Inherited risk for autism through maternal and paternal lineage

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Inherited Risk for Autism Through Maternal and Paternal Lineage

Dan Bai, Natasha Marrus, Benjamin Hon Kei Yip, Abraham Reichenberg, John N. Constantino, and Sven Sandin

ABSTRACT
BACKGROUND: Autism spectrum disorder (ASD) is highly familial, with a positively skewed male-to-female ratio that is purported to arise from the so-called female protective effect. A serious implication of a female protective effect is that familial ASD liability would be expected to aggregate asymptptomatically in sisters of affected probands, who would incur elevated rates of ASD among their offspring. Currently, there exist no data on second-generation recurrence rates among families affected by ASD.

METHODS: We analyzed data from the Swedish National Patient Register and the Multi-Generation Register for a cohort of children born between 2003 and 2012. ASD was ascertained in both the child and parental generations.

RESULTS: Among 847,732 children, 13,103 (1.55%) children in the cohort were diagnosed with ASD. Among their maternal/paternal aunts and uncles, 1744 (0.24%) and 1374 (0.18%) were diagnosed with ASD, respectively. Offspring of mothers with a sibling(s) diagnosed with ASD had higher rates of ASD than the general population (relative risk, 3.05; 95% confidence interval, 2.52–3.64), but not more than would be predicted for second-degree relatives within a generation, and only slightly more than was observed for fathers with siblings with ASD (relative risk, 2.08; 95% confidence interval, 1.53–2.67). Models adjusting for temporal trends and for psychiatric history in the parental generation did not alter the results.

CONCLUSIONS: These findings establish a robust general estimate of ASD transmission risk for siblings of individuals affected by ASD, the first ever reported. Our findings do not suggest female protective factors as the principal mechanism underlying the male sex bias in ASD.

Keywords: Autism, Epidemiology, Female protective effect, Population-based, Psychiatry, Sex bias

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Autism spectrum disorder (ASD) is a heritable, genetically heterogeneous neurodevelopmental disorder (1,2). A vast share of the population-attributable risk for this condition is accounted for by polygenic inheritance, as evidenced by 1) accumulated genetic epidemiological research demonstrating heritability of 0.80 or greater (1,3,4) and 2) several recent molecular genetic analyses indicating that the majority of causal genetic variation for ASD is additive (5,6).

A well-established epidemiological feature of ASD is the elevated prevalence in males, with a usual ratio of approximately triple the number of male versus female patients, depending on the method of ascertainment (7). This phenomenon is not strictly accounted for by classic sex chromosome–linked disease genes because evidence from human genetic studies suggests that a relatively minor proportion of genetic risk for ASD is resolvable to genes on sex chromosomes (8). Although community-based ASD diagnoses are somewhat more likely for male individuals than for female individuals at a given level of symptoms (9), the magnitude of the described diagnostic bias likely accounts for a minority of the observed male predominance of ASD. Several studies of toddlers, including prospective infant sibling studies (10) and general population screening studies (11,12), have confirmed that a true sex disparity manifests long before puberty (13).

Sex-specific modulation of the expression of heritable ASD liability could involve either or both of two general mechanisms: 1) protective factors conferring a higher liability threshold in female individuals or 2) susceptibility factors conferring a lower susceptibility threshold in male individuals, as shown as difference in liability thresholds (Figure 1). Several recent findings have suggested a potential role for female protective factors. For example, highly penetrant autosomal de novo copy number variations causing ASD tend to be larger and to contain more genes in affected female versus male individuals (14,15). Similarly, a recent analysis of exome sequence data from over 27,000 trios affected by neurodevelopmental disorders revealed that 6.5% of affected females harbored a de novo mutation in a gene more commonly disrupted in affected females than in affected males, whereas 2.5% of males harbored a de novo mutation in “male-enriched” genes (16). Some family studies have suggested an elevated genetic burden in cohorts enriched for females with ASD, for example, through greater ASD recurrence rates (17), and higher autistic trait scores in co-twins of affected females than in the
co-twins of affected males (18). While these findings do not directly compare male versus female liability thresholds for ASD, in aggregate, they are consistent with female individuals requiring greater genetic ASD risk to manifest a categorical diagnosis. Should such a female protective effect (FPE) account for the ASD sex ratio, it would imply that unaffected female individuals with a family history of ASD may carry and silently transmit proportionally greater genetic liability than unaffected male family members, amplifying recurrence rates in their male offspring, in particular. This possibility poses a significant public health issue, particularly in light of the international increase in ASD prevalence within the past 2 decades (19,20), specifically rendering sisters of individuals with ASD particularly concerned about risk of transmitting autism to their own future offspring. The prevalence shift has complicated reliable estimation of epidemiological risk to children in the second generation of families affected by ASD, and there exists no preconceptional guidance for the unaffected siblings of individuals with autism who have reached childbearing age.

Given evidence for the sex-specific modulation of ASD and widespread familial liability in the general population, quantifying silent maternal transmission of ASD represents a key step toward specification of second-generation recurrence risk in families, as well as toward risk stratification and identification of children most likely to benefit from early intervention. The complex polygenic nature of most cases of ASD constrains the capacity of molecular genetic methods to specify individual risk, particularly because the most robust associations of single variants with autism have involved highly deleterious mutations that arise de novo in the germline. By definition, de novo variation makes no contribution whatsoever to inherited risk in families, and it cannot be assessed through linkage studies of familial risk by virtue of the fact that different de novo variants may amplify ASD liability in different members of the same multiplex family affected by ASD (21). Thus, population-level quantification of transmitted ASD liability provides a more relevant and actionable appraisal of average inherited risk to offspring of siblings when clinical genetic information is not available or has not been identified or more variants contributing to autism in an affected family member.

The aim was therefore to examine the maternal transmission of ASD liability by testing whether ASD risk differs between maternal and paternal lineage, in which a higher risk from the maternal lineage is hypothesized under an FPE. For the most specific test of the female protective effect hypothesis, we compared whether ASD is more prevalent among second-generation offspring with a maternal uncle diagnosed with ASD than among those with a paternal uncle diagnosed with ASD, noting that, among such families, offspring affection represents an incident of familial recurrence, and such families thereby are comparable to prior research involving multiplex families. This study effectively implements a within-family design within an epidemiologic 2-generation sampling frame, which confers an advantage analogous to the transmission disequilibrium test in molecular genetic research; both are conservative methods for accommodating heterogeneity in genetic causation across families in the estimation of main effects for the population.

METHODS AND MATERIALS

Study Population

The study population includes all children live-born in Sweden between January 1, 2003, and December 31, 2012, identified from the Swedish Medical Birth Register. The register links the children with their mother and covers 99% of all births nationwide since 1973 (22,23). Paternity is assumed to be the husband or the male individual acknowledged by the mother, and adoption or other nonbiological relations are flagged. Fathers, siblings, cousins, uncles, and aunts were identified through linkage with the unique Swedish Multi-Generation Register (24); an example family pedigree is given in Figure S1 to illustrate how offspring and uncle/aunt pairs were identified on maternal and paternal lineage. The Multi-Generation Register includes identifiers for all Swedish citizens 15 years of age or younger and their parents from 1947 and onward. To be included in the register index, individuals had to be alive in 1961 (when the register was computerized) or thereafter. We only included children of parents with a full sibling.

ASD and Psychiatric Diagnoses

In Sweden, all infants and preschool children regularly undergo routine medical and developmental examinations. At 4 years of
age, a mandatory developmental assessment (motor, language, cognitive, and social development) is conducted. Children with suspected developmental disorders are referred for further assessment by a specialized team in a child psychiatry unit or habilitation service. Diagnostic information is reported to the Swedish National Patient Register (NPR) (25). The NPR includes all inpatient psychiatric diagnoses in Sweden since 1973 and outpatient visits from 2001, with almost complete national coverage from 2005. The diagnoses in the NPR are coded using the ICD-8, -9, and -10 and are assigned by clinical specialists. The NPR has been subjected to extensive validation efforts (25) and is frequently used (26). ASD was defined by a clinical diagnosis of autistic disorder (AD), Asperger syndrome, or pervasive developmental disorder not otherwise specified (Table S1). Our database includes diagnoses in the NPR up to December 31, 2017.

**Covariates**

We considered several factors that potentially could bias the results. The prevalence of childhood psychiatric disorders has increased over time. Therefore, to adjust for potential temporal trends, we included birth year and sex from the Medical Birth Register and calculated age at first diagnosis of ASD using the NPR. We defined psychiatric history as presence of a maternal or paternal psychiatric diagnosis of at least 1 diagnosis among 10 ICD disorder categories (Table S1) at the time of offspring birth. Similarly, we defined an ASD-exposed individual as one with an ASD diagnosis (Table S1) in an aunt/uncle at the time of offspring birth. Besides an assessment of any psychiatric diagnosis, we also defined covariates for parental psychiatric history at birth for 10 different psychiatric diagnostic groups and disorders (Table S1) (26). Using the same procedure as for the parents, we also defined aunt and uncle psychiatric history. To adjust for differences in length of follow-up, we used date of death and date of emigration from Sweden using data from Statistics Sweden, the Swedish government bureau for official statistics.

**Statistical Analysis**

A key biological hypothesis tested is whether ASD transmission from one generation to the next occurs proportionately through female individuals, among whom inherited liability is less expressed, or phenotypically “silenced,” in comparison with male individuals. We tested this hypothesis by using the presence or absence of ASD in the parental generation as an indirect measure of parental exposure to genes conferring increased ASD risk (by virtue of relation to probands in Figure 1). The actual degree of transmitted risk was measured by the rate of ASD in the offspring of the parental generation (subjects C1, C2 in Figure S1).

To directly address the underlying biological hypothesis, we estimated the relative risk (RR) of ASD in individuals with an aunt or uncle diagnosed with ASD compared with offspring for whom an aunt or uncle was not diagnosed with ASD, and next estimated the RR for offspring when 1) an aunt (only) or 2) an uncle (only) had ASD, in comparison with offspring for whom neither an aunt nor an uncle was diagnosed with ASD. We estimated the RR for maternal-lineage aunt(s)/uncle(s) and for paternal-lineage aunt(s)/uncle(s) separately. We fitted Cox proportional hazards regression models and calculated hazard ratios as measures of RR, together with 2-sided 95% confidence intervals (CIs) corresponding to tests of statistical hypotheses on the 2-sided 5% significance level. Each child was followed for an outcome of ASD from 2 years of age until death, emigration from Sweden, or end of follow-up on December 31, 2017, whichever came first.

We fitted a sequence of Cox regression models with increasing degree of adjustment for potentially confounding factors. First, we fitted crude models, only including a covariate for the exposure group (maternal aunt or uncle diagnosed with ASD). Thereafter, we adjusted for temporal trends by adding parameters for maternal birth year, birth year of the maternal aunt or uncle, and birth year in the offspring generation using natural cubic splines (27). Further, we included indicators of any mental illness (yes/no) of the mother and the uncle and aunt (28). We repeated the models above separately for paternal offspring. Last, we repeated the approach above by first refitting the models only considering uncles as exposures and then only considering aunts as exposures.

Because each child can occur several times in the calculations and because siblings and cousins can be assumed to be correlated, we estimated the CIs using bootstrap techniques (29,30). In the bootstrap, we resampled each child with replacement, so each child had the probability of being selected more than once as a representative of the overall study population. We examined the assumption of proportional hazards by visual inspection of weighted Schoenfeld residuals (31). Our database, using data from the national registers, was essentially free from any missing values. We used SAS version 14.2 (SAS Institute, Cary, NC) on a Linux Red Hat 7.2 server (Red Hat, Raleigh, NC) for all calculations including PROC PHREG for Cox regression (see Supplemental Notes for SAS computer code).

We also performed several sensitivity analyses. To test the specificity of RR associations, we repeated analyses first with AD in the offspring instead of ASD, and second with male and female offspring separately. Third, we extended the diagnoses corresponding to familial liability for ASD/AD in the parental uncle/aunt to include schizophrenia, intellectual disability, or schizoid personality disorder. In the parental generation, when ASD was less well recognized, these outcomes may have lower specificity but increased sensitivity for inherited risk of ASD. Fourth, in our analyses, data were at offspring-aunt/uncle pair level (see Supplemental Notes for an example), in which some individuals were included in more than one cousin-uncle/aunt comparison, allowing individuals from larger families to contribute more to the sample size. Therefore, as a test of robustness, we fitted additional models in which the oldest cousin pairs were drawn within “families.” Fifth, to test the specificity of the findings relative to overall familial liability to psychiatric illness (parental generation), we repeated analyses on the subgroup of families with no psychiatric history other than ASD. Sixth, while adjusting for covariates, instead of parameters for “any mental health,” we included indicators for specific psychiatric disorders (intellectual disability, depression, anxiety disorders, substance use...
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results, bipolar disorder, compulsive disorder, attention-deficit/hyperactivity disorder, affective disorders, schizophrenia, schizoid personality disorder) of the mother and the uncle/aunt (28). Last, to verify that our results were not biased owing to sparse data, which potentially can result in biased estimates, we performed a supplementary analysis using the Firth correction for monotone likelihood to adjust for the case-control imbalance (32,33).

RESULTS

The study cohort included a total of 847,732 children, 51.43% boys, followed for ASD from 5 to 15 years of age. There were 13,103 (1.55%) children with an ASD diagnosis, of whom 8216 (0.97%) were diagnosed with AD. The median age at onset was estimated to be 7.72 years for ASD. Among the cohort children, 742,813 (87.6%) had a paternal aunt or uncle (Table 1), and 742,125 (87.5%) had a maternal aunt or uncle and was estimated to be 7.72 years for ASD. Among the cohort (0.97%) were diagnosed with AD. The median age at onset

Complementary Analyses

The results remained robust across a set of sensitivity analyses (Supplement): for maternal and paternal uncles and aunts with AD (Table S2A, B), for maternal and paternal uncles and aunts diagnosed with ASD/schizophrenia/intellectual disability or schizoid personality disorder (Table S2A, B), for offspring with AD (Table S3A, B), and for male and female offspring evaluated separately (Table S4). The results were also robust when adjusting for family size, and for potential correlations within families (Tables S5 and S6). The results also remained in the subgroup of families with aunts and uncles free from psychiatric history other than ASD (Table S7), as well as when adjusting for psychiatric history in 8 different psychiatric disorders instead of “any” psychiatric history (Table S8) and when excluding offspring to mothers with ASD from the analysis (Table S9). Our large sample size allowed us to consider

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Female Ratio</th>
<th>Mothers</th>
<th>Fathers</th>
<th>Uncles</th>
<th>Aunts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.06</td>
<td>475,669</td>
<td>379,365</td>
<td>356,244</td>
<td>479,373</td>
<td>386,103</td>
<td>364,467</td>
</tr>
<tr>
<td>Number of Individuals</td>
<td>847,732</td>
<td>1.00</td>
<td>847,732</td>
<td>1.00</td>
<td>847,732</td>
<td>1.00</td>
</tr>
<tr>
<td>ASD, n (%)</td>
<td>13,103 (1.55)</td>
<td>2.89</td>
<td>344 (0.07)</td>
<td>344 (0.07)</td>
<td>1158 (0.31)</td>
<td>586 (0.16)</td>
</tr>
<tr>
<td>AD</td>
<td>8216 (0.97)</td>
<td>3.07</td>
<td>41 (0.01)</td>
<td>41 (0.01)</td>
<td>308 (0.08)</td>
<td>146 (0.04)</td>
</tr>
<tr>
<td>Autism Spectrum Disorders</td>
<td>2596 (0.30)</td>
<td>3.07</td>
<td>219 (0.05)</td>
<td>219 (0.05)</td>
<td>227 (0.06)</td>
<td>125 (0.03)</td>
</tr>
<tr>
<td>PDD-NOS</td>
<td>291 (0.04)</td>
<td>3.07</td>
<td>219 (0.05)</td>
<td>219 (0.05)</td>
<td>227 (0.06)</td>
<td>125 (0.03)</td>
</tr>
<tr>
<td>Age at First ASD Diagnosis, Years</td>
<td>7.72 (2.32–10.32)</td>
<td>0.93</td>
<td>7.72 (2.32–10.32)</td>
<td>0.93</td>
<td>7.72 (2.32–10.32)</td>
<td>0.93</td>
</tr>
</tbody>
</table>
| Prevalence for offspring with uncles and aunts diagnosed with ASD from maternal lineage and paternal lineage is presented in Figure 2.

In analyses without covariate adjustment, presence of ASD diagnosis in a maternal uncle or aunt was associated with an increased risk of ASD, compared with a maternal uncle or aunt without ASD diagnosis (RR, 3.05; 95% CI, 2.52–3.64), while the RR for the paternal side was estimated at 2.08 (95% CI, 1.53–2.67). After adjustment for confounding, including adjustment for temporal trends and family psychiatric history, the RRs were slightly diluted: 1.88 (95% CI, 1.54–2.26) for maternal lineage and 1.44 (95% CI, 1.05–1.86) for paternal lineage (Table 2).

The RRs were similar for ASD in aunts and in uncles, for both the maternal lineage and the paternal lineage (Table 2): for adjusted models, maternal uncles had an RR of 1.92 (95% CI, 1.49–2.37), maternal aunts had an RR of 1.73 (95% CI, 1.19–2.35), paternal uncles had an RR of 1.63 (95% CI, 1.16–2.16), and paternal aunts had an RR of 1.07 (95% CI, 0.56–1.63).
the subgroup of offspring to the 344 mothers with an ASD diagnosis at time of delivery. For this subgroup, the RR of offspring with an uncle or aunt with an ASD diagnosis compared with offspring with uncle or aunt without an ASD diagnosis was estimated at 5.23 (95% CI, 1.94–14.11) (Table S10). The results in Table 2 remained unchanged when applying Firth’s adjustment for monotone likelihood (Table S11).

### Table 2. Relative Risks for ASD Among Participants With ASD-Affected Uncle(s)/Aunt(s) Compared With Participants With Uncle(s)/Aunt(s) Free From ASD Diagnosis

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Person-Years of Follow-up</th>
<th>Rates of ASD per 100,000 Person-Years</th>
<th>Relative Risk (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
<td>Unexposed</td>
<td>Crude</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted 1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted 2***</td>
</tr>
<tr>
<td><strong>Maternal Lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncle(s) affected by ASD</td>
<td>19,662</td>
<td>6,675,534</td>
<td>3.22 (2.54–3.91)</td>
</tr>
<tr>
<td>Aunt(s) affected by ASD</td>
<td>9984</td>
<td>6,337,615</td>
<td>2.73 (1.88–3.73)</td>
</tr>
<tr>
<td>Uncle(s)/aunt(s) affected by ASD</td>
<td>29,646</td>
<td>13,013,149</td>
<td>3.05 (2.52–3.64)</td>
</tr>
<tr>
<td><strong>Paternal Lineage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncle(s) affected by ASD</td>
<td>13,274</td>
<td>6,723,265</td>
<td>2.39 (1.72–3.13)</td>
</tr>
<tr>
<td>Aunt(s) affected by ASD</td>
<td>7342</td>
<td>6,292,408</td>
<td>1.52 (0.79–2.30)</td>
</tr>
<tr>
<td>Uncle(s)/aunt(s) affected by ASD</td>
<td>20,616</td>
<td>13,015,674</td>
<td>2.08 (1.53–2.67)</td>
</tr>
</tbody>
</table>

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*Affected by ASD” refers to an individual with a clinical diagnosis of ASD.
ASD, autism spectrum disorder; CI, confidence interval.
**Bootstrapped 95% CI: 2.5%–97.5% percentiles of estimates from 1000 bootstrapped samples.
***Adjusted for the birth year of the participant, the mother, and the uncle/aunt.
**Adjusted for covariates in Adjusted 1 and any mental illness (yes/no) of the mother and the uncle/aunt.

### Figure 2. Inverse Kaplan-Meier curves for autism spectrum disorder (ASD) probability among participants with uncle(s)/aunt(s) affected by ASD, compared with participants with uncle(s)/aunt(s) free from ASD diagnosis, by maternal and paternal lineage. Note: exposed groups (participants with aunt/uncle free from ASD diagnosis) were plotted in solid lines; unexposed groups (participants with aunt/uncle affected by ASD) were plotted in dashed lines.
DISCUSSION

To our knowledge, this work represents the first epidemiological study comparing transmission of maternally versus paternally mediated ASD risk in second-generation offspring of parents having siblings with ASD.

When estimating second-generation ASD risk, we observed that offspring of female individuals with a sibling diagnosed with ASD did not exceed what would be expected on the basis of autism recurrence rates in large-scale population-based twin and family studies: 0.8 for identical twins, 0.2 for nonidentical siblings, and 0.04 for second-degree relatives (34). Additionally, while point estimates were numerically higher in models examining maternal lineages, overlapping CIs indicated no difference in recurrence risk between offspring of females versus males with a sibling diagnosed with ASD. Within the second generation, ASD relative risk estimates did not differ between male and female offspring, contrary to the expected elevation for males under an FPE. Given these observations, an overarching inference is that the sex disparity in ASD may not be primarily derived from an FPE.

There are other potential models to explain the male predominance in ASD, the first being the corollary that male sex confers heightened sensitivity (in comparison to the population average for first-degree relatives) and therefore greater phenotypic expression of a given inherited liability. This would result in unaffected brothers and sisters of ASD probands carrying comparable levels of subthreshold liability, with male individuals at the upper extreme being affected and not contributing to risk among second-generation offspring. A second possibility relates to a recently published observation among monozygotic twins affected by ASD (predominantly male-male pairs) that there can exist pronounced variability in severity between identical co-twins (35). If such variability is more pronounced in male than in female carriers of ASD liability, this too could contribute to the observed sex ratio. Greater variability among males (GVM) would predict that more males than females would manifest higher severity of autistic features arising from the same level of inherited liability. From a statistical genetic standpoint, the FPE and male sensitivity hypotheses refer to sex-specific genetic thresholds of expression of liability, while GVM relates to sex-specific variance in phenotypic expression. To directly test the GVM hypothesis—which is as yet unproven—a population-level liability modeling and quantification of either genetic risk or phenotypic expression would be required—both are beyond the scope of the data available to this study. It is also important to note that FPE, male sensitivity, and GVM are not mutually exclusive events and could hypothetically coexist; the key conclusion from this analysis is that if FPE exists, it does not do so to an extent that substantially raises risk to second-generation offspring of sisters of ASD probands over what would be expected for second-degree relatives of affected individuals.

We also examined whether these estimates differed based on the time period of ASD diagnosis and family history of psychiatric diagnoses in the parental generation. These adjusted models, which accounted for potential bias due to temporal trends in ASD diagnosis and confounding due to environmental factors correlated with psychiatric illness, exhibited only in a slight attenuation of estimated effects. A sensitivity analysis of individuals without a family history of psychiatric diagnosis confirmed elevated recurrence risk independent of overlapping familial liability for ASD and other psychiatric conditions. Similar recurrence risk estimates were also observed both when restricting to the oldest cousin pair within families with ASD diagnosis and when directly comparing families with and without maternal siblings with ASD, demonstrating the robustness of the findings. In addition, the similar magnitude of risk estimates, whether ascertaining ASD or AD in the parental generation, suggests that this pattern of sex modulation applies across the continuum of ASD severity, consistent with a polygenic, additive genetic model of ASD.

Among neurodevelopmental disorders, ASD is distinguished by a well-documented increase in prevalence over the past 2 decades (19). Despite advances in early screening, the median diagnostic age is long after 2 years (36), an age at onset exhibiting diagnostic stability (37) as illustrated by this sample’s median diagnostic age of 7 years. For pediatricians representing the first-line resource for ASD screening, current guidelines do not articulate standards for evaluating family history of ASD in the parental generation. These findings suggest that irrespective of sex, offspring of parents with a sibling diagnosed with ASD warrant especially diligent screening, including family history, to inform risk stratification and planning. As knowledge of cross-generational ASD risk factors advances, family history in the parental generation may be integrated with genotyping of affected family members, quantitative autistic trait measures, and review of psychosocial factors associated with increased offspring risk (e.g., advanced parental age), leading to more comprehensive risk assessment and improved clinical guidance.

In population-based studies, social and potentially sex-related effects on the assessment of psychiatric diagnoses cannot be ruled out. Especially, there may be a risk for reversed causation if individuals in the parental generation seek psychiatric care after an ASD diagnosis in the offspring generations. To address this issue, we chose to consider ASD and other psychiatric diagnoses in the parental generation, including aunt and uncle, only at the birth of the offspring-generation participants. Therefore, aunt/uncle bias in ASD assessment after an ASD diagnosis of the offspring did not affect our results.

Study strengths include a large epidemiologic sample in a health system with equal access and near-complete follow-up of clinically ascertained diagnoses, which allowed ASD diagnoses in the offspring generation based on up-to-date ICD-10 criteria. Regarding limitations, our multigenerational cohort necessitated a parental generation that matured prior to widespread awareness of ASD and was thus prone to underascertainment, as suggested by that generation’s somewhat low ASD prevalence in our sample. Thus, these results likely underestimate risk for silent parental transmission: future studies that are fully representative of contemporary ASD criteria would be warranted to confirm findings in the context of milder forms of ASD in the parental generation. This concern is mitigated, however, by the lack of differences observed in transmission rates among families of probands diagnosed with ASD versus AD in the parental generation. Furthermore, even
though we present one of the largest and most detailed epidemiological examinations of maternal transmission, a study such as ours cannot detect the true underlying causes and mechanisms of transmission (or nontransmission) of autism. For this purpose, other types of studies are required, e.g., including genomic analysis with single nucleotide polymorphism data or genomic sequencing to obtain both copy number and point mutation data (38). We note, however, that at this stage of science, established molecular genetic correlates of ASD account for only a fraction of known inherited risk, and our study was focused on within-family transmission.

Although we performed a very detailed adjustment for temporal trends and familial psychiatric conditions, we acknowledge that residual confounding may remain. The determination of ASD diagnoses would also likely have been susceptible to sex biases inherent in ASD diagnostic criteria (8). In future studies, quantitative autistic trait measures, which were not widely available early in the parental generation, could be used to identify undiagnosed female individuals with clinically elevated autistic traits.

Conclusions
We found similar ASD risk in offspring from maternal and paternal lineages. The risk of ASD in offspring of siblings of ASD probands equals but does not exceed what has been observed for second-degree relatives within a single generation. These findings do not suggest female protective factors as the principal mechanism underlying the male sex bias in ASD.

While these results mitigate concern for amplification of maternally transmitted ASD risk, they affirm the importance of heightened surveillance for ASD in second-generation offspring. Given the benefits of early intervention, these results support incorporating second-degree family history of ASD in pediatric practice, as well as future studies involving behavioral phenotyping and genotyping to advance individualized estimates of ASD recurrence risk.

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The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

The authors report no biomedical financial interests or potential conflicts of interest.

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