Published in final edited form as:

J Autism Dev Disord. 2019 January ; 49(1): 173–184. doi:10.1007/s10803-018-3688-5.

Cord and Early Childhood Plasma Adiponectin Levels and Autism Risk: A Prospective Birth Cohort Study

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Conflict of interest: None of the authors have a conflict of interest pertaining to this work.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Abstract

Emerging research suggests that adiponectin, a cytokine produced by adipose tissue, may be implicated in ASD. In this prospective birth cohort study (n=847), we assessed the association between cord, early childhood plasma adiponectin and the risk of developing ASD. ASD was defined based on ICD codes of physician diagnosis. Cord adiponectin levels were inversely associated with ASD risk (aOR: 0.50; 95% CI: 0.33, 0.77), independent of preterm birth, early childhood adiponectin and other known ASD risk factors. Early childhood adiponectin, assessed prior to ASD diagnosis, was associated with lower risk of ASD, which attenuated after adjusting for cord adiponectin, indicating the relative importance of cord adiponectin in ASD risk. Further research is warranted to confirm our findings and elucidate biological mechanisms.

Keywords

Autism; adiponectin; preterm birth; cytokines

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by impairments in social interaction and communication, and by the presence of restrictive, repetitive behaviors and interests (American Psychiatric Association 2013). Recent epidemiological studies suggest that one in 59 children are diagnosed with ASD (Baio et al. 2018). While genetic, environmental, and prenatal/perinatal factors have been implicated in ASD (Fiorentino et al. 2016; Wozniak et al. 2017), the etiology of the disorder remains largely unknown and no convincing biomarkers of ASD have been identified (Molloy et al. 2006; Lyall et al. 2017; Akintunde et al. 2015). Disturbances in immunoinflammatory factors and adipocytokines have been observed in subjects with ASD (Molloy et al. 2006; Ghaffari et al. 2016). Aberrant immune activity during vulnerable periods of neurodevelopment could potentially play a role in neural dysfunction associated with ASD (Ashwood et al. 2006). Emerging evidence suggests that immune dysfunction and neuroinflammation may be a common mechanism across the individual ASD risk factors (Erdei and Dammann 2014).

Beyond its energy storing capabilities, adipose tissue has emerged as an endocrine organ that orchestrates inflammatory response through the production of adipocytokines (Hansen-Pupp et al. 2015; Mazaki-Tovi et al. 2009; Villarreal-Molina and Antuna-Puente 2012). The family of adipocytokines includes a variety of highly active molecules including interleukin

(IL)-6, tumor necrosis factor (TNF)- α , leptin, adiponectin, resistin and vistafin, all of which are known to play a critical role in the regulation of inflammatory responses (Hansen-Pupp et al. 2015; Mazaki-Tovi et al. 2009). Adiponectin is the most abundant adipocytokine, mainly produced by brown and white adipose tissues (Hansen-Pupp et al. 2015).

The role of adiponectin in ASD deserves attention for at least two important reasons. First, as an anti-inflammatory cytokine, adiponectin functions as a mediator of inflammatory response and has a protective role against metabolic disturbances (Mazaki-Tovi et al. 2009; Lenz and Diamond 2012; Fujita-Shimizu et al. 2010). Emerging evidence suggests that adiponectin may be implicated in neurological conditions (Lisik et al. 2016; Mansur et al. 2016; Hu et al. 2015) (Burd et al. 2012). To our knowledge, three studies have assessed adiponectin levels in children with ASD (Fujita-Shimizu et al. 2010; Blardi et al. 2010; Rodrigues et al. 2014), of which only one found altered adiponectin levels in subjects with ASD (Fujita-Shimizu et al. 2010). The second study observed no significant difference in adiponectin levels between the ASD subjects and controls (Blardi et al. 2010). Similarly, Rodrigues et al., noted unaltered adiponectin levels; however, they reported a negative correlation between adiponectin levels and severity of symptoms (Rodrigues et al. 2014). In addition to these inconsistent findings, limited sample size, lack of adjustment of numerous confounders, and cross-sectional study designs have precluded elucidation of the role of adiponectin in the development of ASD.

Second, preterm birth has become an established risk factor for ASD (Darcy-Mahoney et al. 2016) (Schendel and Bhasin 2008; Kuzniewicz et al. 2014; Movsas and Paneth 2012; Fezer et al. 2017; Angelidou et al. 2012). Lower serum adiponectin levels are noted in mothers who had preterm labor and delivery (Mazaki-Tovi et al. 2009) as well as in children born preterm when compared to their term counterparts (Saito et al. 2011; Oberthuer et al. 2012; Kajantie et al. 2004; Lindsay et al. 2003; Nakano et al. 2013; Terrazzan et al. 2014; Yoshida et al. 2009; Siahianidou et al. 2007). While the role of adiponectin in prematurity is well characterized (Saito et al. 2011; Oberthuer et al. 2012; Kajantie et al. 2004; Lindsay et al. 2003; Nakano et al. 2013; Terrazzan et al. 2014; Yoshida et al. 2009; Siahianidou et al. 2007), existing research has not jointly looked at adiponectin and gestational age at birth in the context of ASD risk.

To fill these gaps, we conducted a study to longitudinally assess the association between plasma adiponectin, measured in cord blood at birth and early childhood venous blood, and subsequent ASD risk. In addition, we set out to understand whether preterm birth and cord adiponectin had joint effects on ASD risk. We sought to clarify these questions by analyzing longitudinal data from the Boston Birth Cohort (BBC), a predominantly urban low-income minority birth cohort, enriched with preterm births.

Methods

Participants and data collection procedure

This study included 847 mother-child pairs from the BBC, of which 792 children were considered neurotypical and 55 received an ASD diagnosis. Online Resource Figure 1 outlines the enrollment, postnatal follow-up, inclusions and exclusions. Between 1998 and

2009, mothers who delivered a singleton live birth at the Boston Medical Center (BMC), were invited to participate in this study. After obtaining informed consent, mothers were interviewed 24–72 hours after delivery using a standardized postpartum questionnaire. Exclusion criteria for the initial enrollment were multiple-gestation pregnancies, chromosomal abnormalities, major birth defects and preterm deliveries as a result of maternal trauma. Children that continued to receive pediatric care at the BMC were included in this study, and they were followed-up until 2015 (Online Resource Figure 1). The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center.

Exposure measures

Plasma adiponectin in the children was measured at two time points: (1) Umbilical cord blood sample, collected at delivery, and (2) early childhood venous blood, collected during a pediatric visit (median time of measurement: 19.03 months; Interquartile range: 10.4–49.2 months). Plasma samples were stored in a freezer at -80°C . Adiponectin was measured using an immunoassay (ELISA) and had an inter-assay variation of $<5.8\%$ (G. Wang et al. 2016a). The assays were run according to the manufacturer's recommendation. Children that had adiponectin assessed after ASD diagnosis were excluded from the analysis. Unlikely adiponectin levels, defined as greater than 3SD above the mean, were observed in 15 and 18 subjects for cord and early childhood adiponectin, respectively and were reassigned a value of 3 SD.

Gestational age at birth was characterized based on the first day of the last menstrual period data and early ultrasound data (X. Wang et al. 2002; G. Wang et al. 2014). Children with gestational age ≥ 37 completed weeks of gestation were categorized as full-term and those <37 weeks were grouped into late- (≥ 34 to <37 weeks) and early-preterm (<34 weeks).

Outcome measure

Electronic Medical Records (EMR) data were used to identify children with ASD. Children were classified as an ASD case if they were ever diagnosed with autism (ICD-9 code 299.00), Asperger syndrome (299.80) and/or pervasive developmental disorder not otherwise specified (299.90). Children that were never diagnosed with ASD, ADHD (314.0 – 314.9), intellectual disabilities (317 – 319) or other developmental disabilities (315.0 – 315.9) were classified as 'neurotypical.' When children with ASD also had ADHD, intellectual disabilities, or other developmental disabilities, they were still categorized as ASD. Two separate sensitivity analyses were performed using the following criteria: 1) a stringent ASD definition that included only those that were diagnosed with ASD on two separate occasions, and had at least one visit with a specialist such as a developmental behavioral pediatrician, pediatric neurologist or child psychologist; and 2) a stringent definition for neurotypical children which excluded those with competing diagnoses such as ADHD (314.0 – 314.9), intellectual disabilities (317 – 319), other developmental disabilities (315.0 – 315.9), Conduct Disorder (312.0 – 312.9), emotional disturbances of childhood or adolescence including Oppositional Defiant Disorder (313.0 – 313.9) and Congenital Anomalies (740 – 759.9).

Covariates

Based on the existing literature and our earlier work in the BBC, we selected the covariates for adjustment *a priori* (G. Wang et al. 2016a; G. Wang et al. 2014; Raghavan et al. 2017; Li et al. 2016), including maternal pre-pregnancy BMI, maternal diabetes status, maternal race/ethnicity, maternal age at the time of delivery, smoking during pregnancy (ever smoked 3 months before pregnancy/during pregnancy vs. not smoked during preconception/pregnancy), parity (not including the index pregnancy), maternal education (high school or less vs. some college or more), child's sex (female vs. male), year of baby's birth (1998–2006 vs. 2007–2013), mode of feeding (formula only, both formula and breast feeding and breastfeeding only), age at which early childhood blood was drawn and follow up time for each participant. Maternal pre-pregnancy weight and height were collected using a standardized questionnaire 2–3 days after delivery, which was used to calculate maternal BMI, defined as weight in kilograms divided by height in meters squared. Mother's diabetes status was categorized into the following groups: 1) normal (without a pregestational or gestational diabetes diagnosis); 2) gestational diabetes (ever diagnosed with diabetes mellitus complicating pregnancy); and 3) pregestational diabetes (ever diagnosed with diabetes). Race/ethnicity was categorized into black, white, Hispanic and Other. Plasma insulin and leptin, in cord blood and in venous blood during childhood, were measured using a sandwich immunoassay and the interassay coefficient of variation was 4.0% and 4.5%, respectively (G. Wang et al. 2016b). Adiponectin/leptin ratio was calculated and was log-transformed. Based on the WHO reference values, weight-for-age *z*-score was used to calculate first year weight gain and was categorized into slow (weight gain *z*-score <−0.67), on track (−0.67 to 0.67), rapid (>0.67 to 1.28), and extremely rapid (>1.28) (G. Wang et al. 2016b).

Statistical Analyses

Distributional assumptions such as normality of cord and plasma adiponectin levels were assessed using the Shapiro-Wilk test. Since adiponectin distributions were skewed, they were log-transformed for both cord and early childhood adiponectin levels (Online Resource Figure 2). Preliminary data analysis was conducted to compare neurotypical and ASD children using a chi-squared test for categorical variables and ANOVA for continuous variables. Logistic regression was used to assess the relationship between log-transformed cord and early childhood plasma adiponectin levels (independent variables) and ASD (dependent variable), with adjustment for potential confounders listed above. The results are presented as odds ratio. Baron and Kenny mediation analysis was used to explore the relationships between preterm birth, cord adiponectin and ASD risk (Baron and Kenny 1986). The Baron and Kenny approach assessed the indirect association between the exposure (i.e. preterm birth) and outcome (i.e. ASD status), through the mediating variable (i.e. cord adiponectin), by comparing four regression models. The first model assessed the total association between the primary exposure and primary outcome. The second model assessed the association between the primary exposure and potential mediator. The third model assessed the association between the potential mediator and primary outcome. Finally, the fourth model assessed the association between the primary exposure and primary outcome, after accounting for the potential mediator. All 4 models adjusted for the confounders listed above. All statistical analyses were performed using STATA version 13.0

(StataCorp, College Station, TX). Differences were considered statistically significant if the *p* value was <0.05.

Results

The demographic and clinical characteristics of mothers and children in this study are presented in the Table 1, and have also been documented in earlier studies from this cohort (Li et al. 2016; Raghavan et al. 2017; Brucato et al. 2017; G. Wang et al. 2014; G. Wang et al. 2016a). We found that risk factors such as advanced maternal age, maternal diabetes, male sex and preterm birth were more common among children with ASD. In addition, ASD children were more likely to have lower birth weight and lower cord adiponectin levels (Table 1). Mean and median cord adiponectin levels were 15.54 µg/mL and 13.52 µg/mL (interquartile range: 8.76–19.21 µg/mL), and mean and median early childhood adiponectin levels were 10.61 µg/mL and 9.68 µg/mL (interquartile range: 6.75–13.5 µg/mL), respectively. Mean cord adiponectin levels were significantly different across groups defined by gestational age at birth, with early preterm children (8.29 µg/mL) having the lowest levels, followed by late preterm (11.51 µg/mL) and term infants (16.90 µg/mL) (*p* value <0.001). Consistent with other studies (Lenz and Diamond 2012; Mantzoros et al. 2009; Nakano et al. 2013; Yeung et al. 2015; Martos-Moreno et al. 2009; Hellgren et al. 2015), we observed that cord adiponectin was associated with the duration of gestation (Online Resource Table 1). Figure 1 shows the distribution of cord adiponectin by preterm status. As observed in panel A, the cord adiponectin distribution is shifted to the left for early preterm (<34 weeks) and late preterm (34 to <37 weeks) babies, when compared to term infants. However, there were no differences in the distribution of early childhood adiponectin levels based on gestational age at birth (Figure 1, panel B). Cord adiponectin levels were also correlated with birth weight. Children with lower cord adiponectin levels were likely to have rapid or extremely rapid weight gain (Online Resource Table 2), but the association attenuated after adjusting for gestational age at birth (data not shown). Correlation between cord and early childhood adiponectin was weak (*r*=0.18), although children whose cord adiponectin were in low, medium or high tertiles likely continued to stay in their respective categories at early childhood (Online Resource Figure 3).

Adiponectin and ASD risk

A total of 674 children, including 634 neurotypical and 40 ASD children, were part of this analysis. Figure 2 shows the distribution of cord and early childhood adiponectin levels by ASD status. The mean cord adiponectin level is reduced among children later diagnosed with ASD (Table 1) and this is evident by the distinct lower cord adiponectin distribution observed in Figure 2, panel A. On the other hand, there was no difference in the mean early childhood adiponectin levels between neurotypical and ASD children (Figure 2, panel B).

Table 2 presents the association between cord adiponectin and subsequent ASD risk. Higher cord adiponectin levels were associated with a lower risk of ASD (OR: 0.51; 95% CI: 0.35, 0.75). When cord adiponectin levels were categorized into quartiles, an inverse dose-response relationship was observed. Compared to children with the lowest cord adiponectin levels (quartile 1), the highest cord adiponectin (OR_{quartile 4}: 0.23, 95% CI: 0.08, 0.62) was

associated with reduced risk of ASD in unadjusted analyses. This association persisted after adjustment for potential confounders (including child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, maternal diabetes status and follow-up time), ($aOR_{\text{quartile 4}}$: 0.14, 95% CI: 0.04, 0.46; model 1). Sequentially adjusting for gestational age, early childhood adiponectin (Table 2) and additional covariates such as cord insulin, cord leptin and weight gain during first year of life (Online Resource Table 3) did not alter the association between cord adiponectin and ASD. This finding was robust irrespective of how adiponectin was categorized (tertiles, quintiles, extreme levels) (Online Resource Table 4).

A total of 638 children had data on early childhood adiponectin, of which 36 were subsequently diagnosed with ASD. As seen in Online Resource Figure 4, there was no difference in the timing of adiponectin measurement between neurotypical children and those that had ASD. In an unadjusted model, early childhood adiponectin was not associated with ASD risk (OR: 0.71; 95% CI: 0.45, 1.13) (Table 3). After adjusting for potential confounders (including maternal age, diabetes status, pre-pregnancy BMI, sex, race, preterm birth, age of measurement, follow-up time and breastfeeding status; model 1), higher early childhood adiponectin levels, modeled as a continuous variable, was inversely associated with the risk of ASD (aOR : 0.54; 95% CI: 0.33, 0.90). When each of the covariates were added stepwise to the model, age of biomarker measurement turned the association from non-significant to significant (data not shown). This is likely because the age distribution is younger in ASD children compared to neurotypical children.

Similar to cord adiponectin, when early childhood adiponectin was categorized as quartiles, those with the highest levels (quartile 4) were associated with the lowest ASD risk ($aOR_{\text{quartile 4}}$: 0.30; 95% CI: 0.09, 0.98). The association approached significance when adjusted for gestational age at birth (model 2). However, after adjusting for cord adiponectin levels (model 3), the association between early childhood adiponectin and ASD was no longer significant. We repeated the analysis by adjusting for additional covariates such as early childhood insulin, early childhood leptin and weight gain during first year of life (Online Resource Table 5) and categorizing early childhood adiponectin as tertiles, quintiles and extreme values (Online Resource Table 6), and observed similar findings.

Sensitivity analyses using stringent ASD case and neurotypical definitions yielded consistent and stronger associations for both cord and early childhood adiponectin (Online Resource Tables 7–10). Repeating the analysis using ASD subjects that were diagnosed >2 years did not alter the findings.

Since leptin is linked to ASD in several studies (Ashwood et al. 2008; Blardi et al. 2010; Rodrigues et al. 2014; Al-Zaid et al. 2014; Essa M.M. et al. 2011), including ours, and adiponectin-leptin ratio is considered as a marker of metabolic syndrome (Inoue et al. 2005), we assessed the association between log-transformed adiponectin-leptin ratio and subsequent ASD risk. We conducted this analysis for both cord and early childhood ratios (Online Resource Tables 11–12). While the associations were statistically significant, the ratio did not predict ASD risk beyond the independent effects of leptin and/or adiponectin.

Next, the joint effects of preterm birth and adiponectin levels on the risk of ASD were assessed. Children that had term birth (> 37 completed weeks of gestation) and high cord adiponectin levels (defined as > 50th percentile) were the reference group (Table 4). After adjusting for confounders, the risk of ASD was not different between preterm (<37 completed weeks of gestation) and term children, as long as they had high cord adiponectin. ASD risk was elevated among term children with low adiponectin (defined as < 50th percentile) in both unadjusted and adjusted models (aOR: 3.06; 95% CI: 1.27, 7.40). Among preterm children with low adiponectin, the ASD risk was significantly higher in the unadjusted model and approached statistical significance, after adjusting for confounders (aOR: 2.62; 95% CI: 0.99, 6.99).

Mediation analysis: An exploration—Since the temporal ordering of preterm birth and altered adiponectin levels is unclear (Mazaki-Tovi et al. 2009), mediation analysis was explored in two ways: 1) Cord adiponectin as a mediator in the relationship between preterm birth (independent variable) and ASD risk (dependent variable); and 2) Preterm birth as a mediator in the relationship between cord adiponectin (independent variable) and ASD risk (dependent variable). As seen in Online Resource Table 13, early preterm (independent variable) was associated with ASD risk (dependent variable) (step 1), both early and late preterm birth was associated with adiponectin (mediator) (step 2), and cord adiponectin was associated with ASD risk (step 3). In the last regression model (step 4), the association attenuated suggesting that cord adiponectin mediated the relationship between early preterm birth and ASD risk (Online Resource Table 13). In the second mediation analysis, preterm birth was considered as mediator in the association between cord adiponectin levels and ASD. Here, cord adiponectin was associated with ASD risk (step 1), preterm birth was associated with cord adiponectin (step 2) and early preterm birth was associated with ASD risk (step 3). In the regression model, adjusting for preterm birth did not alter the association between cord adiponectin and ASD risk, suggesting that there was no mediation (Online Resource Table 14). The role of early childhood adiponectin mediating the association between preterm birth and ASD risk is presented in Online Resource Table 15.

Discussion

To our knowledge, this is the first study to prospectively assess adiponectin levels in cord blood (a proxy of fetal adiponectin) and in early childhood venous blood in relation to ASD risk. We find cord blood adiponectin levels to be inversely associated with the risk of ASD. In comparison, the association between early childhood adiponectin and ASD was less robust and was further weakened after adjustment of cord adiponectin. These associations remained consistent after additional analyses, including sequential adjustment for potential confounders and other metabolic biomarkers (such as leptin, insulin) and after more stringent ASD and neurotypical classifications of outcome.

Our findings are in line with the observations of Fujita-Shimizu et al. who noted that adiponectin levels are lower in subjects with ASD (Fujita-Shimizu et al. 2010). Specifically, they reported a negative correlation between adiponectin and social development in children, characterized by the Autism Diagnostic Interview Revised (Fujita-Shimizu et al. 2010). Consistent with these findings, Rodrigues et al., noted that adiponectin levels were inversely

correlated with the severity of autism symptoms (Rodrigues et al. 2014). Lisik et al. showed that adiponectin levels were lower in those with Fragile X Syndrome (Lisik et al. 2016). These findings suggest that adiponectin may have a larger role to play in neurodevelopmental processes, rather than merely regulate energy expenditure or serve as a biomarker for the onset of metabolic syndrome (Inami et al. 2007; Blardi et al. 2009).

Adiponectin is detected in cord serum as early as 24 weeks of gestation, after which the concentration rises 20-fold until term (Kajantie et al. 2004). This increase during third trimester mirrors the increase in adiposity (Lenz and Diamond 2012) and is in stark contrast with children and adults in whom adiponectin concentration is inversely associated with body fat percentage (Kajantie et al. 2004; Siahianidou et al. 2007). Cord adiponectin does not correlate with maternal adiponectin suggesting the fetal origins of cord adiponectin (Lenz and Diamond 2012; Saito et al. 2011; Brochu-Gaudreau et al. 2010; Dawczynski et al. 2014; Mazaki-Tovi et al. 2007). In the fetus, adiponectin is secreted by muscle and vascular cells, in addition to adipocytes (Z. Q. Zhang et al. 2016).

While cord adiponectin is associated with ASD, it is intriguing that early childhood adiponectin demonstrates a less robust association. Adiponectin expression and its relationship to growth parameters and fat distribution seem to evolve temporally, from birth to childhood (Meyer et al. 2017; Kotani et al. 2004; Inami et al. 2007). At birth, adiponectin is positively correlated with growth parameters and fat mass (Kotani et al. 2004; Tsai et al. 2004; Pardo et al. 2004; Weyermann et al. 2006; Inami et al. 2007). Subsequent to this initial time window, there is possibly a shift in this relationship noting an inverse association between adiponectin and fat mass (Kotani et al. 2004; Stefan et al. 2002; Inami et al. 2007; Mantzoros et al. 2009). Taken together, these findings suggest that the role of adiponectin *in-utero* may be fundamentally different than early childhood adiponectin (Meyer et al. 2017; Mantzoros et al. 2009). The discrepancy between adiponectin at different time points has been hypothesized to the differential origination of adiponectin, variation in fat storage, secretion and modulation of adiponectin (Z. Q. Zhang et al. 2016; Mantzoros et al. 2009). While the evidence discussed here is in the context of obesity, adiponectin's effect may not be different for neurodevelopmental outcomes such as ASD.

Adiponectin has been previously shown to be altered in preterm babies, however, for the first time we jointly assessed adiponectin and preterm birth in the context of ASD and showed that lower adiponectin levels could increase ASD risk, irrespective of the gestational age. As a next step, we conducted a preliminary mediation analysis. Given the uncertainty in the biological temporal relationship between preterm birth and cord adiponectin (meaning whether preterm birth alters adiponectin levels, or changes in adiponectin triggers preterm birth) (Mazaki-Tovi et al. 2009), our analysis assessed both as mediators. In this exploratory analysis, cord adiponectin mediated the relationship between preterm birth and ASD; whereas, preterm birth did not seem to mediate the association between cord adiponectin and ASD. These findings are preliminary and suggestive of a potential mediating role of adiponectin in relationship between preterm birth and ASD. More research in animal models and human studies is warranted to further clarify this relationship.

Biological plausibility

Several lines of evidence support the biological plausibility of adiponectin's role in ASD. Adiponectin suppresses the macrophage production of pro-inflammatory cytokines such as TNF- α , IL-6 (Brochu-Gaudreau et al. 2010), Interferon (IFN)- γ (Mazaki-Tovi et al. 2009), which are noted to be elevated in children with ASD (Chez et al. 2007; Wei et al. 2013; Ashwood et al. 2011). Consistent findings suggest that IL-6 was significantly increased in the anterior cingulate gyrus, frontal cortices and cerebellum (Wei et al. 2013) and TNF was increased almost 50 times in the cerebrospinal fluid (Angelidou et al. 2012). Similarly, IFN- γ is elevated in the brain of ASD subjects, when compared to controls (Wei et al. 2013). Adiponectin signaling inhibits NF- κ B, an essential transcription factor that mediates and regulates inflammatory and stress-related protein expression (Villarreal-Molina and Antuna-Puente 2012), which is aberrantly expressed in subjects with ASD (Young et al. 2011).

Beyond its role in influencing other cytokines, emerging evidence suggests that adiponectin may be implicated in brain functions (Thundyil et al. 2012). Adiponectin enters the brain from circulation and directly targets neurons (Ng and Chan 2017; Thundyil et al. 2012; D. Zhang et al. 2016; Zhang et al. 2017; Song et al. 2015). Its receptors are widely expressed in the dentate gyrus of hippocampus (D. Zhang et al. 2016), hypothalamus, cortex and pituitary glands (Thundyil et al. 2012; Brochu-Gaudreau et al. 2010). Given this broad distribution of adiponectin receptors in different brain regions, adiponectin's role may be broader than the regulation of metabolic homeostasis and could play an important role in the 'adipocyte-brain cross-talk' (Thundyil et al. 2012). Adiponectin is involved in neurogenesis, dendritic spine remodeling, hippocampal neural stem cell proliferation and dentate gyrus neuronal excitability (Zhang et al. 2017; Ng and Chan 2017; Song et al. 2015; D. Zhang et al. 2016). It also promotes adaptive neuroplasticity and may possess cerebra-protective role, likely mediated through the eNOS signal pathway (Thundyil et al. 2012; Brochu-Gaudreau et al. 2010; Machado-Vieira et al. 2017). Abnormal development of dentate gyrus of hippocampus, an important center for learning and memory, is likely implicated in the pathophysiology of ASD (Ito et al. 2017). Adiponectin may also possess higher brain functions. For example, diminished adiponectin levels are associated with clinically significant affective episodes and subjects with major depressive symptoms are known to have lower adiponectin levels (Machado-Vieira et al. 2017). Adiponectin knockout mice have exhibited depressive-like behavior (Ng and Chan 2017). Further, hypoadiponectinemia is thought to increase sympathetic nervous system activity – which is observed in depression (Lehto et al. 2010).

Limitations and strengths

There are limitations to highlight. First, only total adiponectin and not the distinct forms such as low-molecular weight trimers, medium-molecular-weight hexamers and high-molecular-weight oligomers (HMW) was examined. It is possible that these individual components could have different roles and should be further explored in the context of ASD (Mazaki-Tovi et al. 2009). However, research in pediatric populations has found no difference in the role of total vs. HMW adiponectin (Meyer et al. 2017). Second, ASD assessment relied on EMR data, which can introduce outcome misclassification in unpredictable ways. However, the results of our sensitivity analysis showed consistent

associations when using more stringent outcome classification for both cases and neurotypical children. Third, because of the relatively small sample of ASD children in the cohort, our estimates may be subject to random variation. Fourth, although well-known ASD risk factors were adjusted for, there is a possibility of residual confounding. Finally, this study consisted primarily of urban, low-income minority population with high risk for preterm births. Thus, the findings may not be generalizable to other U.S. populations with different characteristics, although, few ASD studies have focused on this important group.

Despite these limitations, our study has a number of strengths. This study is based on a well-designed prospective birth cohort in an urban under-represented minority population. This is one of the first longitudinal studies to measure adiponectin at two-time points (birth and early childhood) prior to ASD diagnosis. Among studies that have looked at adipocytokines in the context of ASD, most of them were cross-sectional, and very few have used cord and/or newborn samples (Zerbo et al. 2014). Additionally, using a preterm enriched cohort, our study was uniquely poised to explore a novel question on the joint effect and mediating role of adiponectin in explaining the relationship between preterm birth and ASD risk and highlighted research gaps that can be explored by future studies.

Conclusions

In summary, our study found an inverse association between cord adiponectin levels and subsequent ASD risk in childhood. The effects of lower adiponectin on increased ASD risk were demonstrated independent of gestational age, early childhood adiponectin, and other important covariables. However, we emphasize that our findings be regarded as hypothesis generating; additional research is needed to replicate these findings. If confirmed, subsequent research can determine whether cord and/or early childhood adiponectin, along with other known risk factors, can be considered a biomarker for identifying children that are at high risk for ASD and thereby serve as a potential molecular target for developing novel interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

Funding Source: This study is supported in part by the Health Resources and Services Administration (HRSA) of the U.S. Department of Health and Human Services (HHS) under grant number R40MC27443, Autism Field-initiated Innovative Research Studies Program; and grant number UJ2MC31074, Autism Single Investigator Innovation Program. This information or content and conclusions are those of the author and should not be construed as the official position or policy of, nor should any endorsements be inferred by HRSA, HHS or the U.S.

Government. The Boston Birth Cohort (the parent study) was supported in part by the March of Dimes PERI grants (20-FY02-56, #21-FY07-605); and the National Institutes of Health (NIH) grants (R21ES011666, R01HD041702, R21HD066471, U01AI090727, R21AI079872, and 5R01HD086013). Ramkripa Raghavan is supported by John and Alice Chenoweth-Pate Fellowship in her current training.

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Fig 1.

Distribution of plasma adiponectin levels ($\mu\text{g/mL}$) in cord blood (panel A) and in early childhood venous blood (panel B), stratified by preterm status in the Boston Birth Cohort (Total n for cord adiponectin = 674; Total n for early childhood adiponectin = 638)

Fig 2.

Distribution of plasma adiponectin levels ($\mu\text{g/mL}$) in cord blood (panel A) and in early childhood venous blood (panel B), stratified by neurotypical children vs. those with ASD in the Boston Birth Cohort (Total n for cord adiponectin = 674; Total n for early childhood adiponectin = 638)

Table 1:

Maternal and child characteristics by child's case status (neurotypical vs. ASD) in the Boston Birth Cohort

	Neurotypical (n=792)	ASD (n=55)	p value
Characteristics			
Mothers			
Age at birth (yrs), mean (SD)	28.19 (6.56)	30.35 (6.16)	0.02
Parity (%)			0.89
0	333 (42.05)	21 (38.18)	
1 or more	458 (57.83)	34 (61.82)	
Missing	1 (0.16)	0 (0.00)	
Mother's education (%)			0.85
High School or less	510 (64.39)	33 (60.00)	
Some college or more	275 (34.72)	21 (38.18)	
Missing	7 (0.88)	1 (1.82)	
Maternal BMI (%)			0.21
Underweight (<18.5) + Normal Weight (18.5-<25)	385 (48.61)	20 (36.36)	
Overweight (25-29.9)	245 (30.93)	19 (34.55)	
Obesity (>=30)	162 (20.45)	16 (29.09)	
Diabetes (%)			0.04
No	718 (90.66)	47 (85.45)	
Gestational	50 (6.31)	2 (3.64)	
Diabetes mellitus	24 (3.03)	6 (10.91)	
Smoking during & 3 months prior to pregnancy (%)			0.45
No	677 (85.48)	42 (76.36)	
Yes	109 (13.76)	12 (21.82)	
Missing	6 (0.76)	1 (1.82)	
Child			
Gender (%)			<0.001
Male	337 (42.55)	40 (72.73)	
Female	455 (57.45)	15 (27.27)	
Race-ethnicity (%)			0.83
Black	453 (57.20)	34 (61.82)	
White	48 (6.06)	4 (7.27)	
Hispanic	186 (23.48)	11 (20.00)	
Other	100 (12.63)	6 (10.91)	
Missing	5 (0.63)	0 (0.00)	
Gestational age (%)			0.04
Term	601 (75.88)	34 (61.82)	
Late preterm (34 - <37 weeks)	128 (16.16)	10 (18.18)	
Early preterm (<34 weeks)	63 (7.95)	11 (20.00)	

	Neurotypical (n=792)	ASD (n=55)	p value
Characteristics			
Birth weight (g)	3017.85 (713.20)	2803.73 (886.24)	0.03
Year of birth (%)			0.66
1998–2006	429 (54.17)	30 (54.55)	
2007–2013	363 (45.83)	25 (45.45)	
Mean cord blood adiponectin (SD) ^a (µg/mL)	15.80 (10.84)	11.37 (88.27)	0.01
Mean early childhood adiponectin (SD) ^b (µg/mL)	10.67 (62.15)	10.11 (73.59)	0.61
First year weight gain pattern (%) ^c			0.05
On target	182 (33.96)	9 (18.75)	
Slow	65 (12.13)	6 (12.50)	
Rapid weight gain	92 (17.16)	6 (12.50)	
Extremely rapid weight gain	197 (36.75)	27 (56.25)	
Mode of feeding (%)			0.86
Formula	183 (23.11)	11 (20.00)	
Both	549 (69.32)	37 (67.27)	
Breastfeeding	54 (6.82)	6 (10.91)	
Missing	6 (0.76)	1 (1.82)	

^a n=674 (Neurotypical n=634; ASD n=40)

^b n=638 (Neurotypical n=602; ASD n=36)

^c n=584 (Neurotypical n=536; ASD n=48)

Table 2:

Association between cord plasma adiponectin levels and ASD risk in children in the Boston Birth Cohort^a

	Total n	ASD n	Unadjusted		Model 1		Model 2		Model 3	
			OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Continuous	674	40	0.51 (0.35, 0.75)	0.001	0.50 (0.33, 0.77)	0.002	0.47 (0.30, 0.74)	0.001	0.36 (0.21, 0.62)	<0.001
Quartile										
Q1	167	20	Ref		Ref		Ref		Ref	
Q2	170	8	0.36 (0.16, 0.85)	0.02	0.39 (0.15, 1.01)	0.05	0.38 (0.14, 1.03)	0.06	0.32 (0.11, 0.91)	0.03
Q3	169	7	0.32 (0.13, 0.77)	0.01	0.40 (0.15, 1.10)	0.08	0.38 (0.13, 1.12)	0.08	0.23 (0.07, 0.77)	0.02
Q4	168	5	0.23 (0.08, 0.62)	0.004	0.14 (0.04, 0.46)	0.001	0.13 (0.04, 0.45)	0.001	0.08 (0.02, 0.34)	0.001

^a Adiponectin was entered as a continuous variable in one model and categorical variable in another model

Model 1: Adjusted for child's sex, race, maternal education, maternal age, parity, smoking status, pre-pregnancy BMI, diabetes status, follow-up time

Model 2: Model 1 + gestational age

Model 3: Model 1 + early childhood adiponectin

Table 3:

Association between early childhood plasma adiponectin levels and ASD risk in children in the Boston Birth Cohort^a

	Total n	ASD n	Unadjusted		Model 1		Model 2		Model 3	
			OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Continuous	638	36	0.71 (0.45, 1.13)	0.15	0.54 (0.33, 0.90)	0.02	0.59 (0.35, 0.99)	0.05	0.67 (0.34, 1.33)	0.25
Quartile										
Q1	160	11	Ref		Ref		Ref		Ref	
Q2	159	8	0.72 (0.28, 1.83)	0.49	0.68 (0.25, 1.85)	0.45	0.74 (0.26, 2.05)	0.56	0.77 (0.19, 3.14)	0.71
Q3	160	10	0.90 (0.38, 2.19)	0.82	0.71 (0.27, 1.90)	0.50	0.84 (0.31, 2.29)	0.73	0.85 (0.23, 3.11)	0.80
Q4	159	7	0.62 (0.24, 1.65)	0.34	0.30 (0.09, 0.98)	0.05	0.32 (0.10, 1.08)	0.07	0.68 (0.15, 3.10)	0.61

^a Adiponectin was entered as a continuous variable in one model and categorical variable in another model

Model 1: Adjusted for maternal age, diabetes status, maternal BMI, sex, race, age of measurement, follow-up time and breastfeeding status

Model 2: Model 1 + gestational age at birth

Model 3: Model 1 + cord adiponectin

Table 4:

Joint effects of preterm birth and cord adiponectin in predicting ASD risk in the Boston Birth Cohort

Joint effects of preterm birth ^a and adiponectin ^b	Total n	ASD n	Unadjusted	P value	Adjusted ^c	P value
Full term + High adiponectin	309	11	Ref		Ref	
Preterm + High adiponectin	28	1	1.0 (0.12, 8.07)	1.0	1.22 (0.14, 10.87)	0.86
Full term + Low adiponectin	221	17	2.26 (1.04, 4.92)	0.04	3.06 (1.27, 7.40)	0.01
Preterm + Low adiponectin	116	11	2.84 (1.20, 6.74)	0.02	2.62 (0.99, 6.99)	0.05

^a – Full term defined as ≥ 37 completed weeks of gestation; preterm defined as <37 completed weeks of gestation

^b – High cord adiponectin – defined as ≥ 50th percentile; Low cord adiponectin – defined as <50th percentile

^c – Adjusted for child's sex, maternal education, maternal age, parity, maternal BMI, maternal smoking status, diabetes, race ethnicity and follow-up time