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Brief Report: Parental Age and the Sex Ratio in Autism

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Abstract

The male-to-female (M:F) ratio for autism spectrum disorders (ASD), typically about 4:1, appears to decrease with increasing paternal age, but this relationship has not been systematically tested. With 393 ASD cases from families with two or more ASD cases, we categorized paternal age into five age groups (<30, 30–34, 35–39, 40–44, 45+) and found that the M:F ratio was significantly decreased with increasing paternal age groups and remained so after also adjusting for maternal

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age. No significant relationship between maternal age group and the M:F ratio was observed. This study suggests that the M:F ratio is reduced with increasing paternal age consistent with de novo genetic or genomic anomalies arising more frequently as men age and then conceive children.

Keywords

Paternal age; Maternal age; Sex ratio; Genetics; Genomic anomalies; Copy number variants

Introduction

Epidemiological studies of autism consistently show an uneven sex ratio with male-to-female (M:F) ratios of about 4:1(Fombonne 2003). Although the mechanisms explaining this male predominance remain unknown, identifying moderators that affect the M:F ratio may help reveal them. One such moderator may be parental age.

Recently, a population-based Israeli birth cohort using registry-based clinical diagnosis observed a sixfold increased risk for autism spectrum disorders (ASD) among children of fathers aged 40 or greater compared with fathers aged less than 30 (Reichenberg et al. 2006). This relationship has been reported in other independent studies as well (Cantor et al. 2007; Croen et al. 2007; Glasson et al. 2004; Lauritsen et al. 2005; Tsuchiya et al. 2008). Interestingly, in the Israeli sample, the M:F ratio among ASD cases steadily diminished with each older paternal age group, but this observation was not statistically tested due to the relatively small numbers of cases (Reichenberg et al. 2006). In addition, in a large hospital-based historical birth cohort study, associations between ASD and both maternal and paternal age were present and appeared somewhat stronger for girls than boys, indicating a declining sex ratio with parental age though the sex differences were not statistically significant (Croen et al. 2007).

In the present study, we examined possible changes in the M:F ratio by both paternal and maternal age in a large sample of ASD cases recruited for family/genetic studies of ASD.

Methods

Subjects

Families with at least two children meeting criteria for an ASD as specified below were recruited as part of an ongoing family/genetic study (Silverman et al. 2002). Families were recruited primarily from the United States since 1994, at the Seaver Autism Research Center in The Mount Sinai School of Medicine. Many families we assessed were referred to us by the Autism genetic resource exchange (AGRE) and others were ascertained through advertising, word of mouth, and physician referrals. We routinely asked caregivers (almost always mothers) whether their affected child was ever diagnosed with a medical condition associated with autism. This was asked both in an open ended way and with a list of specific disorders (i.e., fragile X, tuberous sclerosis, Angelman Syndrome, Prader Willi syndrome, PKU, Rett syndrome). Children reported with such conditions were excluded from the sample. In addition, all twins were excluded from the sample. Families included in this study were required to have more than one child with autism, Asperger's disorder, or borderline autism (described below).

Diagnosis of Autism

We administered the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al. 1994) to the primary caregiver of any offspring with suspected autism or autism-related traits. When it

became available, the Autism Diagnostic Observational Schedule-Generic (ADOS-G) (Lord et al. 2000) was also routinely administered to the affected child. Parents provided written informed consent. The ADI-R interviewers all had a reliability of better than 90% with either Dr. Lord's group or C.J.S. The information from the ADI-R was used to determine ICD-10/DSM-IV autism diagnoses following the ADI-R algorithm. A research diagnosis of Asperger syndrome was given to those who met criteria for Asperger's disorder, but not autism, according to DSM-IV. Individuals were classified as borderline autism [sometimes called "not quite autism" (International Molecular Genetic Study of Autism Consortium 1998)] if they failed to meet ADI-R algorithm criteria for autism by no more than one point in the social domain as well as no more than one point for either the communication or repetitive behavior domain (but not both) and also did not meet criteria for Asperger's disorder.

Parental Age Determination

The birth date of each family member was routinely collected independently from each parent. With this information we determined the ages of the mother and father when each child with an ASD was born.

Statistical Analyses

Each ASD subject had one or more sibling with ASD also present in the sample, potentially violating the assumption that all observations be uncorrelated (independent). For this reason we used the generalized linear mixed model (GLMM) to examine the associations between parental age group and the ASD case's sex. GLMM is similar to logistic regression in treating a binary variable (in this case, sex) as an outcome variable. It controls for sibship membership by treating all the affected children from a family as a cluster and giving them the family-wise common (random) coefficients (i.e., intercept and/or slope) in the logistic regression model. We chose GLMM as the major analytic tool here, as implemented by the GLIMMIX procedure in SAS, because of its flexibility in handling clustered data and correlation due to clustering. Paternal age was divided into five age categories: less then 30, 30–34, 35–39, 40–44, and 45 years or more. The same procedures were repeated to assess the association between maternal age and the ASD affected child's sex, but four groups were used: less than 30, 30–34, 35–39, and 40 years or more. The odds ratio (OR) provided by GLMMIX is a measure of the increased (>1.0) or decreased (<1.0) chance of that an affected offspring is male versus female. The *t* statistic (with its associated degrees of freedom and *p*-value) provides a two-sided test of the null hypothesis that the OR is one.

Results

From 188 families, we studied 393 individuals with an ASD disorder. The diagnostic and demographic characteristics of ASD males and females and in the total ASD sample are shown on Table 1. If two parents of a case were of different ethnicities or at least was of unknown ethnicity there were included as "other/unknown category". The M:F ratio (Table 1) did not differ among ASD disorders. There was no paternal age group by diagnostic group effect.

Paternal Age and M:F Ratio

Table 1 also gives the raw M:F ratio by paternal and maternal age groups. For paternal age, this ratio descended as the age group increased, from 6.2:1 among offspring of fathers less than age 30 years to 1.2:1 among offspring of fathers aged 45+ years. Using GLMM to account for within sibship relationships, we found evidence for a significant linear relationship between advancing paternal age group and the sex of the ASD case (OR = 0.72, 95% CI: 0.56, 0.93; $t = -2.51$, $df = 204$, $p = 0.01$). In other words, as the paternal age group

increased, the probability of the affected child being male decreased. We then included a linear relationship with maternal age group as a covariate in the model and found essentially no change in the result (OR = 0.72, 95% CI: 0.53, 0.96; $t = -2.20$, $df = 203$, $p = 0.03$). We also examined the OR for each paternal age group treated categorically using the offspring of fathers 45+ years as the reference group. Controlling for all maternal age groups, the ORs descended as the father's age group increased. Compared with the reference group, the M:F ratio was significantly greater in the <30 and the 30–34 paternal age group (offspring of fathers <30: OR = 5.45 [95% CI: 1.15, 25.91], $t = 2.13$, $df = 198$, $p = 0.03$; offspring of fathers 30–34: OR = 4.92 [95% CI: 1.15, 21.08], $t = 2.15$, $df = 198$, $p = 0.03$; offspring of fathers 35–39: OR = 2.96 [95% CI: 0.71, 12.34], $t = 1.49$, $df = 198$, $p = 0.13$; offspring of fathers 40–44: OR = 2.86 [95% CI: 0.64, 12.85], $t = 1.37$, $df = 198$, $p = 0.17$).

Maternal Age and M:F Ratio

For maternal age, the linear trend for the raw M:F ratio across in the four age groups was less pronounced (Table 1). Using GLMM, the relationship between maternal age group and the sex of the ASD case was not statistically significant whether entered alone (OR = 0.82, 95% CI: 0.60, 1.11; $t = -1.31$, $df = 204$, $p = 0.19$) or with paternal age group included as a covariate (OR = 1.02, 95% CI: 0.71, 1.49; $t = 0.13$, $df = 203$, $p = 0.90$). Finally, the OR for each maternal age group treated categorically using the offspring of mothers 40+ years as the reference group were small and not significant (offspring of mothers <30: OR = 1.14 [95% CI: 0.26, 4.98], $t = 0.18$, $df = 198$, $p = 0.90$; offspring of mothers 30–34: OR = 1.00 [95% CI: 0.24, 4.13], $t = 0.00$, $df = 198$, $p = 1.00$; offspring of mothers 35–39: OR = 1.42 [95% CI: 0.33, 6.07], $t = 0.48$, $df = 198$, $p = 0.64$).

Discussion

The present study provides evidence in a large series of ASD cases from multiplex families that the well established M:F ratio in autism favoring males at a rate of about 4:1 diminishes as the age group of the father increases. In contrast, we did not find evidence for a shift in the M:F ratio associated with maternal age group. A reduction in the M:F ratio with increasing paternal age was observed descriptively in two recent population-based studies (Croen et al. 2007; Reichenberg et al. 2006).

These results indirectly argue against an explanation for the association between paternal age and risk of autism that relies on a putative tendency for fathers with more prominent genetic loading for autism to have children at later ages. Underlying that explanation is the notion that a more heavily genetically loaded father will tend to have more autism-related traits than one with a lower genetic load and that such traits, in turn, lead to delayed parenthood (Miller 2006). However, if an increasing genetic load in the father is associated with later paternity, one would predict more cases of autism with increased paternal age but not a change in the sex ratio. Furthermore, a previous study found no evidence for an association between paternal age and autism-related personality traits in the father or in the mother (Puleo et al. 2008). Instead, the shifting M:F ratio in ASD associated with paternal age may suggest a changing balance in the *types* of mechanisms causing ASD.

At present we can only speculate about the potential mechanisms mediating the observed effect of paternal age on the autism M:F ratio. Accumulating evidence suggests that genomic copy number variants (CNVs), i.e., the loss or gain of a small piece of chromosome (micro-deletions and microinsertions, respectively), can play an important role in causing at least some cases of autism (Sebat et al. 2007; Szatmari et al. 2007; Weiss et al. 2008). CNVs can arise as de novo genomic events and, along with de novo Mendelian mutations, are increasingly common in sperm as men age (Wyrobek et al. 2006; Crow 1997). Thus, under the assumption that autism arising from CNVs is less discriminating toward females than

autism that instead arises from the inheritance of liability genes (Miles et al. 2005), a reduced M:F ratio would be expected with later paternal age as an increasing proportion of cases owing to CNVs would likely emerge. Alternatively, overproduction or underproduction of a protein coded on the X-chromosome could increase autism susceptibility. Insertions, deletions, or breaks in the X-chromosome, which have been hypothesized to cause a loss of dosage compensation in females (Brooks 2005), and which appear more frequently in the offspring of older fathers, could cause excess or decreased production of that protein. Thus, with increased paternal age, increasing loss of dosage compensation would heighten vulnerability for autism specifically to girls. This would again decrease the M:F ratio in ASD as fathers aged. Finally, the presence of a paternally genetic imprinted protective gene on the X-chromosome has been proposed as an explanation for the high M:F ratio in autism (Skuse 2000). Such a protective effect may be diminished with increasing paternal age due to increased paternally transmitted X chromosomal abnormalities.

It is of interest that certain phenotypic differences, such as IQ (Volkmar et al. 1993) or abnormalities of early morphogenesis (Miles et al. 2005), appear to be associated with changes in the sex ratio. Also, locus heterogeneity, associated with whether families have affected boys *only* or one or more affected girls, has been observed at several chromosomal regions (Cantor et al. 2005; Ma et al. 2007; Schellenberg et al. 2006; Stone et al. 2004). Such differences, too, are consistent with different genetic or other biologic mechanisms leading to autism in families with affected girls. Thus, our results appear broadly consistent with recent proposals that autism may be divisible into those with inherited pre-existing genetic variants and those with spontaneous mutations, CNVs or other emergent genomic abnormalities that may be less strongly differentiated by sex (Miles et al. 2005; Sebat et al. 2007; Szatmari et al. 2007; Zhao et al. 2007). The proportions of these different causal mechanisms may shift with paternal age. Our results further contribute to the evidence that paternal age may help distinguish between these two types of causes.

Increased paternal age has been previously associated with a shift toward female births in the general population (Ruder 1985). This raises the possibility that the ASD-associated M:F shift with advancing paternal age simply reflects a process at the general population level. However, the difference observed in the general population study was a very subtle one, while ours was not. For example, for those of European or of African ancestry the average paternal age in girls was, for each group, only 0.02 years greater than it was in boys. This general effect is therefore unlikely to explain the far more marked M:F ratio shift we have observed in the present study.

Although our sample is large, a limitation to this study is that cases were ascertained opportunistically and all come from nuclear families with two or more affected children. For this reason, the sample cannot be deemed representative of the ASD population as a whole and it was not possible to assess paternal or maternal age as a risk factor for ASD overall. However, neither the sex of the affected children nor the age of the parents played any role in determining whether a family would be recruited for our studies. Thus, the association observed in this study between M:F ratio and paternal age in ASD is unlikely to be attributable to a selection bias.

However, as the ASD cases are from multiplex families, it is possible that some parents may have opted for selective termination of a male fetus after having one child with autism, given the recognized high risk for autism in male children. To the extent this may have occurred, this would alter the observed sex ratio in the later-born affected and unaffected children in these families and would tend to skew the sex ratio of offspring of older parents. This possibility notwithstanding, it is noteworthy that the diminution of the M:F ratio with

paternal age was found in a sample for which the role of inherited genetic factors is presumably strong. Using cases from multiplex families presumably increases the likelihood that parents carry some pre-existing genetic liability transmitted to their offspring, reducing the likelihood that the disorder has occurred due to a de novo CNV, new mutation, or some other emergent chromosomal abnormalities or changes. The hypothesis that this association may be stronger still in a large independent sample of cases that is less familially loaded than the present one is worthy of testing in a future study.

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Table 1

Demographic characteristics of autism spectrum disorder (ASD) subjects by sex

Characteristic	Males	Females	M:F	Total
N	320	73	4.4	393
Age (at assessment) mean years (SD)	8 (6)	9 (6)	–	8 (6)
ASD disorder *N* (%)				
Autism	285 (89.1)	66 (90.4)	4.3	351 (89.3)
Asperger's	25 (7.8)	4 (5.5)	6.3	29 (7.4)
Borderline Autism	10 (3.1)	3 (4.1)	3.3	13 (3.3)
Ethnicity *N* (%)				
European ancestry (non-Hispanic)	264 (82.5)	62 (84.9)	4.1	326 (83.0)
Hispanic	20 (6.3)	4 (5.5)	5.0	24 (6.1)
African ancestry	9 (2.8)	1 (1.4)	9.0	10 (2.5)
Other/unknown	27 (8.4)	6 (8.2)	4.5	33 (8.4)
Paternal age *N* (%)				
<30	81 (25.3)	13 (17.8)	6.2	94 (23.9)
30–34	132 (41.3)	25 (34.2)	5.0	157 (39.9)
35–39	71 (22.2)	21 (28.8)	3.4	92 (23.4)
40–44	30 (9.4)	9 (12.3)	3.3	39 (9.9)
45+	6 (1.9)	5 (6.8)	1.2	11 (2.8)
Maternal age *N* (%)				
<30	140 (43.8)	26 (35.6)	5.4	166 (42.2)
30–34	124 (38.8)	32 (43.8)	3.9	156 (39.7)
35–39	47 (14.7)	11 (15.1)	4.3	58 (14.8)
40+	9 (2.8)	4 (5.5)	2.3	13 (3.3)