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Severity of ASD symptoms and their correlation with the presence of copy number variations and exposure to first trimester ultrasound

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Abstract

Scientific Abstract—Current research suggests that incidence and heterogeneity of autism spectrum disorder (ASD) symptoms may arise through a variety of exogenous and/or endogenous factors. While subject to routine clinical practice and generally considered safe, there exists speculation, though no human data, that diagnostic ultrasound may also contribute to ASD severity, supported by experimental evidence that exposure to ultrasound early in gestation could perturb brain development and alter behavior.

Here we explored a modified *triple hit hypothesis* (Williams & Casanova 2010) to assay for a possible relationship between the *severity* of ASD symptoms and (1) ultrasound exposure (2) during the first trimester of pregnancy in fetuses with a (3) genetic predisposition to ASD. We did so using retrospective analysis of data from the SSC (Simon's Simplex Collection) autism genetic repository funded by the Simons Foundation Autism Research Initiative. We found that male children with ASD, copy number variations (CNVs) and exposure to first trimester ultrasound had significantly decreased non-verbal IQ and increased repetitive behaviors relative to male children with ASD, with CNVs and no ultrasound.

These data suggest that heterogeneity in ASD symptoms may result, at least in part, from exposure to diagnostic ultrasound during early prenatal development of children with specific genetic vulnerabilities. These results also add weight to on-going concerns expressed by the FDA about non-medical use of diagnostic ultrasound during pregnancy.

Lay Abstract—Variations in autism spectrum disorder (ASD) may arise through both internal and external factors. Diagnostic ultrasound is used in routine clinical practice and is generally considered safe during pregnancy. However animal models suggest that ultrasound could

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Competing Interests

The authors declare that they have no competing interests.

contribute to ASD severity as ultrasound early in prenatal development may alter brain development and behavior.

We test for a possible relationship between the *severity* of ASD symptoms and (1) ultrasound exposure (2) during the first trimester of pregnancy in fetuses with a (3) genetic predisposition to ASD. We used data from the SSC (Simon's Simplex Collection) autism genetic repository funded by the Simons Foundation Autism Research Initiative. We found that male children with ASD and copy number variations (CNVs), which is a type of genetic vulnerability, and exposure to first trimester ultrasound had decreased non-verbal IQ and increased repetitive behaviors compared to male children with ASD and CNVs but no ultrasound.

These data suggest that variation in ASD symptoms may result from exposure to diagnostic ultrasound during early prenatal development of children with specific genetic vulnerabilities. These results also add weight to on-going concerns expressed by the FDA about non-medical use of diagnostic ultrasound during pregnancy.

Background

Heterogeneity of ASD

Current research supports the view that incidence and the heterogeneity of symptoms of autism spectrum disorder (ASD) can arise through multiple factors.

In multifactorial disorders such as ASD, Casanova (Casanova 2007) proposed a *triple hit hypothesis* where the convergence of three factors, (1) the brain of a genetically at risk individual is (2) exposed during a time of critical neurodevelopment to (3) an exogenous stressor, may contribute to the incidence of ASD. First, the stressor occurs during a critical period of brain development. In ASD, it has been proposed that this insult occurs early in gestation (e.g. 1st or 2nd trimester) (Chess 1977; Ivarsson et al. 1990; Rodier 2002; Torrey et al. 1975) but not later (Yamashita et al. 2003). Second, the individual has an underlying (genetic) vulnerability. In ASD, both specific genetic events (e.g., Bernier et al. 2014; O'Roack et al. 2012) and genetic risk factors (e.g., Klei et al. 2012; Werling & geschwind 2013) have been proposed to play a role in the disorder. Third, there is an exogenous gestational stressor. Proposed gestational stressors are varied and have included (but are not limited to): anticonvulsants and other psychotropic medications (Christensen et al. 2013; Wood 2014), maternal infection and immune activation (Malkova et al. 2012; Mazina et al. 2015; Patterson 2011; Zerbo et al. 2013), and environmental toxins such as air pollutants (Ehrenstein et al. 2014), volatile organic compounds (McCanlies et al. 2012), pesticides (Roberts et al. 2007; Roberts & English 2013), and plasticizers (Kalkbrenner et al. 2014; Stein et al. 2015; Testa et al. 2012).

Of interest to the present work is the possibility that diagnostic ultrasound could act as an exogenous stressor (Williams & Casanova 2010) and may be related to ASD severity.

Diagnostic Ultrasound Effects in Animal Models

Effects on Brain Structure, *in vivo*—There exist a number of early studies that link ultrasound exposure *in utero* in rodents to changes in neuro-anatomy, summarized in Stewart et al. (1985). Ellisman et al. (1987) observed that 30 minutes of diagnostic ultrasound

applied to neonatal rat pups disrupted brain myelination compared to sham exposed animals. Ang et al. (2006) found distrupted cortical migratory patterns in mice exposed to a 30+ minutes of diagnostic ultrasound *in utero* relative to shams. Li et al. (2015) exposed rats to 20 minutes of diagnostic ultrasound *in utero* and observed changes in mRNA and protein expression levels of hippocampal N-methyl-D-aspartate (NMDA) receptor units, brain-derived neurotrophic factor (BDNF), and the presence of damaged hippocampal synapses.

Effects on Behavior, *in vivo*—Ultrasound exposure to rodents *in utero* can also alter their subsequent behavior. For example, decreased locomotor and exploratory activities, as well as decreased learning ability was found in mice exposed to prenatal ultrasound (Devi et al. 1995; Suresh et al. 2002; Suresh et al. 2008). Similarly in macaques exposed to prenatal ultrasound, the authors reported reduced levels of physical activity as compared to control monkeys, with all effects normalizing by age 5–6 months (Tarantal et al. 1989). McClintic et al. (2013) found that that 30 minutes of prenatal ultrasound resulted in juvenile mice that were less social and more hyperactive in social situations than sham exposed mice. Finally, Li et al. (2015) observed a reduction in spatial learning and memory abilities of rats after their exposure to prenatal diagnostic ultrasound. It is important to mention that there exist studies showing negative results after prenatal ultrasound exposure. As an example, Jensh et al. (1995) found that rats exposed to 35 minutes of ultrasound *in utero* did not exhibit any changes in memory and anxiety tests.

Diagnostic Ultrasound in Prenatal Clinical Practice

Each decade since the introduction of ultrasound to obstetric medicine, its popularity and use has escalated. In modern obstetrics, it is standard clinical practice to utilize ultrasound to diagnose, date, and monitor the growth of the fetus. From an obstetric perspective, first trimester ultrasound leads to improved prganancy dating, reducing antenatal testing and labor inductions (Caughey et al. 2008; Abramowicz 2013). Socio-emotional concerns may also guide ultrasound requests -- to provide reassurance of fetal health, to increase paternal and familial involvement, and to provide pictorial momentos (Gudex et al. 2006). While rates of use vary based on insurance type and provider (O'Keeffe & Abuhamad 2013), there has been a significant increase in the utilization of obstetric ultrasound in the last 5 years, including increases in multiple general ultrasounds in low-risk pregnancies and targeted ultrasound examinations intended for higher risk concerns (You et al. 2010).

While generally considered safe, recent FDA statements regarding the use of prenatal diagnostic ultrasound as well as on-going attention in the scientific literature (Abramowicz 2013) continue to stress its conservative use for medical purposes. Ultrasound consists of high frequency sound, which when absorbed may lead to thermal (hyperthermia) and non-thermal (cavitation, radiation pressure) effects within tissue (Mourad 2012). Energy absorbtion depends on tissue type, exposure, and ultrasound mode, such as for imaging or blood-flow monitoring. The early FDA restriction on intensity alone was replaced during the 1990s with a nearly 8-fold increase in intensity along with imposition of maximum values of a thermal index (TI) to measure the tendency of ultrasound to warm tissue and a mechanical index (MI) related to the likelihood of ultrasound to produce cavitation. This is particularly concerning as ultrasound technicians and maternal fetal medicine fellows demonstrated poor

knowledge regarding safety levels (Meizner 2012; Houston et al. 2011; Sheiner et al. 2007). For example, although less established in humans than in animal models (reviewed in Edwards 2006), the developing central nervous system, particularly during the 1st trimester may be particularly susceptible to hyperthermic injury (Edwards 2006), with maternal hyperthermia associated with increased neural tube defects (Milunsky et al. 1992; Moretti et al. 2005).

Effects on General Child Outcomes—In humans, a number of non-randomized and randomized studies have investigated ultrasound exposure and found no signifcant (or consistent) relation to congenital anomalies, birth size, cancer/tumors, heart disease, general neonatal and child oucomes, and specific psychopathology such as schizophrenia and psychosis (reviewed in Houston et al. 2009). A few notable differences were found in case-control designs suggesting increased presence of speech delay (Campbell et al. 1993) but not in randomized trials (Kieler et al. 1998; Newnham et al. 2004; Salvesen et al. 1994). There have been several reports of increased left handendess in males in relation to fetal ultrasound exposure (Kieler et al. 1998; Kieler et al. 2002; Salvesen et al. 1993).

ASD Incidence and Ultrasound Exposure—The relation between ultrasound as a primary etiological factor and ASD diagnostic outcome has not been supported (Grether et al. 2010; Stoch et al. 2012). Stoch et al. (2012) did not find a relation between child ASD diagnosis and randomized prenatal exposure to a single second trimester ultrasound versus multiple second and third trimester scans (at 18, 24, 34, 38 weeks); nor to levels of adult autism traits in a primarily “neurotypical” population. Grether et al. (2010) analyzed antenatal ultrasound exposure (primarily 2nd trimester exposure) as a risk factor for ASD using medical data from Kaiser Permanente of Northern California Health Care System; there was no difference in number of exposures during the entire gestation or by trimester in 393 controls and 362 autism cases. Critical to the triple hit hypothesis and not addressed by previous reports, ultrasound as an exogenous stressor would have the most significant impact during the 1st trimester and only in those with specific genetic risk factors.

Current Project

Given the increase in ultrasound intensity and use, extant animal studies, and lack of human data addressing ASD characteristics and ultrasound exposure during the 1st trimester of pregnancy, we sought here to explore a variant of Casanova's triple hit hypothesis, assessing factors that may influence the *severity* of ASD symptoms rather than ASD incidence. Specifically, we sought to study how (1) the presence of ultrasound (2) within a period of high neural vulnerability (1st trimester) in a group (3) with underlying vulnerabilities (males versus females; presence or absence of CNVs) may relate to the severity of ASD symptoms. We used linear regression models with autism severity outcomes (including verbal and non-verbal IQ, adaptive skills, social ability, and repetitive behaviors), to test if the presence of an ultrasound, maleness, and CNVs are significant risk factors. In this analysis we calculated propensity scores to assess the predicted probability of a first trimester ultrasound in order to adjust for differences in baseline factors that influence prenatal care and subsequent ultrasound exposure. We predicted that first trimester ultrasound would be a statistically significant risk factor for increased autism severity in children made genetically vulnerable

by CNVs, even after adjusting for confounding factors due to differences in likelihood of ultrasound.

Methods

Participants

This study uses data from the SSC (Simon's Simplex Collection) funded by the Simons Foundation Autism Research Initiative (Fischbach & Lord 2010). Briefly, 2,644 families were successfully recruited from 12 sites across the USA and included families with one child with ASD aged 4 to 18 years (a “proband”), one unaffected full sibling (>80% of cases), and the biological mother and father. Inclusion/exclusion criteria can be found at http://sfari.org. Approval was obtained at each local human subject's institutional review board and parents and participants completed informed consent.

All probands were assessed on the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al. 1994), the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 2000), and DSM-IV (American Psychiatric Association 2000) as administered by experienced clinicians. A description of the instruments can be found at https://sfari.org/resources/simons-simplex-collection/ssc-instruments.

Variables

Proband descriptive characteristics include measures of cognitive ability (non-verbal and verbal subdomains of the Differential Ability Scale [DAS], Elliott 2007), social ability (ADI-R Social Domain score [ADI-R SD], Lord et al. 1994; ADOS Social Affective Domain score [ADOS SA], Gotham et al. 2007; Social Responsiveness Scale [SRS], Constantino & Gruber 2003), and repetitive behaviors (ADI-R Restrictive and Repetitive Behaviors score [ADI RRB], Lord et al. 1994; ADOS Repetitive Domain score [ADOS REP], Gotham et al. 2007; Repetitive Behavior Scale-Revised [RBS-R], Bodfish et al. 1999; Vineland Adaptive Behavior Scale, Second Edition [VABS-II], Sparrow 2011). Observed child behaviors are derived from the DAS and the ADOS. Parent report of behaviors is derived from the ADI-R, VABS-II, SRS, and RBS. These tests were not applied to the family members of probands.

Information regarding pregnancy and general medical history for all probands was collected by a clinician in a semi-structured interview. Specific to this study, we assessed ultrasound exposure as whether or not the biological mother received an ultrasound during a time of critical neurodevelopment of the fetus: the first trimester.

Genetic data included analysis of the presence or absence of ASD-associated CNVs of a subset (1749) of the SCC children, made available via Girirajan et al. (2013). In order to set the context for the analysis of that subpopulation that is at the heart of this paper, we analyzed (SPSS Statistical Software, IBM Corporation, Armonk, NY) our data for phenotype differences related to the known presence (n=133) or absence (n=1616) of a CNV (Mazina et al. 2015) and for differences related to duplications (n=56) or deletions (n=77) (Girirajan et al. 2013). Children with ASD and an identified CNV compared to those without a CNV had mothers and fathers of similar age ($F(1,147) < .56$, $p > .46$), no differences in

maternal and paternal education ($x^2(3)<4.2$, p >.33) or family income ($x^2(3)=.82$, p=.66), no differences in Verbal IQ (F(1,1747) = 1.55, p =. 21) or Vineland Adaptive Behavior Composite (F(1,1747) = 1.78, p = .18), nor did they differ on any of the other autism symptom variables (Fs <2.36, p >.13). Relative to ASD children without identified CNVs, ASD children with identified CNVs did trend towards a lower Nonverbal IQ (F(1,1747) = 3.73, p = .054). Also, with regard to children with a duplication compared to a deletion, they did not differ in any of the phenotypic variables reported above (Fs(1,131)<1.34, p >.25). This last result differs from those of Girirajan et al who found more impairment in the restricted and repetitive behavior domain in children with duplications compared to deletions.

Therefore, from the total of 2,644 SSC probands with ASD, the final sample for our analysis consisted of 1749 children (66.1% of the total data set) with ASD for whom we had information on the presence or absence of CNVs and the presence or absence of a first trimester ultrasound. Proband characteristics are in Supplemental Table 1; a descriptive list of CNVs by sex and ultrasound exposure is presented in Supplemental Table 2. Rates of ultrasound use in the first trimester did not differ between individuals with specific CNV types (duplications vs deletions or inherited maternal/paternal or de novo mutations) as presented in Supplemental Table 3.

Statistical Analysis

We applied a multiple linear regression model (Stata SE, version 12) for analysis of autism severity with first trimester ultrasound as a dichotomous primary predictor, and the propensity score as an additional covariate, along with child sex and age in months at assessment. Given that we hypothesize that child sex and CNV status may be effective modifiers in the relationship between first trimester ultrasound and ASD severity, we included sub-analyses limited to: (1) all ASD children with identified CNVs and (3) ASD males with identified CNVs, and we formally tested for effect modification using interaction terms and the Wald statistic.

We performed covariate adjustment using propensity scores, as described by Austin (2011a/b) and as employed by other researchers (Arabi et al., 2013; Goldin, Sawin, Garrison & Christakis 2007; Gagliardi, Bellu, Zanini, Dammann, 2009; Lund et al., 2013 but see Brooks & Ohsfeldt, 2013; Weitzen et al., 2004). Propensity scores were developed to adjust all regression analysis for potential confounding due to the likelihood of a mother receiving a first trimester ultrasound. Specifically, maternal and pregnancy characteristics may influence the likelihood of receiving a first trimester ultrasound (Caughey et al. 2008) and may therefore influence child outcomes. Variables included in the development of the propensity scores were limited to those that would have been known during the first trimester such as demographic characteristics (e.g., race, income), maternal characteristics (e.g., age, education), prior pregnancy history (e.g., use of fertility treatments, pregnancy loss, preterm birth), and current pregnancy information (e.g., weight, weight gain, timing of prenatal care initiation, and complications or procedures occurring during the first trimester) (Baxter et al. 2007; Ben Itzchak et al. 2011; Zachor & Ben Itzchak 2011).

Results

For all 1749 SSC children with ASD (Table 1), exposure to 1st trimester ultrasound compared to those with no exposure was related to lower observed social affective symptoms (ADOS SA), but greater parent reported restrictive and repetitive behaviors (ADI-R RRB).

For all 133 SSC children with ASD and identified CNVs (Table 2), exposure to 1st trimester ultrasound compared to those with no exposure was related to statistically significantly lower Nonverbal IQ. In addition, there were trends towards more impaired adaptive behaviors (VABS-II) via parent report, and trends toward increased observed repetitive behaviors (ADOS REP).

For all 111 SSC male children with ASD and identified CNVs (Table 3), exposure to 1st trimester ultrasound compared to those with no exposure was related to statistically significantly lower Nonverbal IQ and significantly increased observed repetitive behaviors (ADOS REP). In addition, there were trends in reduced Verbal IQ and in more parent reported repetitive symptoms (RBS-R).

Wald tests for interaction between ultrasound and gender were statistically significant for parent report of adaptive behaviors (VAB-II: beta −4.26, 95% CI −6.71 to −1.80, p = .001), social ability (SRS: beta 5.3, 95% CI 3.11 to 7.50, p < .001). Wald tests for the interaction between ultrasound and CNVs within males were statistically significant for Verbal IQ (beta −13.3 95% CI −25.8 to −.78, p = .04) and Nonverbal IQ (beta −12.0, 95% CI −21.5 to −2.5, p = .01).

The small sample size of females with identified CNVs precluded us from applying our model to this cohort. Information on measurement means and standard deviations for males or females, with and without identified CNVs, and with and without 1st trimester ultrasound is provided in Supplemental Table 4.

Discussion

Supporting the hypothesis that ultrasound may act as an exogenous stressor related to ASD severity, for male children with ASD and an identified CNV, exposure to 1st trimester ultrasound was associated with poorer Nonverbal IQ and increased repetitive behaviors. In addition, we found that ultrasound may also contribute to variability in outcome in children with ASD in a double hit model. Specifically, exposure to 1st trimester ultrasound in children with ASD, regardless of the known presence or absence of an identified CNV, was associated with less observed social affective severity, but increased parental reports of repetitive behaviors.

Triple Hit Hypothesis

We could not test the original triple hit hypothesis of Casanova, which speaks to the incidence of ASD, because we did not have the requisite medical information for the siblings of our probands.

In support of our modified triple hit hypothesis, however, males with ASD and an identified CNV with first trimester ultrasound exposure had lower Nonverbal IQ and increased repetitive behavior, with trends towards lower Verbal IQ, all relative to males with an identified CNV but not exposed to first trimester ultrasound. Given that lowered IQ and greater repetitive behaviors are found in a number of other neurodevelopmental disorders (Lewis & Kim 2009) and in children with genetic vulnerabilities (Girirajan et al. 2011; Girirajan et al. 2012; Pinto et al. 2014), the specificity to ASD is unclear. Expanding consideration of our modified triple hit hypothesis to other neurodevelopmental disorders is therefore warranted.

As females with ASD with available data for this analyses made up only 12.6% of the sample, and with females with an identified CNV representing only 1.2% of cases, addressing the impact of first trimester ultrasound on girls was not possible. Relative to the entire sample, girls in the SSC sample were found to have higher levels of social and communication symptoms as well as lower verbal and non-verbal IQ (Frazier et al. 2014), and more likely to have CNVs in deleterious regions related to neurodevelopmental disorders (Jacquemont et al. 2014; Robinson et al. 2013; Werling & Geschwind 2013). The extent to which female sex interacts with environmental hits remains to be tested.

All children with ASD, identified absence/presence of CNVs, and 1st trimester ultrasound

All children with ASD known absence or presence of CNVs and exposure to 1st trimester ultrasound had increased repetitive behaviors, suggesting that an exogenous stressor (here, diagnostic ultrasound), delivered during a time of critical neurodevelopment can increase the severity of at least one class of ASD symptom, consistent with a ‘double hit’ hypothesis. This result holds true when considering gender and the presence of CNVs, discussed below.

In contrast to our hypothesis, however, children with ASD, known absence or presence of identified CNVs and exposure to 1st trimester ultrasound had a small but statistically significant reduction in social affective symptoms. The presence of a 1st trimester ultrasound may also be related to numerous maternal variables that influence child outcomes. Specifically, mothers in our sample who received 1st trimester ultrasounds were more likely to be older at time of conception, which has been shown to increase risk for ASD (Sandin et al. 2013), and more likely to have a higher household income, found to be related to increased diagnostic rates but also access to resources (Croen et al. 2002; Johnson et al. 2007; Thomas et al. 2012a; Thomas et al. 2012b). In non-clinical samples, older maternal age, higher parental education, and higher income are strongly associated with better academic achievement, cognitive ability and social competence (Edwards & Roff 2010; Lieu et al. 2015; Pati et al. 2011). Similarly, utilization of prenatal care is more likely in mothers with higher income, more educated, or live in urban settings (Braveman et al. 2003; Chiavarini et al. 2014; Stativa et al. 2014). Preconception and pregnancy health is related to better child outcomes (Liu et al. 2015), with some disagreement (Noonan et al. 2012; for review - Alexander 2001). Thus, it is possible that despite our use of a propensity score to correct for likelihood of 1st trimester ultrasound, there remain residual confounding factors. Disentangling the effect of ultrasound *per se* versus ultrasound as a proxy for other variables related to outcomes will be critical in future studies.

Neural vulnerability and external hits

In this report, we focused on the 1st trimester as a period of neural vulnerability as supported by other studies of environmental factors and their relevance to autism (Dufour-Rainfray et al. 2011). For example, the chemicals valproate (Arndt et al. 2005), thalidomide (Miller et al. 2005), and misoprostol (Bandim et al. 2003) cause up or down regulation of certain genes involved in neural proliferation and migration (Dufour-Rainfray et al. 2011). Maternal infection and fever generally (Atladottir et al. 2010; Lee et al. 2014; Zerbo et al. 2013) and specifically during the first trimester (Atladottir et al. 2010), have also been associated with autism. Supporting a gene by environment interaction, Schwartzer et al. (2013) found maternal immune activation was strain dependent in mice, and male mice born to maternal immune activated mothers had more disrupted social behaviors (Malkova et al. 2012). Similarly, Mazina et al. (2015) found that maternal infection during pregnancy in children with CNVs resulted in greater severity of autism symptoms.

Mechanism

The rodent studies of Li et al. (2015) suggest one potential mechanism by which ultrasound may act. They observed reduced expression of mRNA and proteins associated with the NDMA receptor pathway and BDNF in the hippocampus of rats exposed to three 20-minute applications of ultrasound. Interestingly, a lower dose exposure (4 minutes) resulted in increased expression in NMDA (GluNR1 and GluNR2B subunits) and BDNF. This interaction with dose and expression suggests an overall sensitivity of these systems to ultrasound. The NMDA system has a broad impact on variability in social and cognitive functioning (Burnashev & Szepetowski 2015; Lee et al. 2015). Alterations of NMDA receptors disrupt response inhibition and social interaction in mice (Chung et al. 2015; Finlay et al. 2015). *GRIN1* encodes for the NMDA GluN1 subunit and is related to decreased social interaction in knockout mice (e.g., Gandal et al. 2012; Saunders et al. 2013); *GRIN2B* encodes for the GluN2B subunit, which has been found in rare mutations in autism (O'Roak et al. 2011; Myers et al. 2011; Talkowski et al. 2012; Yoo et al. 2012). This complex pathway is susceptible to disruption via both increases and decreases in protein expression (Zito & Scheuss 2009). Additional animal studies are needed to elucidate the vulnerability of the NDMA system to genetic and environmental disruption and their role in social and non-social learning and memory.

BDNF is a neurotrophic factor related to neuronal maturation and synaptic synthesis. BDNF is elevated in children with ASD (Bryn et al. 2015; Kasarpalkar et al. 2014; Zhang et al. 2014) and genes associated with neurodevelopmental disorders impact BDNF expression (Briz et al. 2013; Koh et al. 2014; Louhivuori et al. 2011; Ouyang et al. 2013). Prenatal exposure to other environmental toxins impact BDNF: Bisphenol A altered DNA methylation in mice (Kundakovic et al. 2014) and valproate increased BDNF mRNA and protein levels in mice and rats (Almeida et al. 2014). A relation between BDNF levels (both hyper and hypo) and neural connectivity in ASD has been proposed (Koh et al. 2014).

Limitations

Ultrasound exposure was assessed via parent report with a 4 to 18 year old sample. Thus, recall error may have influenced our results in regard to reporting on the presence, absence,

and the timing of ultrasounds, although Olson et al. (1997) suggest that memory for pregnancy related medical procedures is relatively high. While presence/absence of an ultrasound is more likely to be reported correctly, reporting an “early” ultrasound as first versus second trimester may have induced random error. Specifically, the first trimester spans weeks 1 through 12 of pregnancy, with first trimester ultrasound recommended between “10 to 13” weeks (Salomon et al. 2012). However, if a systematic recall bias existed, it is unlikely this would differentially impact reporting by child genotype, as genotype was not known at time of ultrasound or at assessment. Future research based on medical records that provide exact dates and nature of ultrasound exams is necessary to determine the influence of ultrasound timing and therefore identify a specific window of vulnerability.

Lastly, there is some controversy regarding the use of propensity scores to adjust for confounding in observational studies (Brooks & Ohsfeldt, 2013; Weitzen et al., 2004). Although it would have been theoretically possible to include all of the covariates used in creation of the propensity score as individual covariates in the final regression model, this would have resulted in even smaller cell sizes throughout the regression matrices for our models, especially given the relative rarity of CNVs in the sample. The small cell size problem is a well-documented rationale for using propensity scores to address confounding in observational studies (Braitman & Rosenbaum, 2002; Cepeda, Boston, Farrar, Stro 2003; Ali, Groenwold, Lungel, 2014; Glynn, Schneeweiss & Sutmer, 2006) as use of propensity scores adds only a single dimension to the regression model. Of note, at least one study found that outliers among the propensity score distribution may considerably skew effect estimates (Kurth et al., 2006). However, post hoc testing found that this was not the case in our analyses. Specifically, sensitivity analyses excluding all subjects whose predicted probability of first trimester ultrasound was less than 20% resulted in no notable differences in regression results. Moreover, the effect size associated with exposure to first trimester ultrasound changed by less than 10% in all cases such that no model predictions shifted between statistically significant and non-significant. We also note that propensity score adjustment does not help reduce bias due to unmeasured confounders (Brooks & Ohsfeldt, 2013; Weitzen et al. 2004). This is, of course, is an inherent limitation of observational studies, one that would remain true if we had chosen regression models that only adjusted directly for measured covariates without the use of propensity scores.

Conclusion

In a national sample of children with ASD that included detailed, quantifiable post-natal childhood outcomes and genetic information, we found that the combination of first trimester ultrasound and presence of CNVs in male children with ASD correlated with poorer cognitive outcomes and increased repetitive behavior. Moreover, across gender and independent of the presence of identified CNVs, first trimester ultrasound correlated with increased repetitive behaviors. Better understanding of the range and types of environmental stressors that may interact with genetic vulnerabilities is needed in order to fully understand contributions to heterogeneity in ASD functioning. If we can better identify the neural development periods most vulnerable to environmental insults, particularly in regard to exposures that can be limited, modified, or regulated by changing practice such as non-

medical use of diagnostic ultrasound imaging, it may be possible to quickly reduce the severity of ASD in future children. Further replication both in larger epidemiological populations and in animal models that can address mechanisms are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Abramowicz JS. Benefits and risks of ultrasound in pregnancy. Seminars in Perinatology. 2013; 37(5): 295–300. http://doi.org/10.1053/j.semperi.2013.06.004. [PubMed: 24176149]

Ali MS, Groenwold RH, Klungel OH. Propensity score methods and unobserved covariate imbalance: comments on “squeezing the balloon”. Health Serv. 2014; 49(3):1074–82. DOI: 10.1111/1475-6773.12152.

Almeida LEF, Roby CD, Krueger BK. Increased BDNF expression in fetal brain in the valproic acid model of autism. Molecular and Cellular Neurosciences. 2014; 59:57–62. DOI: 10.1016/j.mcn.2014.01.007. [PubMed: 24480134]

Ang ESBC, Gluncic V, Duque A, Schafer ME, Rakic P. Prenatal exposure to ultrasound waves impacts neuronal migration in mice. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(34):12903–12910. DOI: 10.1073/pnas.0605294103. [PubMed: 16901978]

Arabi YM, Khedr M, Dara SI, Dhar GS, Bhat SA, Tamim HM, Afesh LY. Use of intermittent pneumatic compression and not graduated compression stockings is associated with lower incident VTE in critically ill patients: a multiple propensity scores adjusted analysis. Chest. 2013; 144(1): 152–9. DOI: 10.1378/chest.12-2028. [PubMed: 23412593]

Arndt TL, Stodgell CJ, Rodier PM. The teratology of autism. International Journal of Developmental Neuroscience : the Official Journal of the International Society for Developmental Neuroscience. 2005; 23(2-3):189–199. DOI: 10.1016/j.ijdevneu.2004.11.001. [PubMed: 15749245]

Atladóttir HO, Thorsen P, Østergaard L, Schendel DE, Lemcke S, Abdallah M, Parner ET. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord. 2010; 40(12):1423–1430. DOI: 10.1007/s10803-010-1006-y. [PubMed: 20414802]

Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav Res. 2011a; 46(3):399–424. DOI: 10.1177/0962280213519716. [PubMed: 21818162]

Austin PC. A Tutorial and Case Study in Propensity Score Analysis: An Application to Estimating the Effect of In-Hospital Smoking Cessation Counseling on Mortality. Multivariate Behav Res. 2011b; 46(1):119–151. DOI: 10.1016/j.cjca.2015.05.015. [PubMed: 22287812]

Bandim JM, Ventura LO, Miller MT, Almeida HC, Costa AES. Autism and Möbius sequence: an exploratory study of children in northeastern Brazil. Arquivos De Neuro-Psiquiatria. 2003; 61(2A):181–185. DOI: 10.1590/S0004-282X2003000200004. [PubMed: 12806493]

Baxter AC, Lotspeich LJ, Spiker D, Martin JL, Grether JK, Hallmayer JF. Brief report: effect of maternal age on severity of autism. J Autism Dev Disord. 2007; 37(5):976–982. DOI: 10.1007/s10803-006-0217-8. [PubMed: 17013673]

Ben Itzchak E, Lahat E, Zachor DA. Advanced parental ages and low birth weight in autism spectrum disorders--rates and effect on functioning. Research in Developmental Disabilities. 2011; 32(5): 1776–1781. DOI: 10.1016/j.ridd.2011.03.004. [PubMed: 21498045]

Bernier R, Golzio C, Xiong B, Stessman HA, Coe BP, Penn O, et al. Disruptive CHD8 Mutations Define a Subtype of Autism Early in Development. Cell. 2014; 158(2):263–276. DOI: j.cell.2014.06.017. [PubMed: 24998929]

Bodfish JW, Symons FJ, Lewis MH. The Repetitive Behavior Scale. 1999

Braitman LE, Rosenbaum PR. Rare outcomes, common treatments: analytic strategies using propensity scores. Ann Intern Med. 2002; 15137(8):693–5. DOI: 10.7326/0003-4819-137-8-200210150-00015.

Braveman, P., Marchi, K., Sarnoff, R., Egerter, S., Rittenhouse, D., Salganicoff, A. Promoting Access to Prenatal Care: Lessons from the California Experience. Henry J Kaiser Family Foundation; 2003. p. 1-86.

Briz V, Hsu Y-T, Li Y, Lee E, Bi X, Baudry M. Calpain-2-mediated PTEN degradation contributes to BDNF-induced stimulation of dendritic protein synthesis. The Journal of Neuroscience : the Official Journal of the Society for Neuroscience. 2013; 33(10):4317–4328. DOI: 10.1523/JNEUROSCI.4907-12.2013. [PubMed: 23467348]

Bryn V, Halvorsen B, Ueland T, Isaksen J, Kolkova K, Ravn K, Skjeldal OH. Brain derived neurotrophic factor (BDNF) and autism spectrum disorders (ASD) in childhood. European Journal of Paediatric Neurology : EJPN : Official Journal of the European Paediatric Neurology Society. 2015 DOI: 10.1016/j.ejpn.2015.03.005.

Burnashev N, Szepetowski P. NMDA receptor subunit mutations in neurodevelopmental disorders. Current Opinion in Pharmacology. 2015; 20:73–82. DOI: 10.1016/j.coph.2014.11.008. [PubMed: 25498981]

Campbell JD, Elford RW, Brant RF. Case-control study of prenatal ultrasonography exposure in children with delayed speech. CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne. 1993; 149(10):1435–1440. DOI: 10.1016/j.ultrasmedbio.2012.01.017.

Casanova MF. The neuropathology of autism. Brain Pathology (Zurich, Switzerland). 2007; 17(4): 422–433. DOI: 10.1111/j.1750-3639.2007.00100.x.

Caughey AB, Nicholson JM, Washington AE. First- vs second-trimester ultrasound: the effect on pregnancy dating and perinatal outcomes. American Journal of Obstetrics and Gynecology. 2008; 198(6):703.e1–5. discussion 703.e5–6. DOI: 10.1016/j.ajog.2008.03.034. [PubMed: 18538160]

Cepeda MS, Boston R, Farrar JT, Strom BL. Comparison of logistic regression versus propensity score when the number of events is low and there are multiple confounders. Am J Epidemiol. 2003; 158(3):280–7. DOI: 10.1093/aje/kwg115. [PubMed: 12882951]

Chess S. Follow-up report on autism in congenital rubella. Journal of Autism and Childhood Schizophrenia. 1977; 7(1):69–81. DOI:10.1007/BF01531116. [PubMed: 576606]

Chiavarini M, Lanari D, Minelli L, Salmasi L. Socio-demographic determinants and access to prenatal care in Italy. BMC Health Services Research. 2014; 14:174. DOI: 10.1186/1472-6963-14-174. [PubMed: 24735757]

Christensen J, Grønborg TK, Sørensen MJ, Schendel D, Parner ET, Pedersen LH, Vestergaard M. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA : the Journal of the American Medical Association. 2013; 309(16):1696–1703. DOI: 10.1001/jama.2013.2270. [PubMed: 23613074]

Chung W, Choi SY, Lee E, Park H, Kang J, Park H, et al. Social deficits in IRSp53 mutant mice improved by NMDAR and mGluR5 suppression. Nature Neuroscience. 2015; 18(3):435–443. DOI: 10.1038/nn.3927. [PubMed: 25622145]

Constantino, JN., Gruber, CP. The Social Responsiveness Scale. Western Psychological Services; Lost Angeles, CA: 2003.

Croen LA, Grether JK, Selvin S. Descriptive epidemiology of autism in a California population: who is at risk? J Autism Dev Disord. 2002; 32(3):217–224. DOI: 10.1023/A:1015405914950. [PubMed: 12108623]

Devi PU, Suresh R, Hande MP. Effect of fetal exposure to ultrasound on the behavior of the adult mouse. Radiation Research. 1995; 141(3):314–317. DOI: 10.2307/3579009. [PubMed: 7871159]

Dufour-Rainfray D, Vourc'h P, Tourlet S, Guilloteau D, Chalon S, Andres CR. Fetal exposure to teratogens: evidence of genes involved in autism. Neuroscience and Biobehavioral Reviews. 2011; 35(5):1254–1265. DOI: 10.1016/j.neubiorev.2010.12.013. [PubMed: 21195109]

Edwards MJ. Review: Hyperthermia and fever during pregnancy. Birth Defects Research. Part a, Clinical and Molecular Teratology. 2006; 76(7):507–516. DOI: 10.1002/bdra.20277.

Edwards RD, Roff J. Negative effects of paternal age on children's neurocognitive outcomes can be explained by maternal education and number of siblings. PloS One. 2010; 5(9):e12157. DOI: 10.1371/journal.pone.0012157. [PubMed: 20856853]

Ehrenstein, von OS, Aralis H, Cockburn M, Ritz B. In utero exposure to toxic air pollutants and risk of childhood autism. Epidemiology (Cambridge, Mass.). 2014; 25(6):851–858. DOI: 10.1097/EDE.0000000000000150.

Elliott, CD. Manual for the Differential Ability Scales. Harcourt Assessment; San Antonio: 2007.

Ellisman MH, Palmer DE, André MP. Diagnostic levels of ultrasound may disrupt myelination. Experimental Neurology. 1987; 98(1):78–92. DOI: 10.1016/0014-4886(87)90073-2. [PubMed: 3308504]

Finlay JM, Dunham GA, Isherwood AM, Newton CJ, Nguyen TV, Reppar PC, et al. Effects of prefrontal cortex and hippocampal NMDA NR1-subunit deletion on complex cognitive and social behaviors. Brain Research. 2015; 1600:70–83. DOI: 10.1016/j.brainres.2014.10.037. [PubMed: 25452020]

Fischbach GD, Lord C. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. Neuron. 2010; 68(2):192–195. DOI: 10.1016/j.neuron.2010.10.006. [PubMed: 20955926]

Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. J Am Acad Child Adolesc Psychiatry. 2014; 53(3):329–40.e1–3. DOI: 10.1016/j.jaac.2013.12.004. [PubMed: 24565360]

G R Alexander MK. Assessing the role and effectiveness of prenatal care: history, challenges, and directions for future research. Public Health Reports. 2001; 116(4):306. DOI: 10.4088/JCP.09m05087. [PubMed: 12037259]

Gagliardi L, Bellù R, Zanini R, Dammann O, Network Neonatale Lombardo Study Group. Bronchopulmonary dysplasia and brain white matter damage in the preterm infant: a complex relationship. Paediatr Perinat Epidemiol. 2009; 23(6):582–90. DOI: 10.1111/j.1365-3016.2009.01069.x. [PubMed: 19840295]

Gandal MJ, Anderson RL, Billingslea EN. Mice with reduced NMDA receptor expression: more consistent with autism than schizophrenia? Genes. 2012; 11(6):740–750. DOI: 10.1111/j.1601-183X.2012.00816.x.

Girirajan S, Brkanac Z, Coe BP, Baker C, Vives L, Vu TH, et al. Relative burden of large CNVs on a range of neurodevelopmental phenotypes. PLoS Genetics. 2011; 7(11):e1002334. DOI: 10.1371/journal.pgen.1002334. [PubMed: 22102821]

Girirajan S, Johnson RL, Tassone F, Balciuniene J, Katiyar N, Fox K, et al. Global increases in both common and rare copy number load associated with autism. Human Molecular Genetics. 2013; 22(14):2870–2880. DOI: 10.1093/hmg/ddt136. [PubMed: 23535821]

Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, Goldstein A, et al. Phenotypic heterogeneity of genomic disorders and rare copy-number variants. The New England Journal of Medicine. 2012; 367(14):1321–1331. DOI: 10.1056/NEJMoa1200395. [PubMed: 22970919]

Glynn RJ, Schneeweiss S, Stürmer T. Indications for propensity scores and review of their use in pharmacoepidemiology. Basic Clin Pharmacol Toxicol. 2006; 98(3):253–9. DOI: 10.1111/j.1742-7843.2006.pto_293.x. [PubMed: 16611199]

Goldin AB, Sawin RS, Garrison MM, Zerr DM, Christakis DA. Aminoglycoside-based triple-antibiotic therapy versus monotherapy for children with ruptured appendicitis. Pediatrics. 2007; 119(5):905–11. DOI: 10.1542/peds.2006-2040. [PubMed: 17473090]

Gotham K, Risi S, Pickles A, Lord C. The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. Journal of Autism and Developmental Disorders. 2007; 37(4):613–627. DOI: 1010.1007/s10803-009-0915-0. [PubMed: 17180459]

Grether JK, Li SX, Yoshida CK, Croen LA. Antenatal ultrasound and risk of autism spectrum disorders. J Autism Dev Disord. 2010; 40(2):238–245. DOI: 10.1007/s10803-009-0859-4. [PubMed: 19728066]

Gudex C, Nielsen BL, Madsen M. Why women want prenatal ultrasound in normal pregnancy. Ultrasound in Obstetrics & Gynecology : the Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2006; 27(2):145–150. DOI: 10.1002/uog.2646.

Houston LE, Allsworth J, Macones GA. Ultrasound is safe... right?: Resident and maternal-fetal medicine fellow knowledge regarding obstetric ultrasound safety. Journal of Ultrasound in Medicine : Official Journal of the American Institute of Ultrasound in Medicine. 2011; 30(1):21–27. DOI:10.1213/ANE.0b013e31821c36d4. [PubMed: 21193701]

Houston LE, Odibo AO, Macones GA. The safety of obstetrical ultrasound: a review. Prenatal Diagnosis. 2009; 29(13):1204–1212. DOI: 10.1002/pd.2392. [PubMed: 19899071]

Ivarsson SA, Bjerre I, Vegfors P, Ahlfors K. Autism as one of several disabilities in two children with congenital cytomegalovirus infection. Neuropediatrics. 1990; 21(2):102–103. http://doi.org/10.1055/s-2008-1071471. [PubMed: 2163029]

Jacquemont S, Coe BP, Hersch M, Duyzend MH, Krumm N, Bergmann S, et al. A higher mutational burden in females supports a “female protective model” in neurodevelopmental disorders. American Journal of Human Genetics. 2014; 94(3):415–425. DOI: 10.1016/j.ajhg.2014.02.001. [PubMed: 24581740]

Jensh RP, Lewin PA, Poczobutt MT, Goldberg BB, Oler J, Goldman M, Brent RL. Effects of prenatal ultrasound exposure on adult offspring behavior in the Wistar rat. Proc Soc Exp Biol Med. 1995; 210(2):171–179. DOI: 10.3181/00379727-210-43937. [PubMed: 7568288]

Johnson CP, Myers SM, American Academy of Pediatrics Council on Children With Disabilities. Identification and evaluation of children with autism spectrum disorders. Pediatrics. 2007; 120(5): 1183–1215. DOI: 10.1542/peds.2007-2361. [PubMed: 17967920]

Kalkbrenner AE, Schmidt RJ, Penlesky AC. Environmental chemical exposures and autism spectrum disorders: a review of the epidemiological evidence. Current Problems in Pediatric and Adolescent Health Care. 2014; 44(10):277–318. DOI: 10.1016/j.cppeds.2014.06.001. [PubMed: 25199954]

Kasarpalkar NJ, Kothari ST, Dave UP. Brain-Derived Neurotrophic Factor in children with Autism Spectrum Disorder. Annals of Neurosciences. 2014; 21(4):129–133. DOI: 10.5214/ans.0972.7531.210403. [PubMed: 25452672]

Kieler H, Axelsson O, Haglund B, Nilsson S, Salvesen KA. Routine ultrasound screening in pregnancy and the children's subsequent handedness. Early Human Development. 1998a; 50(2):233–245. DOI:10.1016/S0378-3782(97)00097-2. [PubMed: 9483394]

Kieler H, Cnattingius S, Palmgren J, Haglund B. First trimester ultrasound scans and left-handedness. Epidemiology and Psychiatric Sciences. 2002; 13(3):370. DOI: 10.1002/uog.8962.

Kieler H, Hellberg D, Nilsson S, Waldenström U, Axelsson O. Pregnancy outcome among non-participants in a trial on ultrasound screening. Ultrasound in Obstetrics & Gynecology : the Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology. 1998b; 11(2):104–109. DOI: 10.1046/j.1469-0705.1998.11020104.x.

Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willsey AJ, et al. Common genetic variants, acting additively, are a major source of risk for autism. Molecular Autism. 2012; 3(1):9. DOI: 10.1186/2040-2392-3-9. [PubMed: 23067556]

Koh J-Y, Lim JS, Byun H-R, Yoo M-H. Abnormalities in the zinc-metalloprotease-BDNF axis may contribute to megalencephaly and cortical hyperconnectivity in young autism spectrum disorder patients. Molecular Brain. 2014; 7:64. DOI: 10.1186/s13041-014-0064-z. [PubMed: 25182223]

Kundakovic M, Gudsnuk K, Herbstman JB, Tang D, Perera FP, Champagne FA. DNA methylation of BDNF as a biomarker of early-life adversity. Proceedings of the National Academy of Sciences. 2014 201408355. DOI: 10.1073/pnas.1408355111.

Kurth T, Walker AM, Glynn RJ, Chan KA, Gaziano JM, Berger K, Robins JM. Results of multivariable logistic regression, propensity matching, propensity adjustment, and propensity-

based weighting under conditions of nonuniform effect. Am J Epidemiol. 2006; 163(3):262–70. DOI: 10.1093/aje/kwj047. [PubMed: 16371515]

Lee BK, Magnusson C, Gardner RM, Blomström S, Newschaffer CJ, Burstyn I, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain, Behavior, and Immunity. 2014; 44(5):100–105. DOI: 10.1016/j.bbi.2014.09.001.

Lee E-J, Choi SY, Kim E. NMDA receptor dysfunction in autism spectrum disorders. Current Opinion in Pharmacology. 2015; 20:8–13. DOI: 10.1016/j.coph.2014.10.007. [PubMed: 25636159]

Lewis M, Kim SJ. The pathophysiology of restricted repetitive behavior. Journal of Neurodevelopmental Disorders. 2009; 1(2):114–132. DOI: 10.1007/s11689-009-9019-6. [PubMed: 21547711]

Li P, Wang P-J, Zhang W. Prenatal exposure to ultrasound affects learning and memory in young rats. Ultrasound in Medicine & Biology. 2015; 41(3):644–653. DOI: 10.1016/j.ultrasmedbio.2014.09.015. [PubMed: 25638314]

Liu Y, Li X-N, Sun X-R, Liu Q-L, Zha S-W, Chen Y-H, et al. Prenatal and neonatal risk factors associated with children's developmental status at ages 4-7: lessons from the Jiangsu China birth defects prevention cohort. Child: Care, Health and Development. 2015 DOI: 10.1111/cch.12225.

Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord. 2000; 30(3):205–223. DOI: 10.1023/A:1005592401947. [PubMed: 11055457]

Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord. 1994; 24(5):659–685. DOI: 10.1007/BF02172145. [PubMed: 7814313]

Louhivuori V, Vicario A, Uutela M, Rantamäki T, Louhivuori LM, Castrén E, et al. BDNF and TrkB in neuronal differentiation of Fmr1-knockout mouse. Neurobiology of Disease. 2011; 41(2):469–480. DOI: 10.1016/j.nbd.2010.10.018. [PubMed: 21047554]

Lund LH 1, Svennblad B, Melhus H, Hallberg P, Dahlström U, Edner M. Association of spironolactone use with all-cause mortality in heart failure: a propensity scored cohort study. Circ Heart Fail. 2013; 6(2):174–83. DOI: 10.1161/CIRCHEARTFAILURE.112.000115. [PubMed: 23386667]

Malkova NV, Yu CZ, Hsiao EY, Moore MJ, Patterson PH. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain, Behavior, and Immunity. 2012; 26(4):607–616. DOI: 10.1016/j.bbi.2012.01.011.

Mazina V, Gerdts J, Trinh S, Ankenman K, Ward T, Dennis MY, et al. Epigenetics of Autism-related Impairment: Copy Number Variation and Maternal Infection. J Dev Behav Pediatr. 2015; 36(2):61–67. DOI: 10.1097/DBP.0000000000000126. [PubMed: 25629966]

McCanlies EC, Fekedulegn D, Mnatsakanova A, Burchfiel CM, Sanderson WT, Charles LE, Hertz-Picciotto I. Parental occupational exposures and autism spectrum disorder. J Autism Dev Disord. 2012; 42(11):2323–2334. DOI: 10.1007/s10803-012-1468-1. [PubMed: 22399411]

McClintic AM, King BH, Webb SJ, Mourad PD. Mice Exposed to Diagnostic Ultrasound In Utero Are Less Social and More Active in Social Situations Relative to Controls. Autism Research : Official Journal of the International Society for Autism Research. 2013; 7(3):295–304. DOI: 10.1002/aur.1349. [PubMed: 24249575]

Meizner I. What do doctors understand regarding ultrasound safety during pregnancy? Harefuah. 2012; 151(4):234–6–252. DOI: 10.1002/pd.2392. [PubMed: 22616153]

Miller MT, Strömland K, Ventura L, Johansson M. Autism associated with conditions characterized by developmental errors in early embryogenesis: a mini review. International Journal of Developmental Neuroscience. 2005; 23(2):201–2019. DOI: 10.1016/j.ijdevneu.2004.06.007. [PubMed: 15749246]

Milunsky A, Ulcickas M, Rothman KJ, Willett W, Jick SS, Jick H. Maternal heat exposure and neural tube defects. JAMA : the Journal of the American Medical Association. 1992; 268(7):882–885. DOI: 10.1001/jama.1992.03490070064043. [PubMed: 1640616]

Moretti ME, Bar-Oz B, Fried S, Koren G. Maternal hyperthermia and the risk for neural tube defects in offspring: systematic review and meta-analysis. Epidemiology (Cambridge, Mass.). 2005; 16(2): 216–219. DOI: 10.1097/01.ede.0000152903.55579.15.

Mourad, PD. Therapeutic Ultrasound, with an Emphasis on Applications to the Brain.. In: Nakamura, K., Ueha, S., editors. Ultrasounic Transducers - Material Desgin and Applications. Woodhead Publishing Ltd; 2012. p. 545-568.

Myers RA, Casals F, Gauthier J, Hamdan FF, Keebler J, Boyko AR, et al. A population genetic approach to mapping neurological disorder genes using deep resequencing. PLoS Genetics. 2011; 7(2):e1001318. DOI: 10.1371/journal.pgen.1001318. [PubMed: 21383861]

Newnham JP, Doherty DA, Kendall GE, Zubrick SR, Landau LL, Stanley FJ. Effects of repeated prenatal ultrasound examinations on childhood outcome up to 8 years of age: follow-up of a randomised controlled trial. Lancet. 2004; 364(9450):2038–2044. DOI: 10.1016/S0140-6736(04)17516-8. [PubMed: 15582061]

Noonan K, Corman H, Schwartz-Soicher O, Reichman NE. Effects of Prenatal Care on Child Health at Age 5. Maternal and Child Health Journal. 2012; 17(2):189–199. DOI: 10.1007/s10995-012-0966-2.

O'Keeffe DF, Abuhamad A. Obstetric ultrasound utilization in the United States: data from various health plans. Seminars in Perinatology. 2013; 37(5):292–294. DOI: 10.1053/j.semperi.2013.06.003. [PubMed: 24176148]

O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. Nature Genetics. 2011; 43(6):585–589. DOI: 10.1038/ng.835. [PubMed: 21572417]

O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science. 2012; 338(6114): 1619–1622. DOI: 10.1126/science.1227764. [PubMed: 23160955]

Ouyang Q, Lizarraga SB, Schmidt M, Yang U, Gong J, Ellisor D, et al. Christianson syndrome protein NHE6 modulates TrkB endosomal signaling required for neuronal circuit development. Neuron. 2013; 80(1):97–112. DOI: 10.1016/j.neuron.2013.07.043. [PubMed: 24035762]

Pati S, Hashim K, Brown B, Fiks AG, Forrest CB. Early identification of young children at risk for poor academic achievement: preliminary development of a parent-report prediction tool. BMC Health Services Research. 2011; 11:197. DOI: 10.1186/1472-6963-11-197. [PubMed: 21851586]

Patterson PH. Maternal infection and immune involvement in autism. Trends in Molecular Medicine. 2011; 17(7):389–394. DOI: 10.1016/j.molmed.2011.03.001. [PubMed: 21482187]

Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, et al. Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. American Journal of Human Genetics. 2014; 94(5):677–694. DOI: 10.1016/j.ajhg.2014.03.018. [PubMed: 24768552]

Roberts EM, English PB. Bayesian modeling of time-dependent vulnerability to environmental hazards: an example using autism and pesticide data. Statistics in Medicine. 2013; 32(13):2308–2319. DOI: 10.1002/sim.5600. [PubMed: 22961924]

Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environmental Health Perspectives. 2007; 115(10):1482–1489. DOI: 10.1289/ehp.10168. [PubMed: 17938740]

Robinson EB, Lichtenstein P, Anckarsäter H, Happé F, Ronald A. Examining and interpreting the female protective effect against autistic behavior. Proceedings of the National Academy of Sciences. 2013; 110(13):5258–5262. DOI: 10.1073/pnas.1211070110.

Rodier PM. Converging evidence for brain stem injury in autism. Development and Psychopathology. 2002; 14(3):537–557. DOI: 10.1017.S0954579402003085. [PubMed: 12349873]

Salomon LJ, Alfirevic Z, Bilardo CM, Chalouhi G, Ghi T, Kagan K, et al. ISUOG Practice Guidelines: performance of first-trimester fetal ultrasound scan. Ultrasound in Obstetrics & Gynecology : the Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2012; 41(1):102–113. DOI: 10.1002/uog.12342.

Salvesen KA, Vatten LJ, Bakketeig LS, Eik-Nes SH. Routine ultrasonography in utero and speech development. Ultrasound in Obstetrics & Gynecology : the Official Journal of the International

Society of Ultrasound in Obstetrics and Gynecology. 1994; 4(2):101–103. DOI: 10.1046/j.1469-0705.1994.04020101.x.

Salvesen KA, Vatten LJ, Eik-Nes SH, Hugdahl K, Bakketeig LS. Routine ultrasonography in utero and subsequent handedness and neurological development. BMJ (Clinical Research Ed.). 1993; 307(6897):159–164. DOI: 10.1136/bmj.307.6897.159.

Sandin S, Nygren K-G, Iliadou A, Hultman CM, Reichenberg A. Autism and mental retardation among offspring born after in vitro fertilization. JAMA : the Journal of the American Medical Association. 2013; 310(1):75–84. DOI: 10.1001/jama.2013.7222. [PubMed: 23821091]

Saunders JA, Leitman VT, Suh J. Knockout of NMDA Receptors in Parvalbumin Interneurons Recreates Autism-Like Phenotypes. Autism. 2013; 6(2):69–77. DOI: 10.1002/aur.1264.

Schwartzer JJ, Careaga M, Onore CE, Rushakoff JA, Berman RF, Ashwood P. Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. Translational Psychiatry. 2013; 3:e240. DOI: 10.1038/tp.2013.16. [PubMed: 23481627]

Sheiner E, Shoham-Vardi I, Abramowicz JS. What do clinical users know regarding safety of ultrasound during pregnancy? Journal of Ultrasound in Medicine : Official Journal of the American Institute of Ultrasound in Medicine. 2007; 26(3):319–25. quiz 326–7. DOI: 10.1002/pd.2392. [PubMed: 17324981]

Sparrow SS. Vineland Adaptive Behavior Scales. Encyclopedia of Clinical Neuropsychology. 2011

Stativa E, Rus AV, Suciu N, Pennings JS, Butterfield ME, Wenyika R, Webster R. Characteristics and prenatal care utilisation of Romanian pregnant women. The European Journal of Contraception & Reproductive Health Care : the Official Journal of the European Society of Contraception. 2014; 19(3):220–226. DOI: 10.3109/13625187.2014.907399.

Stein TP, Schluter MD, Steer RA, Guo L, Ming X. Bisphenol A Exposure in Children With Autism Spectrum Disorders. Autism Research : Official Journal of the International Society for Autism Research. 2015 DOI: 10.1002/aur.1444.

Stewart HD, Stewart HF, Moore RM, Garry J. Compilation of reported biological effects data and ultrasound exposure levels. Journal of Clinical Ultrasound. 1985; 13(3):167–186. DOI: 10.1002/jcu.1870130304. [PubMed: 3920278]

Stoch YK, Williams CJ, Granich J, Hunt AM, Landau LI, Newnham JP, Whitehouse AJO. Are prenatal ultrasound scans associated with the autism phenotype? Follow-up of a randomised controlled trial. J Autism Dev Disord. 2012; 42(12):2693–2701. DOI: 10.1007/s10803-012-1526-8. [PubMed: 22456820]

Suresh R, Ramesh Rao T, Davis EM, Ovchinnikov N, Mc Rae A. Effect of diagnostic ultrasound during the fetal period on learning and memory in mice. Annals of Anatomy = Anatomischer Anzeiger : Official Organ of the Anatomische Gesellschaft. 2008; 190(1):37–45. DOI: 10.1016/j.aanat.2007.04.008. [PubMed: 18342141]

Suresh R, Uma Devi P, Ovchinnikov N, McRae A. Long-term effects of diagnostic ultrasound during fetal period on postnatal development and adult behavior of mouse. Life Sciences. 2002; 71(3):339–350. DOI: 10.1016/S0024-3205(02)01642-9. [PubMed: 12034351]

Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell. 2012; 149(3):525–537. http://doi.org/10.1016/j.cell.2012.03.028. [PubMed: 22521361]

Tarantal AF, Hendrickx AG. Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (Macaca fascicularis): I. Neonatal/infant observations. Teratology. 1989; 39(2):137–147. DOI: 10.1002/tera.1420390206. [PubMed: 2648634]

Testa C, Nuti F, Hayek J, De Felice C, Chelli M, Rovero P, et al. Di-(2-ethylhexyl) phthalate and autism spectrum disorders. ASN Neuro. 2012; 4(4):223–229. DOI: 10.1042/AN20120015. [PubMed: 22537663]

Thomas KC, Parish SL, Rose RA, Kilany M. Access to care for children with autism in the context of state Medicaid reimbursement. Maternal and Child Health Journal. 2012a; 16(8):1636–1644. DOI: 10.1007/s10995-011-0862-1. [PubMed: 21833759]

Thomas P, Zahorodny W, Peng B, Kim S, Jani N, Halperin W, Brimacombe M. The association of autism diagnosis with socioeconomic status. Autism. 2012b; 16(2):201–213. DOI: 10.1177/1362361311413397. [PubMed: 21810908]

Torrey EF, Hersh SP, McCabe KD. Early childhood psychosis and bleeding during pregnancy. A prospective study of gravid women and their offspring. Journal of Autism and Childhood Schizophrenia. 1975; 5(4):287–297. DOI: 10.1007/BF01540676. [PubMed: 1243134]

Werling DM, Geschwind DH. Understanding sex bias in autism spectrum disorder. Proceedings of the National Academy of Sciences. 2013; 110(13):4868–4869. DOI: 10.1073/pnas.1301602110.

Williams EL, Casanova MF. Potential teratogenic effects of ultrasound on corticogenesis: implications for autism. Medical Hypotheses. 2010; 75(1):53–58. DOI: 10.1016/j.mehy.2010.01.027. [PubMed: 20149552]

Wood A. Prenatal exposure to sodium valproate is associated with increased risk of childhood autism and autistic spectrum disorder. Evidence-Based Nursing. 2014; 17(3):84. DOI: 10.1136/eb-2013-101422. [PubMed: 23999195]

Yamashita Y, Fujimoto C, Nakajima E, Isagai T, Matsuishi T. Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord. 2003; 33(4):455–459. DOI:10.3109/13550281003685839. [PubMed: 12959425]

Yoo HJ, Cho IH, Park M, Yang SY, Kim SA. Family based association of GRIN2A and GRIN2B with Korean autism spectrum disorders. Neuroscience Letters. 2012; 512(2):89–93. DOI: 10.1016/j.neulet.2012.01.061. [PubMed: 22326929]

You JJ, Alter DA, Stukel TA, McDonald SD, Laupacis A, Liu Y, Ray JG. Proliferation of prenatal ultrasonography. CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne. 2010; 182(2):143–151. DOI: 10.1503/cmaj.090979.

Zachor DA, Ben Itzchak E. Assisted reproductive technology and risk for autism spectrum disorder. Research in Developmental Disabilities. 2011; 32(6):2950–2956. DOI: 10.1016/j.ridd.2011.05.007. [PubMed: 21658904]

Table 1

Proband Age, mean Standard Test scores (SD), and regression results for all children with ASD with known absence or presence of identified CNV, and absence (‘No U1’) or presence (‘U1’) of first trimester ultrasound.

Total N = 1749	**ASD No U1**	**ASD U1**	**Beta**	**95%CI**	**p-value**
N	668	1081			
Age (mos)	117.7 (44.2)	101.6 (40.3)			
Adaptive and Cognitive					
VABS-II	72.6 (11.5)	73.6 (12.1)	−0.73	−1.87 to 0.43	0.22
Verbal IQ	77.5 (31.4)	78.5 (30.4)	0.13	−3.02 to 3.28	0.94
Nonverbal IQ	85.3 (26.3)	84.9 (26.1)	−1.28	−3.94 to 1.38	0.35
Social Affective Behaviors					
ADOS SA	**11.3 (4.0)**	**11.0 (4.0)**	**−0.45**	**−0.86 to −0.04**	**0.03**
ADI-R SD	20.4 (5.6)	20.1 (5.8)	0.28	−0.29 to 0.85	0.33
SRS	79.9 (9.8)	79.5 (10.5)	0.53	−0.50 to 1.56	0.31
Repetitive Behaviors					
ADOS REP	3.9 (2.1)	4.0 (2.0)	−0.02	−0.23 to 0.18	0.84
ADI-R RRB	**6.4 (2.5)**	**6.6 (2.5)**	**0.34**	**0.09 to 0.60**	**0.008**
RBS-R	26.7 (17.0)	27.7 (17.0)	1.36	−0.38 to 3.10	0.13

Table 2

Proband Age, mean Standard Test scores (SD), and regression results for all children with ASD, with an identified CNV, and absence (‘No U1’) or presence (‘U1’) of first trimester ultrasound.

ASD with CNV N = 133	**ASD+CNV No U1**	**ASD+CNV U1**	**Beta**	**95%CI**	**p-value**
N	49	84			
Age (mos)	118.5 (42.5)	111.4 (41.4)			
Adaptive and Cognitive					
VABS-II	*73.5 (10.1)*	*71.0 (13.4)*	*−4.11*	*−8.56 to 0.35*	*0.07*
Verbal IQ	81.8 (32.6)	73.6 (30.5)	−9.39	−21.37 to 2.59	0.12
Nonverbal IQ	**87.7 (27.6)**	**76.8 (25.8)**	**−11.16**	**−21.08 to −1.24**	**0.03**
Social Affective Behaviors					
ADOS SA	10.7 (3.8)	11.4 (3.9)	0.90	−0.59 to 2.38	0.23
ADI-R SD	20.9 (5.3)	20.9 (5.9)	0.42	−1.73 to 2.56	0.70
SRS	77.9 (12.3)	78.6 (11.0)	2.24	−2.13 to 6.62	0.31
Repetitive Behaviors					
ADOS REP	*3.5 (2.1)*	*4.2 (1.9)*	*0.69*	*−0.04 to 1.43*	*0.06*
ADI-R RRB	6.5 (2.6)	7.0 (2.8)	0.71	−0.36 to 1.77	0.19
RBS-R	23.2 (14.1)	26.6 (15.2)	4.01	−1.72 to 9.74	0.17

Table 3

Proband Age, mean Standard Test scores (SD), and regression results for male children with ASD, with an identified CNV, and absence (‘No U1’) or presence (‘U1’) of first trimester ultrasound.

Males with CNV N = 111	**Male CNV No U1**	**Male CNV U1**	**Beta**	**95%CI**	**p-value**
N	38	73			
Age (mos)	115.8 (39.6)	113.7 (42.2)			
Adaptive and Cognitive					
VABS-II	74.7 (10.3)	71.5 (13.0)	−4.15	−9.04 to 0.75	0.10
Verbal IQ	*86.5 (32.5)*	*75.1 (29.7)*	*−11.29*	*−24.32 to 1.73*	*0.09*
Nonverbal IQ	**92.6 (26.8)**	**78.6 (24.4)**	**−12.43**	**−23.11 to −1.76**	**0.02**
Social Affective Behaviors					
ADOS SA	10.6 (3.6)	11.5 (3.6)	1.14	−0.39 to 2.67	0.14
ADI-R SD	20.7 (5.0)	20.7 (6.0)	−.052	−2.39 to 2.89	0.97
SRS	77.1 (11.8)	78.0 (11.2)	1.87	−2.94 to 6.68	0.44
Repetitive Behaviors					
ADOS REP	**3.4 (2.2)**	**4.2 (1.8)**	**0.90**	**0.12 to 1.69**	**0.02**
ADI-R RRB	6.3 (2.6)	7.1 (2.8)	0.92	−0.25 to 2.09	0.12
RBS-R	*22.0 (12.9)*	*26.8 (15.4)*	*5.47*	*−0.73 to 11.67*	*0.08*