicaid) (Leviton et al. 2016b). Thus, confounding by social class is not an issue. Similarly, other potential confounders such as sex were also not associated with systemic inflammation. On the other hand, fetal growth restriction, defined as a birth weight Z-score < -1, is associated in this sample with elevated concentrations of some proteins, (Leviton et al. 2016b) and with ADHD symptoms (Korzeniewski et al. 2017). All analyses have been adjusted for gestational age 23–24 and 25–26 weeks as well as birth weight Z-score < -1.

We examined high and low concentrations of neuroprotective proteins in relation to high and low concentrations of the three inflammatory proteins (TNF- α , IL-8, and ICAM-1) most consistently associated with brain damage and/or adverse neurodevelopmental outcomes in the ELGAN study cohort. High concentrations of IL-8, and ICAM-1 were associated with enlarged cerebral ventricles (when the infant was in the intensive care nursery), and low mental development index (MDI) and microcephaly at age 2 years, (Leviton et al. 2016a) while high concentrations of TNF-alpha, IL-8, ICAM-1 were associated with diparetic cerebral palsy at age 2 years (Kuban et al. 2014) and low IQ at age 10 years (Kuban et al. 2017).

Results

Sample Description (Table 1)

Of the 864 children who provided two blood specimens a week apart during the first postnatal month and whose parent

Table 1 Sample description: number of children

	Yes
Enrolled at birth	1506
Candidates for re-enrollment at age 10 years	966
Re-enrolled at age 10 years	889
Assessed at age 10 years	864
IQ \geq 70	793
Identified as any ADD by parent	151
Identified as any ADD by teacher	128
Identified as any ADD by parent and teacher	52
Identified as any ADD by physician	181
Identified as any ADD by any 2	130

completed a CSI-4 when the child was 10 years old, 793 had an IQ \geq 70. Of these children, the numbers identified as having ADHD symptoms were 151 by the parent, 128 by the teacher, 52 by both the parent and teacher, 181 by a physician, and 130 by any 2 observers.

Summary of Individual Proteins on Individual Days (Table 2)

The [appendix](#) contains 7 tables. Each of first 5 are for the concentrations on the day the blood was collected (i.e., days 1, 7, 14, 21, and 28), and the last 2 are for the early and late epochs. These tables contain 945 odds ratios for ADHD symptoms (27 proteins X 5 observers X 7 times). The findings are summarized in Table 2, which identifies the 40 statistically-significant instances of increased risk and the 9 instances of statistically-significant decreased risk.

By and large the associations summarized in this table are scattered by day, protein, and observer. The most consistent associations were for VEGF-family-associated increased risks for ADHD symptoms identified by the teacher, as well as by both teacher and parent.

Early Epoch Sets of Proteins: Teacher-Identified ADHD Symptoms (Table 3)

Because the associations in Table 2 are more abundant for ADHD symptoms identified by the teacher than by the parent, we limit additional analyses to screening positive according to the teacher. We attach the name “pattern A” to the pattern of a significantly elevated odds ratio of screening positive for ADHD when the concentrations of both proteins are in the top quartile. This pattern is seen when high concentrations of TNF- α occurred with high concentrations of MMP-9, EPO, VEGF, and Ang-1. It also occurred with high concentrations of both IL-8 and MMP-9.

“Pattern B” is characterized by increased risks of ADHD symptoms when a top quartile concentration of the

neurotrophic protein is associated with increased risks of ADHD symptoms even when concentrations of the inflammation-related protein are low. This pattern was seen with IL-6R regardless of the concentrations of TNF- α , IL-8, and ICAM-1, and with VEGF-R2 regardless of the concentrations of these proteins.

Pattern C is characterized by significantly increased risk associated with high concentrations of the inflammation-related protein, regardless of the concentration of the neurotrophic protein. This pattern is seen with top quartile concentrations of IL-8 regardless of the concentration of IL-6R, EPO, and VEGF.

We assign the name Pattern D to the increased risk of ADHD symptoms associated with high concentrations of the inflammation-related protein, but only when concentrations of the neurotrophic protein are lower. This pattern was seen for the combinations of TNF- α with NT-4 and bFGF, and combinations of IL-8 with RANTES, VEGF-R1, VEGF-R2, NT-4, BDNF, bFGF, PIGF, Ang-1, and Ang-2. We identify this pattern as “protection in the face of adversity.”

Late Epoch Sets of Proteins: Teacher-Identified ADHD Symptoms: Late Epoch (Table 4)

Pattern D is seen with VEGF and IL-6R, RANTES, EPO, IGF-1, PIGF, and Ang-2, as well as with VEGF-R1 and EPO, and with VEGF-R2 and EPO, VEGF-R2 and Ang-2. Pattern A is seen with VEGF and MMP-9, VEGF-R1, bFGF, and Ang-1, as well as with VEGF-R1 and VEGF, and Ang-1, and with VEGF-R2 and IL-6R, RANTES, VEGF-R1, and PIGF. Pattern C was seen with VEGF and VEGF-R2, as well as with NT-4 and VEGF, VEGF-R1, and VEGF-R2, and with Ang-1 and VEGF, VEGF-R1, and VEGF-R2.

Early Epoch Sets of Proteins: Parent and Teacher-Identified ADHD Symptoms (Table 5)

Pattern C was seen with VEGF and the three inflammation-related proteins that head the columns (i.e., TNF- α , IL-8, and ICAM-1), as well as with NT-4 and IL-8. Pattern D was exemplified by top quartile concentrations of VEGF-R2, Ang-1, and Ang-2, but only in the setting of top quartile concentrations of IL-8.

Discussion

We found 40 instances of increased risk and 9 instances of decreased risk among 27 proteins assessed by 5 observers on 7 occasions (5 days and 2 epochs) = 945 non-independent opportunities. This fraction of 49/945 = 5%, leads us to infer that what we found might reflect random phenomena. On the other hand, the 20 statistically significant associations of the VEGF family (VEGF, VEGF-R1, and VEGF-R2) out of 105

Table 3 Odds ratios (95% Confidence Intervals) of teacher-identified ADHD symptoms at age 10 years associated with concentrations in the top quartile on 2 days a week apart during the early epoch (days 1, 7, 14)

		TNF- α		IL-8		ICAM-1	
		+	–	+	–	+	–
IL-6R	+	2.6 (1.2, 5.4)	1.8 (1.03, 3.1)	2.6 (1.2, 5.8)	2.0 (1.2, 3.4)	1.6 (0.8, 3.1)	1.9 (1.1, 3.4)
	–	1.7 (0.9, 3.0)	1.0	2.6 (1.4, 4.7)	1.0	0.9 (0.4, 1.6)	1.0
MMP-9	+	2.4 (1.1, 5.1)	1.1 (0.6, 2.1)	2.8 (1.4, 5.9)	0.9 (0.5, 1.9)	1.8 (0.9, 3.8)	1.0 (0.5, 2.0)
	–	1.5 (0.8, 2.6)	1.0	1.9 (1.00, 3.5)	1.0	0.7 (0.3, 1.3)	1.0
RANTES	+	1.5 (0.7, 3.4)	0.9 (0.5, 1.7)	1.5 (0.6, 3.5)	1.0 (0.6, 1.9)	0.4 (0.2, 2.5)	2.3 (0.7, 2.1)
	–	1.7 (0.97, 3.0)	1.0	2.7 (1.5, 4.8)	1.0	1.3 (0.7, 2.3)	1.0
EPO	+	2.0 (1.1, 3.8)	1.2 (0.6, 2.7)	2.3 (1.2, 4.4)	1.2 (0.6, 2.5)	1.3 (0.6, 2.9)	1.8 (0.9, 3.2)
	–	1.4 (0.7, 2.8)	1.0	2.2 (1.1, 4.5)	1.0	0.9 (0.5, 1.8)	1.0
VEGF	+	2.4 (1.2, 4.8)	1.4 (0.8, 2.4)	2.4 (1.2, 5.0)	1.5 (0.8, 2.6)	1.4 (0.7, 3.1)	1.5 (0.9, 2.6)
	–	1.5 (0.8, 2.8)	1.0	2.4 (1.2, 4.5)	1.0	0.9 (0.4, 1.7)	1.0
VEGF-R1	+	1.7 (0.7, 4.0)	0.8 (0.4, 1.6)	1.5 (0.6, 3.7)	1.0 (0.5, 1.9)	1.1 (0.4, 2.6)	0.9 (0.5, 1.7)
	–	1.6 (0.9, 2.8)	1.0	2.6 (1.4, 4.5)	1.0	0.9 (0.5, 1.7)	1.0
VEGF-R2	+	2.2 (1.1, 4.5)	2.0 (1.1, 3.4)	1.8 (0.8, 3.8)	2.5 (1.5, 4.3)	1.4 (0.8, 2.6)	2.3 (1.2, 4.1)
	–	1.8 (1.00, 3.4)	1.0	3.7 (2.0, 6.8)	1.0	0.6 (0.2, 1.6)	1.0
NT-4	+	1.6 (0.6, 4.1)	1.4 (0.8, 2.6)	1.0 (0.3, 3.8)	1.7 (0.99, 3.1)	1.2 (0.4, 3.9)	1.3 (0.7, 2.3)
	–	1.8 (1.04, 3.1)	1.0	2.9 (1.7, 5.0)	1.0	1.0 (0.5, 1.7)	1.0
BDNF	+	1.9 (0.9, 3.9)	1.3 (0.7, 2.4)	1.9 (0.8, 4.2)	1.5 (0.9, 2.7)	1.3 (0.5, 3.0)	1.4 (0.8, 2.4)
	–	1.6 (0.9, 2.9)	1.0	2.7 (1.5, 5.0)	1.0	0.9 (0.5, 1.7)	1.0
bFGF	+	1.1 (0.5, 2.7)	0.8 (0.4, 1.6)	1.7 (0.7, 4.1)	0.6 (0.3, 1.4)	0.5 (0.1, 1.8)	0.9 (0.5, 1.7)
	–	1.8 (1.1, 3.2)	1.0	2.3 (1.3, 4.0)	1.0	1.1 (0.6, 1.9)	1.0
IGF-1	+	1.6 (0.6, 4.2)	0.8 (0.4, 1.6)	2.8 (0.96, 8.3)	0.8 (0.4, 1.5)	0.6 (0.1, 2.6)	0.9 (0.5, 1.7)
	–	1.6 (0.9, 2.7)	1.0	2.0 (1.1, 3.4)	1.0	1.0 (0.6, 1.8)	1.0
PIGF	+	1.7 (0.7, 4.3)	1.4 (0.7, 2.7)	1.9 (0.6, 5.7)	1.5 (0.8, 2.8)	0.9 (0.2, 3.5)	1.4 (0.8, 2.6)
	–	1.7 (1.01, 3.0)	1.0	2.5 (1.4, 4.3)	1.0	1.0 (0.6, 1.8)	1.0
Ang-1	+	2.3 (1.1, 4.9)	1.2 (0.6, 2.1)	1.9 (0.8, 4.2)	1.6 (0.9, 2.9)	1.6 (0.7, 3.7)	1.2 (0.7, 2.2)
	–	1.4 (0.8, 2.6)	1.0	2.8 (1.6, 5.1)	1.0	0.8 (0.4, 1.5)	1.0
Ang-2	+	1.6 (0.7, 3.6)	1.3 (0.7, 2.6)	1.8 (0.8, 4.2)	1.4 (0.7, 2.6)	1.4 (0.7, 3.1)	1.1 (0.6, 2.2)
	–	1.8 (1.00, 3.1)	1.0	2.6 (1.4, 4.7)	1.0	0.7 (0.4, 1.5)	1.0

Bolded items are statistically significant at $p < .05$

+ indicates concentrations in the top quartile

– indicates concentrations below the top quartile

Children who consistently had concentrations of both proteins below the top quartile (–/–) comprise the referent group (odds ratio = 1.0)

Adjustment is made for gestational age 23–24 and 25–26 weeks and for birth weight Z-score < -1

Another possibility is that damage results in the release of both proteins into the circulation and reflects not damage causation, but rather damage consequence. This inference is supported by observations that brain damage can be followed by increased concentrations peripherally of inflammation-related proteins, such as TNF, (Ross et al. 1994; Stover et al. 2000; Sotgiu et al. 2006; Sriram and O’Callaghan 2007; Wiseman et al. 2014) IL-6, (Wiseman et al. 2014) IL-8, (Whalen et al. 2000) ICAM-1, (Sotgiu et al. 2006; Wiseman et al. 2014) and MMP-9 (Sotgiu et al. 2006). Brain damage can also be followed by the release

and/or upregulated expression of IGF-1, (Wildburger et al. 2001; Aberg et al. 2011; Mangiola et al. 2015) BDNF, (Yang et al. 1996; Korhonen et al. 1998; Kazanis et al. 2004; Rodier et al. 2015) bFGF, (Lin et al. 1997; Guo et al. 2006) NT-4, (Royo et al. 2006) VEGF, (Slevin et al. 2000; Shore et al. 2004; Matsuo et al. 2013) EPO, (Sakanaka et al. 1998; Juul et al. 1999; Brines et al. 2000; Aberg et al. 2016).

A third possibility is that high concentrations of one protein contribute to the damage, which then results in the release of the other protein. In the fourth, high concentrations of one

Table 4 Odds ratios (95% Confidence Intervals) of teacher-identified ADHD symptoms at age 10 years associated with concentrations in the top quartile on both days 21 and 28 of the late epoch

		VEGF		VEGF-R1		VEGF-R2	
		+	–	+	–	+	–
IL-6R	+	2.0 (0.6, 6.0)	1.4 (0.6, 3.2)	2.4 (0.7, 8.8)	1.2 (0.6, 2.8)	2.8 (1.1, 7.2)	0.9 (0.4, 2.3)
	–	2.5 (1.3, 4.9)	1.0	2.2 (1.00, 4.6)	1.0	2.1 (0.96, 4.7)	1.0
MMP-9	+	2.7 (1.03, 7.1)	0.9 (0.3, 3.0)	10 (2.0, 55)	0.9 (0.3, 2.2)	2.8 (0.6, 13)	1.5 (0.7, 3.5)
	–	2.0 (1.01, 4.1)	1.0	1.4 (0.7, 3.1)	1.0	2.4 (1.2, 4.9)	1.0
RANTES	+	2.3 (0.9, 6.3)	0.6 (0.2, 1.8)	2.3 (0.6, 8.4)	0.9 (0.4, 2.0)	5.1 (1.4, 19)	0.6 (0.2, 1.5)
	–	2.1 (1.04, 4.2)	1.0	2.1 (0.96, 4.4)	1.0	1.8 (0.9, 3.7)	1.0
EPO	+	0.6 (0.1, 5.2)	0.6 (0.3, 1.6)	1.0 (0.2, 5.0)	0.5 (0.2, 1.4)	0.8 (0.1, 6.9)	0.6 (0.2, 1.6)
	–	2.4 (1.3, 4.6)	1.0	2.3 (1.1, 4.9)	1.0	2.5 (1.3, 4.9)	1.0
VEGF	+			6.0 (1.9, 19)	1.7 (0.9, 3.5)	3.5 (1.03, 12)	2.4 (1.2, 4.6)
	–			1.5 (0.6, 3.5)	1.0	2.6 (1.2, 5.3)	1.0
VEGF-R1	+	6.0 (1.9, 19)	1.5 (0.6, 3.5)			9.3 (1.7, 49)	2.0 (1.00, 4.1)
	–	1.7 (0.9, 3.5)	1.0			1.8 (0.8, 3.9)	1.0
VEGF-R2	+	3.5 (1.03, 12)	2.6 (1.2, 5.3)	9.3 (1.7, 49)	2.0 (1.00, 4.1)		
	–	2.4 (1.2, 4.6)	1.0	1.8 (0.8, 3.9)	1.0		
NT-4	+	3.2 (0.9, 11)	1.1 (0.5, 2.6)	3.2 (0.9, 11)	1.1 (0.5, 2.6)	3.2 (0.9, 11)	1.1 (0.5, 2.6)
	–	2.1 (1.1, 4.1)	1.0	2.1 (1.1, 4.10)	1.0	2.1 (1.1, 4.05)	1.0
BDNF	+	2.1 (0.8, 5.3)	0.5 (0.2, 1.4)	2.7 (0.7, 10)	0.7 (0.3, 1.7)	2.5 (0.8, 3.4)	0.7 (0.3, 1.6)
	–	1.8 (0.8, 3.9)	1.0	1.9 (0.9, 4.1)	1.0	2.2 (1.01, 4.5)	1.0
bFGF	+	3.2 (1.1, 9.4)	0.7 (0.3, 1.9)	2.5 (0.7, 9.4)	1.0 (0.4, 2.3)	2.7 (0.8, 9.0)	1.0 (0.4, 2.3)
	–	1.9 (0.9, 3.7)	1.0	2.0 (0.9, 4.4)	1.0	2.3 (1.1, 4.7)	1.0
IGF-1	+	1.2 (0.2, 5.8)	1.1 (0.6, 2.3)	2.9 (0.6, 13)	0.9 (0.4, 1.8)	–	1.3 (0.7, 2.4)
	–	2.6 (1.3, 4.9)	1.0	2.0 (0.9, 4.2)	1.0	2.9 (1.5, 5.6)	1.0
PIGF	+	1.3 (0.4, 3.8)	0.7 (0.2, 2.2)	2.3 (0.4, 14)	0.8 (0.3, 1.8)	3.7 (1.01, 13)	0.5 (0.2, 1.4)
	–	2.7 (1.4, 5.5)	1.0	2.0 (1.00, 4.2)	1.0	1.9 (0.9, 4.0)	1.0
Ang-1	+	3.1 (1.3, 7.0)	0.8 (0.3, 2.1)	3.7 (1.1, 12)	1.3 (0.6, 2.6)	3.4 (1.1, 11)	1.3 (0.6, 2.8)
	–	1.7 (0.7, 3.7)	1.0	1.8 (0.8, 4.0)	1.0	2.2 (1.03, 4.6)	1.0
Ang-2	+	1.0 (0.3, 3.7)	0.8 (0.3, 1.7)	2.5 (0.6, 9.7)	0.5 (0.2, 1.3)	1.0 (0.3, 3.0)	0.7 (0.3, 1.7)
	–	2.7 (1.4, 5.2)	1.0	1.9 (0.9, 4.0)	1.0	3.6 (1.7, 7.7)	1.0

Bolded items are statistically significant at $p < .05$

+ indicates concentrations in the top quartile

– indicates concentrations below the top quartile

Children who consistently had concentrations of both proteins below the top quartile (–/–) comprise the referent group (odds ratio = 1.0)

Adjustment is made for gestational age 23–24 and 25–26 weeks and for birth weight Z-score < -1

protein contribute to the damage, which then promotes the synthesis of the other protein.

The B pattern of increased risks of ADHD symptoms when concentrations of the neurotrophic protein are high and the concentrations of the inflammation-related protein are lower was seen with three proteins thought to have neurotrophic properties, IL-6R, VEGFR-2, and NT-4. Each of these is characterized by pleiotropy (Tjwa et al. 2003; Kishimoto 2010; Bothwell 2014). Thus, it is possible, that the high concentrations of these proteins allow or contribute to the increased risk of screening positive for ADHD.

The C pattern, characterized by significantly increased risk associated with high concentrations of the inflammation-related protein, regardless of the concentration of the neurotrophic protein, can be viewed as what would be expected if the neurotrophic protein failed to protect against brain adversities associated with inflammation.

The D pattern of increased risk of screening positive for ADHD associated with high concentrations of the inflammation-related protein, but only when concentrations of the neurotrophic protein are lower is exactly what would be expected if the neurotrophic protein modulated

Table 5 Odds ratios (95% Confidence Intervals) of parent-and-teacher-identified ADHD symptoms at age 10 years associated with concentrations in the top quartile on 2 of the first 3 postnatal days (early epoch)

		TNF- α		IL-8		ICAM-1	
		+	–	+	–	+	–
IL-6R	+	1.1 (0.3, 3.8)	1.6 (0.8, 3.5)	1.7 (0.6, 5.3)	1.3 (0.6, 3.0)	1.1 (0.4, 3.1)	1.3 (0.6, 2.8)
	–	1.8 (0.8, 4.0)	1.0	1.8 (0.8, 4.3)	1.0	0.5 (0.2, 1.8)	1.0
MMP-9	+	2.1 (0.7, 5.8)	1.5 (0.6, 3.5)	2.4 (0.9, 6.3)	1.3 (0.5, 3.2)	1.6 (0.6, 4.4)	1.4 (0.6, 3.3)
	–	1.2 (0.5, 2.8)	1.0	1.4 (0.5, 3.5)	1.0	0.4 (0.1, 1.4)	1.0
RANTES	+	1.4 (0.5, 4.3)	1.2 (0.5, 2.7)	2.2 (0.8, 6.3)	0.9 (0.4, 2.2)	0.8 (0.2, 3.6)	1.2 (0.6, 2.6)
	–	1.4 (0.6, 3.2)	1.0	1.4 (0.6, 3.4)	1.0	0.8 (0.3, 2.0)	1.0
EPO	+	1.6 (0.7, 3.8)	0.8 (0.2, 2.8)	1.3 (0.5, 3.5)	1.5 (0.5, 4.0)	0.9 (0.2, 3.0)	1.4 (0.6, 3.3)
	–	1.1 (0.4, 3.0)	1.0	2.4 (0.99, 6.0)	1.0	0.8 (0.3, 1.0)	1.0
VEGF	+	2.4 (0.9, 6.3)	2.5 (1.2, 5.1)	2.5 (0.96, 6.6)	2.6 (1.2, 5.3)	1.4 (0.5, 4.2)	2.7 (1.4, 5.3)
	–	1.3 (0.5, 3.4)	1.0	1.8 (0.7, 4.8)	1.0	0.7 (0.2, 2.2)	1.0
VEGF-R1	+	0.4 (0.05, 2.7)	0.6 (0.2, 1.9)	0.8 (0.2, 3.4)	0.5 (0.1, 1.6)	–	0.7 (0.3, 2.0)
	–	1.7 (0.8, 3.5)	1.0	1.9 (0.9, 4.2)	1.0	1.0 (0.5, 2.3)	1.0
VEGF-R2	+	1.3 (0.4, 3.8)	1.4 (0.6, 3.1)	0.7 (0.2, 3.1)	1.9 (0.9, 4.0)	1.1 (0.5, 2.6)	1.2 (0.5, 3.1)
	–	1.6 (0.7, 3.7)	1.0	3.0 (1.4, 6.8)	1.0	0.3 (0.03, 2.0)	1.0
NT-4	+	1.7 (0.5, 6.0)	1.7 (0.8, 3.9)	–	2.5 (1.2, 5.3)	2.3 (0.6, 8.8)	1.3 (0.6, 2.8)
	–	1.5 (0.7, 3.3)	1.0	2.7 (1.3, 5.7)	1.0	0.6 (0.2, 1.6)	1.0
BDNF	+	1.9 (0.7, 5.0)	1.2 (0.5, 2.9)	2.1 (0.8, 6.1)	1.3 (0.6, 3.1)	1.5 (0.5, 4.7)	1.3 (0.6, 2.8)
	–	1.1 (0.4, 2.8)	1.0	1.6 (0.7, 3.9)	1.0	0.6 (0.2, 1.6)	1.0
bFGF	+	0.7 (0.1, 3.3)	1.0 (0.4, 2.8)	0.9 (0.2, 4.1)	1.0 (0.4, 2.6)	0.4 (0.1, 3.4)	0.9 (0.4, 2.4)
	–	1.6 (0.8, 3.5)	1.0	2.0 (0.9, 4.4)	1.0	0. (0.4, 2.3)	1.0
IGF-1	+	1.5 (0.4, 5.4)	0.7 (0.3, 1.8)	2.7 (0.7, 10)	0.6 (0.2, 1.6)	–	1.0 (0.4, 2.2)
	–	1.2 (0.5, 2.7)	1.0	1.4 (0.6, 3.0)	1.0	0.9 (0.4, 2.1)	1.0
PIGF	+	2.4 (0.8, 7.5)	1.6 (0.7, 3.8)	1.8 (0.4, 8.3)	1.2 (0.9, 4.6)	0.8 (0.1, 6.3)	1.9 (0.9, 4.2)
	–	1.2 (0.5, 2.8)	1.0	2.0 (0.9, 4.3)	1.0	0.9 (0.4, 2.1)	1.0
Ang-1	+	1.7 (0.6, 4.6)	0.7 (0.3, 1.8)	0.8 (0.2, 3.6)	1.3 (0.5, 2.9)	1.3 (0.4, 4.0)	0.7 (0.3, 1.8)
	–	1.1 (0.5, 2.6)	1.0	2.3 (1.1, 5.2)	1.0	0.5 (0.2, 1.5)	1.0
Ang-2	+	0.6 (0.2, 3.9)	2.0 (0.4, 2.7)	0.7 (0.2, 3.2)	1.0 (0.4, 2.6)	0.8 (0.2, 2.7)	0.7 (0.2, 2.0)
	–	1.7 (0.8, 3.7)	1.0	2.3 (1.1, 5.0)	1.0	0.7 (0.3, 1.9)	1.0

Bolded items are statistically significant at $p < .05$

+ indicates concentrations in the top quartile

– indicates concentrations below the top quartile

Children who consistently had concentrations of both proteins below the top quartile (–/–) comprise the referent group (odds ratio = 1.0)

Adjustment is made for gestational age 23–24 and 25–26 weeks and for birth weight Z-score < -1

inflammation-associated risk. It is this hope that has prompted the recommendation that growth factors (with neurotrophic properties) be considered therapeutic (Hassell et al. 2015; Larphaveesarp et al. 2015).

Definition of ADHD Symptoms

The CSI-4 does not provide DSM-IV diagnoses. Rather it identifies children as screening positive for the inattentive and/or hyperactive/impulsive domains. Because of poor

agreement between parent and teacher on these domains, and in light of concern about the validity (Bernfeld 2012), and the stability of subtypes (Lahey et al. 2005; Valo and Tannock 2010; Willcutt et al. 2012; Srebnicki et al. 2013), we chose to view ADHD symptoms as “an important unitary component to ADHD symptoms” (Toplak et al. 2009).

We acknowledge that the CSI-4 is not routinely used to assess the existence of ADHD symptoms (Chang et al. 2016). On the other hand, it has the advantage of providing information about other disorders that can influence

manifestations of ADHD, or pose diagnostic problems (DeVincent and Gadow 2009; Ondrejka et al. 2010; Nazari et al. 2016).

Observers

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) requires “several inattentive or hyperactive-impulsive symptoms ... in two or more settings” (American PA 2013), while The American Academy of Pediatrics (AAP) clinical practice ADHD guideline recommends that reports of behaviors be collected from a classroom teacher and from parents (American Academy of Pediatrics 2000).

The different profiles of high concentrations of proteins associated with parent-identified ADHD symptoms and teacher-identified symptoms adds to the literature about how teachers differ from parents in the behaviors they identify under the rubric of attention-related symptoms (Dirks et al. 2012). Having agreement between teacher and parent, however, did not increase the ability to detect an association between postnatal inflammation and ADHD symptoms.

Age 2 or Age 10; CBCL or CSI-4; Parent or Teacher

At age 2 years, an attention problem was defined on the basis of a Child Behavior Checklist (CBCL) completed by the parent or caregiver (O’Shea et al. 2014). Then the inflammation signal was comprised of top quartile concentrations on 2 of the 3 sampling days (days 1, 7, and 14) of MPO, IL-6, IL-8, ICAM-3, VEGF-R1, and VEGF-R2.

We do not view a parent-completed CBCL-defined attention problem at age 2 years as equivalent to one defined by a teacher-completed CSI-4. Three differences need to be emphasized, the age at assessment, the assessor, and the instrument.

The Importance of Context (or the Limits of Univariable Analyses)

RANTES, VEGF-R1, NT-4, BDNF, bFGF, PIGF, Ang-1 and Ang-2 are not identified in Table 2 as reducers of the risk of teacher-identified ADHD symptoms. In Table 3, however, each of these proteins displays a neurotrophic effect when the concentration of IL-8 is in the top quartile. Similarly, IL-8 is not identified in Table 2 as associated with increased risk of teacher-identified ADHD symptoms. In essence, evaluating the risk is limited when we have no sense of the context that might modify the risk. We strongly encourage that context be considered.

VEGF

The neurotrophic and angiogenic capabilities of VEGF and similar proteins are so interrelated that the term “angioneurin” was created to emphasize these dual sets of capabilities (Zacchigna et al. 2008). VEGF (Raab et al. 2004) and VEGFR-2 (Flk-1)(Jin et al. 2002; Sun et al. 2003) appear to be involved in neurogenesis, as well as repair of the brain after damage (Sun et al. 2003; Madri 2009). Of the multiple VEGF receptors found in CNS tissue, VEGF-2 appears to mediate almost all of the known cellular responses to VEGF. VEGF-R1 (Flt-1) is not normally expressed on mature neurons, but can be strongly upregulated after nerve crush injury (Islamov et al. 2004), and even transient CNS ischemia (Jin et al. 2001; Choi et al. 2007), suggesting a role in brain injury, and or repair.

Despite its neuroprotective effects, VEGF can exacerbate brain damage (Ma et al. 2012). Some of this exacerbation has been attributed to “the promotion of blood-brain barrier breakdown that inevitably accompanies vascular growth.” If this is the case, VEGF and VEGF-R2 can be seen not as primary agents of brain damage, but as contributors to secondary brain damage.

BDNF

Of all the neurotrophic proteins we evaluated, BDNF appears to have been the one most often considered a candidate for a role in the occurrence of ADHD (Shim et al. 2008; Scassellati et al. 2014; Liu et al. 2015). And yet, the data are conflicting, with some studies reporting higher peripheral concentrations (Yeom et al. 2016), and others failing to find any association (Scassellati et al. 2014; Zeni et al. 2016). Gene studies have also failed to produce a coherent relationship between BDNF-related genes and the risk of ADHD (Cho et al. 2010; Zeni et al. 2016).

We found only one instance (Table 3: Teacher-identified ADHD symptoms and early epoch protein concentrations with IL-8) among 9 opportunities for top-quartile BDNF concentrations to show a beneficial effect on the risk of ADHD symptoms, even when other proteins with neurotrophic capabilities did (e.g., NT-4, IGF-1, PIGF, as well as VEGF-R2, Ang-1, and Ang-2). We do not equate our assessment of BDNF concentrations shortly after birth with studies during childhood. Nevertheless, our failure to find a convincing relationship between top-quartile BDNF concentrations and ADHD symptoms can be viewed as adding support to the “no-association” side of the balance.

ICAM-1

On occasion, we have found that high concentrations of a protein thought to be capable of damaging the brain are not so identified when the concentrations of neurotrophic proteins

are not known, but are so identified when the concentrations of neurotrophic proteins are known to be low. Because elevated concentrations of ICAM-1 have been associated with indicators of brain damage in our cohort (Leviton et al. 2011a; O'Shea et al. 2013; Leviton et al. 2016a), we reasoned that since ADHD does have brain structural correlates (Rubia et al. 2014), perhaps ICAM-1, which was not associated with increased risk of ADHD symptoms on univariable analyses, would be identified when evaluated in light of the concentrations of neurotrophins. We confirm that even under such scrutiny, high concentrations of ICAM-1 are not consistently or prominently associated with increased risk of teacher-identified ADHD symptoms.

Synthesis

Although we evaluated several individual proteins, we prefer to view each inflammation-related protein as a surrogate for many other proteins in its class (Zhao et al. 2016), and each neurotrophic protein as a surrogate for the class of proteins that possess neurotrophic characteristics (Oliveira et al. 2013). In addition, the more “mature” the newborn (along the developmental regulation schedule), the more likely s/he should be able to resolve inflammation, and minimize/repair damage (Clapp 2006; Zasada et al. 2014; van Tilborg et al. 2016).

The maturation level is likely to influence the synthesis of many proteins, even if not all to the same extent. Consequently, we urge caution in drawing inferences about any individual protein, and instead encourage seeing our data as indicating the need to evaluate multiple proteins in light of biomarkers of biologic processes that might moderate their effects.

Strengths and Limitations

Our analyses had sufficient power to detect as statistically significant odds ratios as low as 1.7 attests to our power, at least for the association of early epoch protein profiles with teacher identified ADHD symptoms. Other strengths include modest attrition, and protein measurements of high quality (Fichorova et al. 2008), and high content validity (Fichorova et al. 2011; Hecht et al. 2011; Leviton et al. 2011b; McElrath et al. 2011).

We measured a limited number of the many proteins involved in inflammation, a broad and complex phenomenon (Zak and Aderem 2009; Xiao et al. 2011). We are also limited by the small number of proteins measured, and inadequate power for assessments of ADHD symptoms by both parent and teacher.

We relied on blood specimens obtained for clinical indications. As they became more stable, healthier and more mature, infants were less likely than their sicker peers to have blood drawn on days 14, 21, and 28. Consequently, statistical power decreased and selection bias probably occurred.

Our bivariate orientation of two proteins simultaneously assumes that everything else is unimportant. Obviously, that is a severe limitation. The child classified as not exposed to a high concentrations of the inflammation-associated protein identified at the top of the column may very well be exposed to high concentrations of another, potentially very important, brain-damaging protein we did not measure. We acknowledge the complexity of inflammation and our inability to provide a useful measure of the severity of the (potentially damaging) exposure to inflammation.

We have limited our vision to the first postnatal month. In the ELGAN cohort, newborns whose placenta was inflamed were more likely to have systemic inflammation, but only on the first postnatal day. (Hecht et al. 2011) Similarly, disorders associated with placenta insufficiency were associated with low concentrations of growth factors in the newborn's blood during the first postnatal day only (unpublished observations). Thus, the effects of inflammation and neurotrophic capabilities associated with maternal-fetal interactions might have had influences we could not identify (Hsiao and Patterson 2012; Instanes et al. 2017).

Conclusions

In this high-risk cohort of children born extremely preterm, systemic inflammation is associated with increased risk of ADHD symptoms, while high concentrations of proteins with neurotrophic properties appear able to modulate this increased risk.

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Compliance with Ethical Standards

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