Research in Autism Spectrum Disorders
Do risk factors for autism spectrum disorders affect gender representation?

Ditza A. Zachor a,b,1,*, Shay Ben-Shachar c,1, Esther Ben-Itzchak a,d

A R T I C L E  I N F O

Article history:
Received 2 July 2013
Received in revised form 18 August 2013
Accepted 19 August 2013

Keywords:
Autism spectrum disorder
Gender
Male:female ratio
Risk factors
Low birth weight
Multiplex families

A B S T R A C T

To examine the M:F ratio in several known risk factors to demonstrate insights regarding autism spectrum disorders (ASD) etiology and sex. The study included 615 participants aged 18 months to 18 years age (mean = 49.8 months, SD = 28.4 months) diagnosed with ASD. Cognitive, adaptive and assessment of ASD were obtained using standardized tests. The study included 615 participants aged 18 months to 18 years age (mean = 49.8 months, SD = 28.4 months) diagnosed with ASD. Cognitive, adaptive and assessment of ASD were obtained using standardized tests. Detailed birth, familial, medical and developmental histories were obtained from the parents. Risk factors included ASD in the family (having a first-order family member with ASD); advanced maternal age (≥35 years); advanced paternal age (≥38 years); birth order (first-born versus third-born); low birth weight (LBW) (<2500 g); prematurity (gestational age <36 weeks). The M:F ratio (4.4:1) in the LBW group was lower than the M:F ratio (7.1:1) in the >2500 g group; however the difference showed only a statistical trend. No significant differences in M:F ratio were found between the ASD groups with and without the other examined risk factors. It is possible that the absence of a major association between most of the examined risk factors and sex representation points to the relatively minor role of these risk factors in ASD.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders defined by social and communication deficits and repetitive and stereotyped behaviors (Johnson & Myers, 2007). The current estimated prevalence of ASD is approximately 1:100–150 (Kogan et al., 2009), which reflects a 15-fold increase from studies published a half-century ago. ASD is a highly heritable disorder, with heritability indices estimated at 60–92% (Bailey et al., 1995), suggesting a major role for genetic factors in the etiology of ASD (reviewed in Schaal & Zoghbi, 2011). However, the exact cause of ASD is still unknown in most cases. It is now believed that environmental factors may modulate phenotypical expression associated with the genetic predisposition (Johnson & Myers, 2007; Kogan et al., 2009).

Single gene disorders, such as Fragile X syndrome and others, explain only about 5–7% of cases of ASD. In recent years significant genetic etiologies have been discovered. It was found that de novo genomic copy number variations (CNVs – gains or losses of genomic material that do not exist in the parents) may explain an additional 7–20% of ASD cases (Schaal & Zoghbi, 2011). In addition, inherited CNVs may serve as risk factors for ASD. However, the disease etiology is still elusive in the great majority of cases. It is believed that both genetic and environmental risk factors play a role in ASD.

* Corresponding author at: The Autism Center, Assaf Harofeh Medical Center, Zerifin 70300, Israel. Tel.: +972 506382593; fax: +972 97289206311.
E-mail addresses: dzachor@smile.net.il, dzachor@assaf.health.gov.il (D.A. Zachor).
1 These authors have equal contribution to this manuscript.

1750-9467/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.rasd.2013.08.0008
The association between specific risk factors and ASD has been documented in some studies. Genetic predisposition for ASD as previously described has long been associated with an increased risk for ASD in siblings (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Lintas & Persico, 2009; Ozonoff et al., 2011; Persico & Bourgeron, 2006). Advanced parental age has been documented in many studies as a risk factor for ASD (Grether, Anderson, Croen, Smith, & Windham, 2009; Kolevzon, Gros, & Reichenberg, 2007). Most of the studies that looked at advanced paternal age as a risk factor found an increased risk for ASD with increasing paternal age (reviewed in Guichat et al., 2012). Several studies described maternal age as a risk factor for ASD but the results are more conflicting than the reports on the effect of paternal age. The biological mechanisms underlying the relationship between parental ages and autism are still unknown, but they may be associated with the increased rate in de novo mutations that appears in advanced paternal age. Several studies have reported that higher birth order (≥3rd) might be a risk factor for ASD (Deykin & MacMahon, 1980; Lord, Mulloy, Wendelboe, & Schopler, 1991; Tsai & Stewart, 1983). Low birth weight (LBW) with or without prematurity is a risk factor for many neurodevelopmental disorders, including cerebral palsy and intellectual disability. Inconsistent results were documented regarding the association of LBW (<2500 g) and very low birth weight (<1500 g) with ASD (Ben-Itzchak, Lahat, & Zachor, 2011; Eaton, Mortensen, Thomsen, & Frydenberg, 2001; Hultman, Sparén, & Cnattingius, 2002; Kolevzon et al., 2007; Schendel & Bhasin, 2008). The main risk factor for ASD is, by far, male sex. Male predominance in ASD has been noted in all epidemiological studies and is a well-established fact in the field of ASD. Male:female (M:F) ratio has been reported in many studies in the range of 4–7:1 (Ben-Itzchak, Ben-Shachar, & Zachor, 2013; Fombonne, 2003; Holtmann, Bolte, & Poustka, 2007; Newschaffer et al., 2007). Moreover, it has been found that, in families having a single child with ASD, the recurrence risk is about 8–21% in male pregnancies but only about 1–7% when the fetus is a female (Miles et al., 2005; Ozonoff et al., 2011).

Interestingly, the M:F ratio in ASD is not constant. For example, in regard to parental age, one of the known risk factor for ASD, it has been reported that a less skewed M:F ratio was observed as the paternal age increased in a cohort of children with ASD. The M:F ratio changed from 6:1 for fathers less than 30 years of age to 1:2:1 for fathers over 45 years (Anello et al., 2009). In addition, it was reported that a low birth weight (LBW < 2500 g) served as a risk factor for autism, with a greater effect in girls (Schendel & Bhasin, 2008). There is no clear explanation for the male predominance in ASD. Given the high M:F ratio and the heritability of ASD, it has been speculated that genetic disorders that are linked to the X or Y chromosomes play a role in the disease etiology. However, so far no X/Y linked disorder can explain more than a fraction of ASD cases. The recent finding that females with ASD and de novo CNVs have a higher number of altered genes than males (Levy et al., 2011), implying again that females are more protected from ASD compared to males and need to have a more severe aberration or an increased number of etiological factors in order to show the autistic phenotype.

Recently, we have shown (Ben-Itzchak et al., 2013) that there is an increased representation of females in ASD with additional neurological phenotypes, such as microcephaly, minor neuromuscular deficits, and a history of developmental regression. These findings suggest that ASD in females is associated with a more complicated presentation as compared to males.

The male predominance in prototypical ASD suggests that females are more protected from ASD than males (Levy et al., 2011). Our findings that females with ASD have more neurological phenotypes support this notion, and imply that additional neurological insults may be required in females to have ASD. Therefore, we hypothesized that ASD in females and males are associated with different mechanisms and etiologies, including risk factors for ASD. Because females compose only a small portion of the ASD population, previous research on risk factors for ASD was conducted on the general ASD population and, therefore, represented the effect of these risk factors mainly on males. We hypothesized that risk factors for ASD will therefore occur more frequently in females than in males. In this study we aimed to examine the M:F ratio in several known risk factors in order to demonstrate insights regarding ASD etiology and sex.

2. Methods

2.1. Participants

The study included 615 participants, 532 males and 83 females (M:F = 6.4:1), diagnosed with ASD, with an age range of 18 months to 18 years and a mean age of 49.8 ± 28.4 months.

2.2. Procedure

Participants were referred to The Autism Center for a comprehensive assessment of a possible diagnosis of ASD. The evaluation included a neurological assessment, and behavioral, cognitive and functional evaluations. Assessments were conducted by a skilled interdisciplinary team. Pediatric neurologists obtained medical, developmental and familial histories from the parents and conducted a comprehensive neurological examination of all the participants.

The diagnosis of autism/ASD was obtained by using two standardized tests, the autism diagnosis interview-revised (ADI-R) (Rutter, LeCouteur, & Lord, 2003) and the autism diagnosis observation schedule (ADOS) (Lord, Rutter, DiLavore, & Risi, 1999) and by meeting criteria for autism/ASD based on DSM-IV criteria (APA, 1994). All the professionals involved in the diagnostic process established reliability as required. Cognitive and developmental abilities (IQ/DQ) were assessed using The Mullen scales of early learning (Mullen, 1995), Bayley scales of infant development (Bayley, 1993), Wechsler preschool and primary scale of intelligence (Wechsler, 1989), Stanford-Binet intelligence scales (Thorndike, Hagen, & Sattler, 1986).
Kaufman assessment battery for children-II (Kaufman, 1983), or Wechsler intelligence scale for children III and IV (Wechsler, 2003), according to the child’s age and language level. Since 2006, developmental and cognitive tests have been obtained and thus DQ/IQ scores were available on 287 participants.

Adaptive skills were assessed in all the participants using the Vineland adaptive behavior scales (VABS) (Sparrow, Balla, & Cicchetti, 1984).

Risk factors: the information regarding the different risk factors for ASD was obtained by the pediatric neurologist during the comprehensive neurological assessment:

Genetic predisposition was defined as having a first-order family member (parent, sibling) with ASD. Advanced maternal age was defined based on previous studies as ≥35 years. Advanced paternal age was defined as ≥38 years, three years older than the definition for advanced maternal age, as the mean paternal age of the entire group (38.0 ± 6.3) was about three years older than the mean maternal age (35.4 ± 5.5).

Birth order: M:F ratio was examined in subgroups of children who were first-born versus a subgroup who were third-born.

Low birth weight (LBW) was defined as birth weight <2500 g. Prematurity was defined as a gestational age <36 weeks.

2.3. Data analysis

To investigate the differences in M:F ratio between the ASD subgroups with and without the examined risk factors (advanced maternal and paternal ages, ASD in the family, higher birth order, low birth weight and prematurity), Chi Square tests were used.

3. Results

The characteristics of the ASD group were examined in autism severity, adaptive skills and developmental/cognitive abilities. For the entire ASD group, meanADOS severity scale score was 7.4 (SD = 2.1), mean VABS composite score was 70.8 (SD = 11.6), and mean DQ/IQ score was 77.2 (SD = 22.5). No significant differences were noted between males and females in all the examined measures (Table 1).

As the different risk factors thought to be associated with ASD may have different effects on different sexes, we first assessed the M:F ratios in these risk factors:

3.1. Parental ages

To investigate the M:F ratio in relation to the maternal age, we first divided the ASD group into two subgroups based on maternal age below and above 35 years. In the ASD group, 20.9% of the participants had mothers with an advanced age (≥35). The M:F ratio (6.1:1) in the ≥35 years maternal age group was similar to the M:F ratio (6.4:1) in the younger <35 years maternal age group \(\chi^2(1) = 0.035, p > 0.05\).

To investigate the sex ratio in relation to paternal age, the ASD group was divided into two subgroups based on paternal age below and above 38 years. Regarding paternal age, 20.2% of the ASD individuals had fathers with an advanced age (≥38 years). The M:F ratio (4.7:1) in the ≥38 years paternal age group was not significantly different from the M:F ratio (6.7:1) in the <38 years paternal age group \(\chi^2(1) = 1.7, p > 0.1\).

3.2. ASD in the family

We assessed whether the M:F ratio in multiplex families (history of relatives with ASD in the family) was different from the ratio in simplex families. Of the ASD participants, 8.0% had a first-order relative diagnosed within the autism spectrum disorders. In the group of multiplex families with ASD, the M:F ratio (3.9:1) was lower than the M:F ratio (6.7:1) in the group of simplex cases (without ASD in first-order relatives), but the difference did not reach statistical significance \(\chi^2(1) = 0.4, p > 0.1\).

3.3. Birth order

We investigated sex ratio in regard to birth order to assess whether this risk factor is associated with sex differences. Comparing the sex ratio between the firstborn \((n = 298)\) and the ≥3rd born \((n = 98)\) groups revealed a M:F ratio of (7.4:1) in the first-born group and 8.8:1 for the ≥3rd group. This difference was not statistically significant \(\chi^2(1) = 0.2, p > 0.05\).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Males, M (SD) (N)</th>
<th>Females, M (SD) (N)</th>
<th>F</th>
<th>(\chi^2(1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADOS severity scale</td>
<td>7.4 (2.1) (526)</td>
<td>7.3 (2.1) (82)</td>
<td>0.238</td>
<td>0.000</td>
</tr>
<tr>
<td>VABS</td>
<td>71.2 (11.5) (532)</td>
<td>68.7 (12.3) (83)</td>
<td>3.2</td>
<td>0.005</td>
</tr>
<tr>
<td>DQ/IQ</td>
<td>77.8 (22.7) (246)</td>
<td>73.5 (20.7) (41)</td>
<td>1.3</td>
<td>0.004</td>
</tr>
</tbody>
</table>
3.4. Low birth weight and prematurity

We assessed the M:F ratio in subgroups with birth weights below and above 2500 g. Of the ASD group, 12.2% had a birth weight ≤2500 g. The M:F ratio (4.4:1) in the LBW group was lower than the M:F ratio (7.1:1) in the >2500 g group; however, the difference showed only a statistical trend \( \chi^2(1) = 2.1, p = 0.07 \).

3.5. Gestational age

Of the ASD group, 21.6% had a gestational age (GA) <36 weeks. In this group, the M:F ratio (5.9:1) did not differ significantly from the M:F ratio in the GA ≥36 weeks group (6.6:1).

4. Discussion

Multiple risk factors associated with ASD have been reported. The most prominent risk factor for ASD is male sex. Numerous studies have demonstrated that ASD is 5–6 times more prevalent in males compared to females. We have shown that the presence of neurological phenotypes in ASD was associated with more female representation (Ben-Itzhak et al., 2013). The relationship between sex and the other risk factors for ASD has not yet been thoroughly examined. Sex representation in various risk factors for ASD may increase the understanding of the pathogenesis of this complex disorder. We have hypothesized that if some risk factors, which equally affect both genders, are associated with normalization of the M:F ratio, then these risk factors may have a significant effect that overcomes, at least partially, the prominent sex ratio bias. However, the present study, conducted on a large series of children with ASD, found only a modest relationship between specific risk factors in ASD and sex representation. These findings generally imply a minor role for the examined risk factors on ASD.

Previous studies reported that LBW and prematurity are risk factors for ASD (Ben-Itzhak et al., 2011; Kolevzon et al., 2007). In the current study, a trend of relatively more female representation was noted in the group with ASD and LBW. Schendel and Bhasin (2008) reported that the magnitude of the risk for ASD was higher for girls with LBW. One explanation for the association of ASD with LBW might be a shared genetic mechanism for both conditions, as well as genetic susceptibility. A recent study reported a similar risk for ASD in monozygotic and dizygotic twin-pairs with LBW. Therefore, the authors suggested that a non-genetic influence, associated with low birth weight, may contribute to the development of ASD (Losh, Esserman, Ancharsater, Sullivan, & Lichtenstein, 2012). Both, the possible genetic and non-genetic factors associated with low birth weight etiology might affect both sexes similarly and raise the risk for ASD in females.

The current study examined the M:F ratio in multiplex families versus simplex families as one would expect the M:F ratio to diminish significantly in multiplex families that are likely carrying that autosomal mutations. The current study found that there was a less prominent M:F ratio, 3.9:1 vs 6.7:1, in multiplex families compared with singlet families. However, this M:F ratio difference did not reach statistical significance, which can be explained by the small number of multiplex families in the study population.

Advanced parental age has been documented in multiple studies to increase the risk for autism (Grether et al., 2009; Shelton, Tancerdi, & Hertz-Picciotto, 2010). When we tested the M:F ratio in older and younger parental age groups, we did not find differences in M:F ratios to be associated with advanced parental age. Hypothetically, if advanced paternal age is associated with de novo mutations, then these single gene mutations should affect both sexes equally. If true, these gene mutations may have a significant role in the etiology of autism. In this case, the M:F ratio would be expected to diminish with advanced paternal age. On the contrary, the current study results support the finding that the mechanism by which advanced paternal age causes ASD might not be related to de novo mutations, as previously suggested (Croen, Najjar, Fireman, & Grether, 2007; Hultman, Sandin, Levine, Lichtenstein, & Reichenberg, 2011). Another explanation for the study results is that de novo mutations might have only a minor contribution to the risk of ASD, and therefore the advanced paternal age did not affect the M:F ratio.

Increased birth order has been described as a risk factor for ASD (Deykin & MacMahon, 1980; Lord et al., 1991; Tsai & Stewart, 1983). A recent study indicated that closely-spaced pregnancies are associated with an increased risk for ASD (Cheslack-Postava, Liu, & Bearman, 2011). In the current study, the M:F ratio was examined in first – versus third-born child. Birth order was not associated with sex representation in ASD.

Overall, by looking for ASD risk factors in a sex-specific manner, in a large, well characterized population of individuals with ASD, we observed that the existence of certain risk factors namely advanced parental age and birth-order, do not increase the representation of females in ASD. Our data suggest that it is possible that the sex ratio is lower in multiplex families with ASD and in LBW with ASD.

The data suggest that these risk factors previously detected to be associated with ASD do not affect the strong male predominance in ASD. The minor changes in M:F ratio in ASD with risk factors are in contrast to the more significant association of neurological phenotypes, such as microcephaly, developmental regression, and minor neuromuscular findings with increased female representation in ASD. Of the examined risk factors, only LBW was associated with a slight increase in female representation. LBW is known to be related to adverse neurodevelopmental outcome and nonspecific brain injury that might increase the risk for ASD for females, similar to our previous findings regarding the other neurological
phenotypes. It is possible that the absence of a major association between the other examined risk factors and sex representation points to the relatively minor role of these risk factors in ASD. Alternatively, these risk factors might only increase the occurrence of primary autism, and therefore M:F ratio is basically unchanged from the male predominance seen in idiopathic ASD. However, more extensive CNS involvement, in addition to the primary risk for ASD, increases the presentation of ASD in female.

Since the number of females with a specific risk factor is relatively small even in a large cohort, further examination of the impact of risk factors for ASD on sex representation is needed. Future research should include a meta-analysis of studies with large and well-diagnosed ASD populations, which examine risk factors in ASD. Such a meta-analysis will enable investigation of a larger number of females in each group of risk factors to confirm the current research conclusions and to shed more light on the pathogenesis of ASD.

**Conflict of interest statement**

No author has any financial disclosure or conflict of interest to declare.

**References**


