J Autism Dev Disord (2017) 47:2621–2627
DOI 10.1007/s10803-017-3169-2

BRIEF REPORT

Brief Report: Association Between Autism Spectrum Disorder, Gastrointestinal Problems and Perinatal Risk Factors Within Sibling Pairs

Johan Isaksson[1,2,3] · **Erik Pettersson**[4] · **Elzbieta Kostrzewa**[1] · **Rochellys Diaz Heijtz**[5] · **Sven Bölte**[1,6]

Published online: 23 May 2017

Abstract Autism spectrum disorder (ASD) has been associated with gastrointestinal (GI) problems, but the nature of this association is unclear. Parents to siblings, concordant or discordant for ASD ($N=217$), participated in a web survey covering mother's weight gain during pregnancy, maternal viral/bacterial infection and use of antibiotics, duration of breastfeeding, mode of delivery, birth weight and child GI problems. ASD was associated with GI problems and perinatal environmental risk, based on a summation of maternal infection and antibiotic use during pregnancy and/or the breastfeeding period. The association between GI problems and ASD remained within the sibling pairs ($\beta=1.23$; $p<.001$) in the adjusted model. Our results indicate non-shared environmental effects on the ASD/GI association, but none of the factors examined explained the link.

Keywords Autism spectrum disorder · Gastrointestinal problems · Siblings · Infections · Breastfeeding · Cesarean section

Introduction

In recent years, there has been an increasing interest on the role of gut microbiota in brain development and behavior, including the development of brain circuitries that control learning, emotional and social behavior (Diaz Heijtz 2016). One neuro developmental disorder that exemplifies this association, with an increasing number of gastrointestinal (GI) problems and an altered composition of the gut microbiota, is autism spectrum disorder (ASD). Children with ASD have been reported to be six to eight times more susceptible to GI problems, such as abdominal pain, diarrhea and flatulence, than typically developing individuals (Adams et al. 2011; Chaidez et al. 2014) and the prevalence of GI problems among children with ASD varies between 24–70%, depending on study design, participants' age and assessments applied (Krajmalnik-Brown et al. 2015). Furthermore, an altered gut microbial composition has been described in children with ASD, with lower gut bacterial diversity and an underrepresentation of potentially beneficial bacteria, such as Bifidobacterium, Prevotella and Desulfovibrio, as well as a reduction of short-chain fatty acids (Vuong and Hsiao 2017; Kang et al. 2017). These findings indicate that the gut–brain axis may be of importance in ASD.

The gut microbiota is a complex system with a variety of microbial species including bacteria, fungi and archaea

Electronic supplementary material The online version of this article (doi:10.1007/s10803-017-3169-2) contains supplementary material, which is available to authorized users.

✉ Johan Isaksson
johan.isaksson@ki.se

1 Department of Women's and Children's Health, Pediatric Neuropsychiatry Unit, Center for Neurodevelopmental Disorders at Karolinska Institutet (KIND), Karolinska Institutet, Stockholm, Sweden

2 Center for Psychiatry Research, Stockholm County Council, Stockholm, Sweden

3 Department of Neuroscience, Child and Adolescent Psychiatry Unit, Uppsala University, Uppsala, Sweden

4 Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

5 Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

6 Child and Adolescent Psychiatry, Center for Psychiatry Research, Stockholm County Council, Stockholm, Sweden

(Dinan and Cryan 2016; Li and Zhou 2016). There is a bidirectional communication between the gut and the brain through immune, neuronal or endocrine pathways (gut–brain axis) and the gut microbiota development coincides with critical windows of brain development (Diaz Heijtz 2016; Dinan and Cryan 2016). The assembly of human gut microbiota typically occurs during the first 2 to 3 years of postnatal life and is influenced by genetic factors as well as perinatal factors. Also, there is evidence that bacteria are present before birth in the amniotic fluid (Jimenez et al. 2008) and in the placenta (Wassenaar and Panigrahi 2014). During birth, the mother's vaginal and fecal microbial microbiota colonizes the child's microbiota, while children born by cesarean section (C-section) instead are colonized with bacteria from their mother's skin and, to a larger extent than children born vaginally, from the delivery location (Adlerberth and Wold 2009; Shin et al. 2015). Furthermore, exposure to antibiotics, infections, diet (formula), preterm birth and fever during pregnancy has been shown to affect the assembly of the gut microbiota (Adlerberth and Wold 2009; Marques et al. 2010; Diaz Heijtz 2016).

There is a general consensus that heritability is high in ASD, with estimates ranging between 64–91% (Tick et al. 2016). However, the exact genetic factors driving ASD phenotypes are still largely unknown and environmental and epigenetic factors have been suggested to contribute to risk for ASD (Risch et al. 2014). Animal studies suggest that the gut microbiota may act as an "environmental agent" that modulates the development of brain circuitries involved in emotion, cognition and social behavior, thus raising the possibility that disturbances in gut microbiota during early life might contribute to the pathophysiology of ASD (Diaz Heijtz et al. 2011; Arentsen et al. 2015, 2017). In line with this speculation, factors that are known to alter the infant gut microbiota such as C-section (Curran et al. 2015a) and absence of breastfeeding (Schultz et al. 2006) have been associated with an increased risk of ASD. Still, findings are inconclusive, with some reports showing no association of breastfeeding and ASD (Husk and Keim 2015) or C-section and ASD when using siblings as controls, indicating genetic familial or shared environment influences (Curran et al. 2015b).

Using a sibling control approach, this study sought to further investigate the associations between GI problems and ASD, also taking into consideration non-shared and specific early environmental factors that have been suggested to moderate this association. These include C-section, exposure to antibiotics, maternal infections, diet, and birthweight. Previous studies have shown that non-shared factors represent a major environmental source of variance for problem behavior (Plomin and Daniels 2011). Therefore, by using siblings close in age, an adjustment is introduced as approximately 50% of the genome. During meiosis, parents' genes are combined randomly such that there is an equal likelihood that siblings will share the same genes from the mother and father. Also, several factors that can be attributed to shared environment are controlled for in such a design (for a review on sibling-comparisons design, see Lahey and D'Onofrio 2010). Most studies on ASD and GI problems have been conducted in North America and predominantly used classical case–control designs. Independent cross-cultural studies applying siblings design are lacking. Thus, we aimed to investigate whether previous results of associations between GI problems and ASD can be replicated in a Scandinavian sample of siblings, concordant or discordant for ASD, and furthermore, whether perinatal risk factors contribute to this relationship.

Methods

Participants and Procedure

Parents with at least two children (closest in age) under age 10 years, where one or both of the children had been diagnosed with a neurodevelopmental disorder, or both offspring were typically developed, were invited to participate in this survey-based study. Participants were recruited via advertisements posted at various websites, newsletters, journals, Facebook pages or local offices of the Karolinska Institutet Center of Neurodevelopmental Disorders (KIND), autism center for young children (Habilitation and Health services, Stockholm County Council), and the two major interest organizations for neurodevelopmental conditions, the Autism and Asperger Association in Stockholm County and the National Association Attention. The study was approved by the Regional Ethical Review Board in Stockholm.

In total, $N=217$ families were included ($N=434$ children). The informants were mothers ($n=197$), fathers ($n=4$), both parents ($n=15$), and unspecified ($n=1$). After excluding participants where diagnosis was reported as unclear (i.e. under evaluation or traits), $n=415$ children remained for the analysis, of which $n=206$ had an ASD diagnosis ($n=162$, 78% males, *Md* age 7 years) and $n=209$ had no ASD diagnosis ($n=209$, 52.7% males, Md age 6 years) as reported by the parents. With regard to sibling status, 20 pairs of siblings were concordant for typical development, 160 sibling pairs were discordant for ASD, and 18 were concordant for ASD. Almost all of the participating parents were born in Europe (97.1% of the mothers and 95.2% of the fathers); a majority had studied at university (65.7 and 51%, respectively) and still lived together (80%). Data on diagnosis, perinatal information and GI problems are presented in Table 1. For C-section delivery, there were

Table 1 Data on sex, age, diagnosis, perinatal data and GI-symptoms

	ASD n = 206	non ASD n = 209
Sex Males n (%)	162 (78.6)	108 (52.7)
Age (in years)		
Median (Q1–Q3)	7 years (5–9)	6 years (4–8.5)
Diagnoses %		
Autism	54.9	–
Asperger's syndrome	19.9	–
Atypical autism/PDD-NOS	25.2	–
NDD other[a]	46.8	10.2
Birthweight %		
<2 kg	2.4	1.0
2–3 kg	16	15.1
3–4 kg	63.1	62.0
>4 kg	18.4	22.0
Maternal weight gain during pregnancy		
Median (Q1–Q3)	14–16 kg (11–20 kg)	14–16 kg (11–20 kg)
Maternal viral/bacterial infection%		
Prenatal	47.6	40.4
Postnatal	25.5	24.8
Maternal use of antibiotics%		
Prenatal	16.9	14.0
Postnatal	13.8	13.5
Breastfeeding duration		
<6 Months %	36.3	31.6
Born with C-section %	22.8	26.8
GI-symptoms[b]	2.12	1.67

[a]*NDD* neurodevelopmental disorders other than ASD, including ADHD, learning disorders, intellectual disability, motor disorders (incl tic disorders)

[b]GI-symptoms were scored as the mean of six symptoms (diarrhea, abdominal pain, flatulence, constipation, vomiting, other intestinal problems), each of which were rated on a five-point scale

27 discordant pairs. Median age was 7 years for the siblings with ASD and 6 years for siblings without ASD diagnosis.

Web Survey

Participants completed a web survey with 114 closed items and open questions covering the socio-economic (e.g. parent educational level) and medical background of the parents, health and diet of the mother during pregnancy, breastfeeding, and the child's medical history and nutrition during infancy. There was also a separate section with questions on the sibling closest by age to the index child with ASD. Items shared by both children are presented in Supplementary Table S1. The web survey was designed using the Textalk AB websurvey program.

In this study, diagnoses of autism, Asperger's syndrome, and pervasive developmental disorder not otherwise specified (PDD-NOS) were combined to ASD. Other neurodevelopmental disorders, including ADHD, learning disorders, intellectual disability, and motor disorders including tic disorders were categorized as presence of neurodevelopmental disorder comorbidity.

Diarrhea, abdominal pain, flatulence, constipation, vomiting, and other intestinal problems were classified as GI problems. The parent rated the presence of GI problems on a five-point scale (1 = almost never, 2 = seldom, 3 = every month, 4 = every week, 5 = every day). Data on prenatal factors included the presence of maternal viral or bacterial infection (rated as yes or no), use of antibiotics during pregnancy (yes, no) and maternal weight gain during pregnancy on a five-point scale (<10, 11–13, 14–16, 17–20, >20 kg). Birth data consisted of information on mode of delivery and birthweight (scored as <2, 2–3, 3–4 and >4 kg). Postnatal factors included length of breastfeeding on a three-point scale (never, up to 6 months, >6 months), maternal viral or bacterial infection (yes, no) and use of antibiotics during breastfeeding (yes, no).

Statistical Analysis

Since the outcome (ASD diagnosis) was dichotomous, we used logistic regression. Because the observations were nested within individuals, we used the sandwich estimator to estimate unbiased standard errors. In order to control all time-invariant confounds within sibling pairs, we used fixed effects regression (Allison 2009). Unmeasured time-invariant confounds are controlled by allowing the pair outcome intercept to correlate with the predictor(s). All analyses were performed with the R software (R Core Team 2015) using the drgee package (Zetterqvist et al. 2016).

In order to reduce the number of predictors and thereby protect against multiple testing and p-hacking, we combined several variables into scales. The parents rated six GI problems (diarrhea, abdominal pain, gas, constipation, vomiting and other intestinal problems) on a five-point Likert scale and the ratings on the six items were averaged into a single scale score, ranging from 1 to 5, where a higher score indicates more GI problems (internal consistency: *Cronbach's alpha* = 0.76). Four items measuring environmental risk, including maternal viral/bacterial infection and use of antibiotics during pregnancy and/or breastfeeding, were averaged into an environmental risk scale. This scale ranged from 0 (no infection/antibiotics) to 2 (both infection and antibiotics were present during pregnancy as well as during breastfeeding; internal consistency: *Cronbach's alpha* = 0.63).

In the first model, we investigated the association between GI problems and ASD using a regular linear regression. Next, we used the fixed within-pair analysis, including environmental predictors and also controlling for sex, age, birthweight and mother's weight gain during pregnancy. Interactions between GI problems and environmental risk, breastfeeding and C-section were tested, and we re-ran the model excluding non-significant interactions. We also included comorbidity, which had a correlation with ASD ($r = .64$). Finally, as a post-hoc analysis, we re-calculated the model with the environmental risk factors included individually in order to investigate whether any environmental risk were more associated with ASD. Furthermore, as a post-hoc analysis, we also investigated whether the association between GI problems and ASD split into DSM-IV subgroups.

Because we used logistic regression, the betas are in log odds form such that when the predictors increase one unit, the outcome increases log odds. These, in turn, can be exponentiated into odds ratios such that when the predictors increase one unit, the expected change in the outcome is described in terms of odds.

Results

Among the ASD cases, 12% reported diarrhea, 19.9% abdominal pain, 24.8% gas, 18.7% constipation, 0.5% vomiting and 6.8% other GI symptoms every week or every day. Among the non-ASD siblings, the prevalence of GI symptoms weekly or daily were 6.1, 9.9, 10.8, 4.5, 1.5 and 5.8% respectively.

There was an association between GI problems and ASD, both in the regular linear regression ($\beta = 0.70$; $p < .001$) and in the fixed effect regression within sibling pairs ($\beta = 1.17$; $p < .001$). The within-pair association between GI problems and ASD is presented in Fig. 1.

Both in the regular regression and in the fixed regression, the association between GI problems and ASD remained ($\beta = 0.78$, $p < .001$; and $\beta = 1.23$, $p < .001$, respectively) after adjusting for sex, age, birthweight and mother's weight gain during pregnancy and including perinatal environmental risk factors (consisting of a combined risk score representing the additive effects of maternal infection and use of antibiotics during pregnancy and/or breastfeeding), duration of breastfeeding, and mode of delivery. In the regular regression, C-section was negatively associated with ASD ($\beta = -0.80$; $p = .003$) and there was a trend that environmental risk was positively associated with ASD ($\beta = 0.66$; $p = .08$). The association between C-section and ASD disappeared in the within-pairs regression. However, the association between environmental risk and ASD was strengthened ($\beta = 2.03$; $p = .004$) (Table 2). No interaction effects were found between environmental factors, breastfeeding, C-section and GI problems on risk of belonging to the ASD group. When adding comorbidity into the model, the association between environmental risk and ASD was lost.

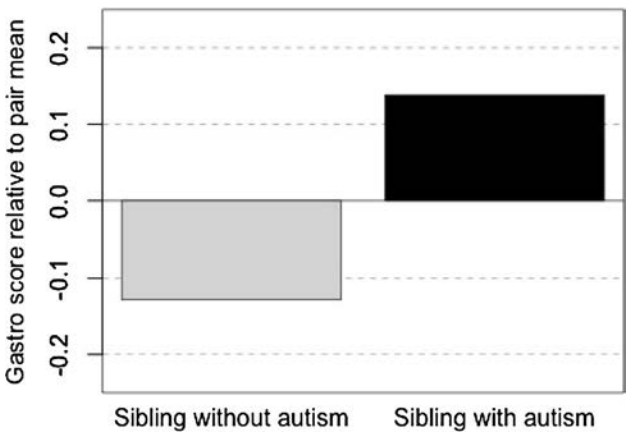

Fig. 1 Siblings discordant for autism as a function of gastrointestinal problems

Table 2 Autism regressed on GI problems and environmental variables, after removing non-significant interactions

	Predictor							
	Regular regression				Fixed effects regression			
OUTCOME	Gastro problems	Environmental risk	Length of nursing	C-section	Gastro problems	Environmental risk	Length of nursing	C-section
Autism	**0.78 (0.17)**	0.66 (0.37)	−0.18 (0.15)	**−0.80 (0.27)**	**1.23 (0.29)**	**2.03 (0.70)**	−0.26 (0.31)	−0.83 (0.54)

Bolded are significant at $p < .05$ (without correction for multiple testing)

Controlling age, sex, birthweight, and mother's weight gain during pregnancy

Logistic regression betas. Standard errors presented in parentheses

Environmental risk scale consisted of sum of viral and bacterial infection and antibiotic treatment during pregnancy and nursing

In the post-hoc analysis, with the environmental risk factors included individually, only maternal viral or bacterial infection during breastfeeding predicted belonging to the ASD group ($\beta = 1.91$; $p = .004$); see Supplementary Table S2. The association between GI problems and autism remained when split into DSM-IV subgroups, with the exception of Asperger's syndrome, possibly due to limited power in the model; see Supplementary Table S3.

Discussion

This study examined the relation between pre- and postnatal factors, GI problems and ASD within sibling pairs with and without ASD. It is to our knowledge one of the first larger studies using such a design and the first to examine a cohort outside North America. We found an association between GI problems and ASD. There was also an association between an environmental risk index based on the presence of maternal viral/bacterial infection and use of antibiotics during pregnancy and/or the breastfeeding period and ASD. However, these perinatal environmental factors did not moderate the association between GI problems and ASD. Thus, neither maternal infection, nor use of antibiotics, nor mode of delivery nor duration of breastfeeding could explain the higher ratings of symptoms of diarrhea, abdominal pain, flatulence, constipation and vomiting among the autistic siblings. Importantly, we are the first to demonstrate that the ASD/GI problems association is independent of specific non-shared environmental factors. The association between environmental risk and ASD was, however, lost when adjusting for comorbidity, implying that environmental risk is not specific for ASD, but perhaps broader, comprising neurodevelopmental conditions in general.

Our results confirm previous research indicating that GI problems in children and adults with ASD are frequent (Adams et al. 2011; Chaidez et al. 2014; Krajmalnik-Brown et al. 2015). In addition, we found that the association between GI problems and ASD remained between siblings, showing that the association is not driven by shared familial (environmental) influences. Rather the association appears to be causal and driven by non-shared environmental factors. Previous studies have described several non-shared environmental factors to be associated with both an altered gut microbiota and ASD. These include premature birth, C-section delivery, absence of breastfeeding, and exposure to antibiotics or infections during the perinatal period (Adlerberth and Wold 2009; Curran et al. 2015a; Marques et al. 2010; Schultz et al. 2006). Also, within the post-hoc analyses, the association between GI problems and autism seemed to be comparable between the autism subgroups.

Several of these non-shared environmental factors were included in our model. In contrast to what was expected from previous literature (Curran et al. 2015a), being born by C-section was associated with a lower risk of belonging to the ASD group in the regular regression. The negative association disappeared, however, when we adjusted for familial effects using within-pair statistics. The latter highlights the importance of sibling design. Our results are consistent with a recent study by Curran et al. (2015b) demonstrating that the positive association between C-section and ASD described in previous studies disappears when using siblings as controls. Thus, the link between mode of delivery and ASD is probably not causal, but rather reflects either shared environmental or genetic effects. Interestingly, the association between maternal infection (viral or bacterial) and/or use of antibiotics (pregnancy or breastfeeding period) and ASD remained and was also strengthened by using a sibling design. However, the duration of breastfeeding was not associated with ASD in our model. Our findings are in agreement with prior research linking extensive antibiotic use (Bölte 1998; Niehus and Lord 2006), a higher burden of mercury (Adams et al. 2007) and a higher incidence of ear infections (Konstantareas and Homatidis 1987; Niehus and Lord 2006) with ASD, and with well-known associations between perinatal factors

such as maternal infection during pregnancy and autism (Gardener et al. 2011).

In this study, the association between GI problems and ASD was independent of common perinatal risk factors (i.e., maternal viral or bacterial infection and use of antibiotics during pregnancy and/or the breastfeeding period). Thus, the underlying reasons for why ASD is associated with an increased number of GI problems are likely to involve other factors. One such potential factor is maternal stress during pregnancy, which may alter the gut microbiota and barrier function (For a review, see Jašarević et al. 2015). Although we made an adjustment for shared familial effects to the association by including siblings, the adjustment is incomplete and genetic effects cannot be ruled out. A twin difference design would be more appropriate to explore this matter further. Moreover, it would be interesting to investigate potential association with polymorphisms in the MET (proto-oncogene receptor tyrosine kinase), where the C allele has been reported to be more frequent in a subset of ASD patients with GI problems (Campbell et al. 2009). The association between GI problems and ASD may also have implications for future treatment options. In a recent study by Kang et al. (2017), a microbiota transfer therapy (FMT) including antibiotic treatment, bowel cleanse and extended FMT resulted in an increased bacterial diversity and a reduction in GI problems as well as in autism symptoms in children with ASD.

There are several limitations to this study that deserve to be addressed. Our study exclusively relies on parent reports, which may be biased for several reasons. For example, there is a risk of recall bias (under-reporting or over-reporting) in case–control studies where the disease has already occurred when exposure information is obtained. Parents may be prone to over-report when seeking for an explanation for the disease or when they have an assumption about its underlying cause. For a focus on neurodevelopmental diagnoses it would be preferable to use data from other information sources besides the parents, such as medical records and structured diagnostic interviews. The sibling without diagnosis at the time of the survey may receive a diagnosis later in life. Nevertheless, we adjusted for age and sex in the analyses. Due to the design of the survey, several items were divided into categories and, hypothetically, the results could differ if, for example, duration of breastfeeding was measured on a monthly basis instead of in 6-month categories. The decision to use a categorization at 6 months of breastfeeding is in line with previous findings on the effect of breastfeeding up to 6 months on child cognitive development (Quigley et al. 2012). We also lack more detailed information regarding the timing of perinatal infection and treatment with antibiotics. The GI problems investigated were chosen based on the ROME criteria for functional gastrointestinal disorders (Rome foundation). However, today there are established scales with good psychometric properties that could have been used instead, such as the Gastrointestinal Symptom Rating Scale (Dimenas et al. 1995) and the questionnaire on pediatric gastrointestinal symptoms.

In summary, we found an association between ASD and GI problems, which was independent of shared familial confounders, thus indicating influences of non-shared environmental effects on the association. However, none of the environmental factors examined in our study explained the association. Future studies on the GI/ASD association investigating twins, which would enable the analysis of differential effects of genetic, shared- and non-shared environmental effects, are desirable.

Acknowledgments This work was supported by Swedish Research Council (SB, RDH), Strategic Research Program in Neuroscience at Karolinska Institutet (RDH and SB) and by EU-AIMS (European Autism Interventions), which receives support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no. 115300, the resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (Grant FP7/2007–2013), from the European Federation of Pharmaceutical Industries and Associations companies' in-kind contributions, and from Autism Speaks. We are grateful to all the parents who participated in the survey, and to the Autism and Asperger Association in Stockholm County and the National Association Attention for support in recruitment. We also want to thank Varvara Sjölander Gogulan for her part in the study.

Author Contributions JI drafted the manuscript; EP performed the statistical analyses; EK had responsibility for the data collection and coordination; RHD and SB were responsible for the planning and design of the study and its data collection. All authors contributed to the interpretation of results, reviewed the manuscript critically, and approved the final version.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest related to this study. Bölte discloses that he has in the last 3 years acted as an author, consultant or lecturer for Shire, Medice, Roche, Eli Lilly, Prima Psychiatry, GLGroup, System Analytic, Kompetento, Expo Medica, Prophase, and receives royalties for text books or diagnostic instruments from Huber/Hogrefe, Kohlhammer and UTB.

Ethical Approval All procedures 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 after the nature of the procedure had been fully explained.

References

Adams, J. B., Johansen, L. J., Powell, L. D., Quig, D., & Rubin, R. A. (2011). Gastrointestinal flora and gastrointestinal status in children

with autism—comparisons to typical children and correlation with autism severity. *BMC Gastroenterology, 11*, 22.

Adams, J. B., Romdalvik, J., Ramanujam, V. M., & Legator, M. S. (2007). Mercury, lead, and zinc in baby teeth of children with autism versus controls. *Journal of Toxicology and Environmental Health A*, *70*(12), 1046–1051.

Adlerberth, I., & Wold, A. E. (2009). Establishment of the gut microbiota in Western infants. *Acta Paediatrica, 98*(2), 229–238.

Allison, P. (2009). *Fixed effects regression models*. Los Angeles: Sage Publications.

Arentsen, T., Qian, Y., Gkotzis, S., Femenia, T., Wang, T., Udekwu, K., Forssberg, H., & Diaz Heijtz, R. (2017). The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior. *Molecular Psychiatry, 22*, 257–266.

Arentsen, T., Raith, H., Qian, Y., Forssberg, H., & Diaz Heijtz, R. (2015). Host microbiota modulates development of social preference in mice. *Microbial Ecology in Health and Disease, 15*(26), 29719.

Bölte, E. R. (1998). Autism and Clostridium tetani. *Medical Hypotheses, 51*(2), 133–144.

Campbell, D. B., Buie, T. M., Winter, H., Bauman, M., Sutcliffe, J. S., Perrin, J. M., & Levitt, P. (2009). Distinct genetic risk based on association of MET in families with co-occurring autism and gastrointestinal conditions. *Pediatrics, 123*(3), 1018–1024.

Chaidez, V., Hansen, R. L., & Hertz-Picciotto, I. (2014). Gastrointestinal problems in children with autism, developmental delays or typical development. *Journal of Autism and Developmental Disorders, 44*(5), 1117–1127.

Curran, E. A., Dalman, C., Kearney, P. M., Kenny, L. C., Cryan, J. F., Dinan, T. G., & Khashan, A. S. (2015b). Association between obstetric mode of delivery and autism spectrum disorder: A population-based sibling design study. *JAMA Psychiatry, 72*(9), 935–942.

Curran, E. A., O'Neill, S. M., Cryan, J. F., Kenny, L. C., Dinan, T. G., Khashan, A. S., & Kearney, P. M. (2015a). Research review: Birth by caesarean section and development of autism spectrum disorder and attention-deficit/hyperactivity disorder: A systematic review and meta-analysis. *Journal of Child Psychology and Psychiatry, 56*(5), 500–508.

Diaz Heijtz, R. (2016). Fetal, neonatal, and infant microbiome: Perturbations and subsequent effects on brain development and behavior. *Seminars in Fetal Neonatal Medicine, 21*(6), 410–417.

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., & Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America, 108*(7), 3047–3052.

Dimenas, E., Glise, H., Hallerback, B., Hernqvist, H., Svedlund, J., & Wiklund, I. (1995). Well-being and gastrointestinal symptoms among patients referred to endoscopy owing to suspected duodenal ulcer. *Scandinavian Journal of Gastroenterology, 30*(11), 1046–1052.

Dinan, T. G., & Cryan, J. F. (2016). Microbes, immunity, and behavior: psychoneuroimmunology meets the microbiome. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 42*(1), 178–192.

Gardener, H., Spiegelman, D., & Buka, S. L. (2011). Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. *Pediatrics, 128*(2), 344–355.

Husk, J. S., & Keim, S. A. (2015). Breastfeeding and autism spectrum disorder in the National Survey of Children's Health. *Epidemiology, 26*(4), 451–457.

Jašarević, E., Rodgers, A. B., & Bale, T. L. (2015). A novel role for maternal stress and microbial transmission in early life programming and neurodevelopment. *Neurobiology of Stress, 1*, 81–88.

Jimenez, E., Marin, M. L., Martin, R., Odriozola, J. M., Olivares, M., Xaus, J., & Rodriguez, J. M. (2008). Is meconium from healthy newborns actually sterile? *Research in Microbiology, 159*(3), 187–193.

Kang, D. W., Adams, J. B., Gregory, A. C., Borody, T., Chittick, L., Fasano, A., & Krajmalnik-Brown, R. (2017). Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome, 5*, 10.

Konstantareas, M. M., & Homatidis, S. (1987). Ear infections in autistic and normal children. *Journal of Autism and Developmental Disorders, 17*(4), 585–594.

Krajmalnik-Brown, R., Lozupone, C., Kang, D. W., & Adams, J. B. (2015). Gut bacteria in children with autism spectrum disorders: challenges and promise of studying how a complex community influences a complex disease. *Microbial Ecology in Health and Disease, 26*, 26914. doi:10.3402/mehd.v26.26914.

Lahey, B. B., & D'Onofrio, B. M. (2010). All in the family: Comparing siblings to test causal hypotheses regarding environmental influences on behavior. *Current Direction in Psychological Science, 19*(5), 319–323.

Li, Q., & Zhou, J. M. (2016). The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder. *Neuroscience, 324*, 131–139.

Marques, T. M., Wall, R., Ross, R. P., Fitzgerald, G. F., Ryan, C. A., & Stanton, C. (2010). Programming infant gut microbiota: influence of dietary and environmental factors. *Current Opinion in Biotechnology, 21*(2), 149–156.

Niehus, R., & Lord, C. (2006). Early medical history of children with autism spectrum disorders. *Journal of Developmental and Behavioral Pediatrics, 27*(2 Suppl), S120–S127.

Plomin, R., & Daniels, D. (2011). Why are children in the same family so different from one another? *International Journal of Epidemiology, 40*(3), 563–582.

Quigley, M. A., Hockley, C., Carson, C., Kelly, Y., Renfrew, M. J., & Sacker, A. (2012). Breastfeeding is associated with improved child cognitive development: A population-based cohort study. *The Journal of Pediatrics, 160*(1), 25–32.

R Core Team (2015). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Rome foundation. Citing source: http://theromefoundation.org.

Risch, N., Hoffmann, T. J., Anderson, M., Croen, L. A., Grether, J. K., & Windham, G. C. (2014). Familial recurrence of autism spectrum disorder: evaluating genetic and environmental contributions. *The American Journal of Psychiatry, 171*(11), 1206–1213.

Schultz, S. T., Klonoff-Cohen, H. S., Wingard, D. L., Akshoomoff, N. A., Macera, C. A., Ji, M., & Bacher, C. (2006). Breastfeeding, infant formula supplementation, and autistic disorder: The results of a parent survey. *International Breastfeeding Journal, 1*, 16. doi:10.1186/1746-4358-1-16.

Shin, H., Pei, Z., Martinez, K. A. II, Rivera-Vinas, J. I., Mendez, K., Cavallin, H., & Dominguez-Bello, M. G. (2015). The first microbial environment of infants born by C-section: The operating room microbes. *Microbiome, 3*, 59. doi:10.1186/s40168-015-0126-1.

Tick, B., Bolton, P., Happé, F., Rutter, M., & Rijsdijk, F. (2016). Heritability of autism spectrum disorders: A meta-analysis of twin studies. *Journal of Child Psychology and Psychiatry, 57*(5), 585–595.

Vuong, H. E., & Hsiao, E. Y. (2017). Emerging roles for the gut microbiome in autism spectrum disorder. *Biological Psychiatry, 81*(5), 411–423.

Wassenaar, T. M., & Panigrahi, P. (2014). Is a foetus developing in a sterile environment? *Letters in Applied Microbiology, 59*(6), 572–579.

Zetterqvist, J., Vansteelandt, S., Pawitan, Y., & Sjolander, A. (2016). Doubly robust methods for handling confounding by cluster. *Biostatistics, 17*(2), 264–276.