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Review

Can the "female protective effect" liability threshold model explain sex differences in autism spectrum disorder?

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SUMMARY

Male sex is a strong risk factor for autism spectrum disorder (ASD). The leading theory for a "female protective effect" (FPE) envisions males and females have "differing thresholds" under a "liability threshold model" (DT-LTM). Specifically, this model posits that females require either a greater number or larger magnitude of risk factors (i.e., greater liability) to manifest ASD, which is supported by the finding that a greater proportion of females with ASD have highly penetrant genetic mutations. Herein, we derive testable hypotheses from the DT-LTM for ASD, investigating heritability, familial recurrence, correlation between ASD penetrance and sex ratio, population traits, clinical features, the stability of the sex ratio across diagnostic changes, and highlight other key prerequisites. Our findings reveal that several key predictions of the DT-LTM are not supported by current data, requiring us to establish a different conceptual framework for evaluating alternate models that explain sex differences in ASD.

INTRODUCTION

The female protective effect

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social function, restricted interests, and repetitive behaviors. ASD is currently diagnosed in 1:44 children and is reported ~4 times more often in boys than in girls (Maenner et al., 2021), a ratio that has been remarkably stable over time (Kanner, 1943). Despite studies focused on sex chromosomes, hormonal influences, and diagnostic biases, the etiology of this sexual dimorphism remains poorly understood. A popular model to explain this sex bias invokes the existence of a "female protective effect" (FPE). Although FPE is not always defined as a testable hypothesis, the most common theoretical model to explain FPE involves males and females having differing thresholds for ASD in a liability threshold model (DT-LTM). To critically evaluate the evidence for a DT-LTM, we first describe the prerequisites and testable predictions of this model across multiple domains and then summarize the evidence for and against the DT-LTM using published data.

The differing thresholds-liability threshold model

The prevalence of many complex traits varies with age, sex, or other risk factors. The DT-LTM, as initially proposed by Carter and subsequently mathematically modeled by Falconer (Carter, 1961; Falconer, 1965), posits that for categorical conditions (e.g., ASD diagnosis), there is a continuous distribution of underlying liability (see glossary, Box 1) within the population. This theoretical concept, liability, is the sum of every risk factor a person has (for example, highly penetrant *de novo* variants (Box 1), polygenic risk (Box 1), and environmental exposures). The DT-LTM posits that individuals whose total liability exceeds a certain threshold are affected (e.g., diagnosed with ASD). Furthermore, known risk factors, such as sex, can be modeled to increase or decrease the liability threshold for males relative to females. The implications are similar, whether this is conceptualized as involving sex differential thresholds for one liability distribution (Figure 1A) or sex differences in mean liability for two populations

> **Box 1. Definitions of human genetics terms**
>
> **Carter effect**: the prediction that in a complex genetic condition with differing prevalence by sex, familial recurrence will be higher when an affected family member is of the less commonly affected sex. For ASD, higher familial recurrence is predicted for females relative to males with ASD.
>
> **Constrained gene**: a gene which strongly influences the likelihood of survival or reproduction and in which mutations are rapidly selectively removed from the human population.
>
> **Constraint**: limitation on the prevalence of gene mutations in the context of selective pressures related to the gene's influence on survival and/or reproduction.
>
> **Epistatic model**: a model involving risk that cannot be added up across independent risk factors (or genetic loci) but involves statistical interaction between loci, or between loci and sex.
>
> **Heritability**: the proportion of variation in a trait or diagnosis that is due to genetic variation. This ranges from 0 to 1 and can be estimated using information about trait similarity and genetic similarity, where genetic similarity is estimated either from family relationships or SNP genotyping.
>
> **Liability**: the sum of the effects of all risk factors for a given condition. This is assumed to be normally distributed across the population.
>
> **Liability threshold**: the amount of liability needed to develop a condition.
>
> **Multiplex**: a family with multiple individuals with ASD.
>
> **Odds ratio**: a statistical measure of how much more likely a person is to be diagnosed with a condition if they carry a particular genetic variant.
>
> **Penetrance**: the likelihood of a clinical condition in the presence of a particular genetic variant, generally considered in terms of the proportion of individuals with that genetic variant who have the condition.
>
> **Polygenic**: a trait or diagnosis that is influenced by many genes or genetic loci.
>
> **Polygenic risk score (PRS)**: an estimate of an individual's genetic liability for a trait or disease based on genetic data from many loci. The PRS is based on the sum of risk alleles weighted by risk allele effect sizes, as estimated by genome-wide association study (GWAS) data.
>
> **Proband**: the initial family member identified with a trait or disease.
>
> **Quantitative autistic trait (QAT)**: an ASD-related feature measured according to ratings along a continuous scale, allowing detection of relevant variation in individuals with and without ASD. Higher scores indicate a greater level of ASD-related features and a stronger association with ASD diagnosis. Well-known QAT metrics include the social responsiveness scale, broad autism phenotype questionnaire, and autism spectrum quotient.
>
> **Risk variants**: differences in nucleotide sequences that demonstrate a statistical association with increased risk of disease across a population.
>
> - *de novo* variant: a variant found in the child, but not in either parent, indicating a mutation that arose in a germ cell or in the fertilized egg
> - **rare variant**: variants found in <1% of a population
> - **common variant**: variants found in >1% of a population
>
> **Sex heterogeneity**: differences by sex. A genetic model of sex heterogeneity would be differing genetic risk factors for males and females.
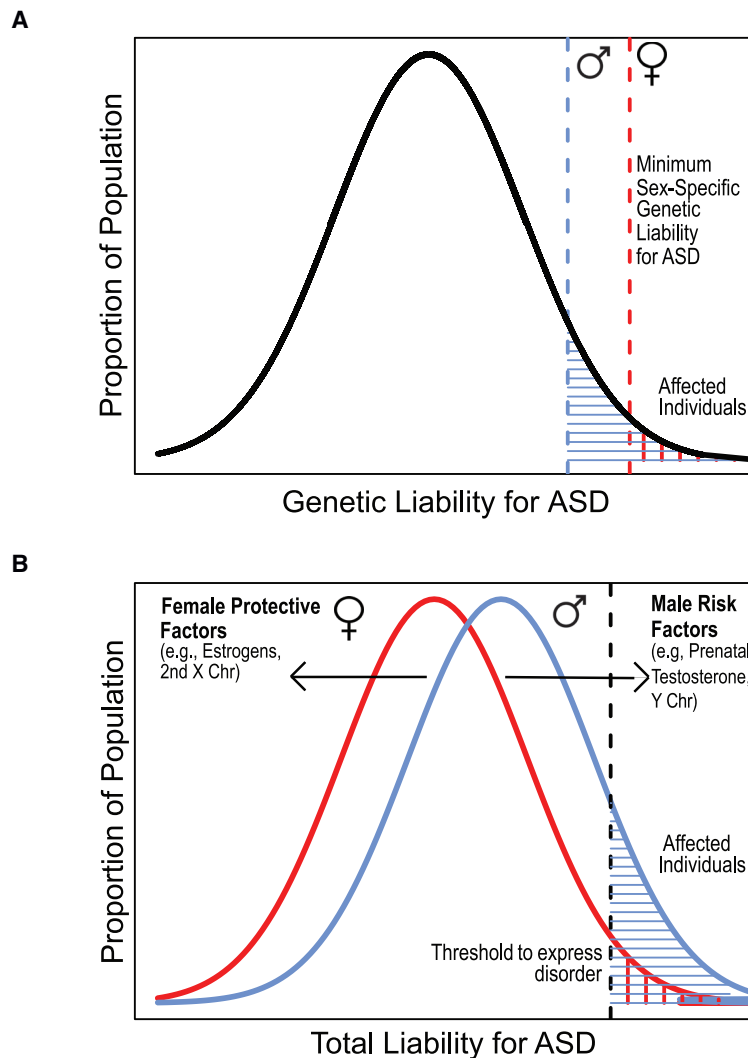>
> **Simplex**: a family with a single individual with ASD and no family history of ASD.
>
> **Single-nucleotide polymorphism (SNP)**: a genetic variant involving a single-nucleotide difference across individuals with frequency of at least 1%. SNP is often used as a synonym for a common variant.

with a single threshold (Figure 1B). Sex-specific genetic (e.g., X or Y chromosome loci) and non-genetic (e.g., hormonal exposures or diagnostic bias) risk factors can influence this threshold but are required to have a uniform effect within each sex (i.e., no interaction). The DT-LTM fits characteristics of several diseases with differences by sex and age of onset (e.g., renal stone disease, pyloric stenosis, club foot, and peptic ulcer) (Falconer, 1965). In each of these diseases, there was no evidence for different risk loci across groups, only different thresholds, as expected under the DT-LTM.

As with all models, formal prerequisite assumptions underlie the DT-LTM (Figure 1C). (1) Other than a single threshold-modifying factor (i.e., sex), all genetic variants (including those on the X or Y chromosome) are expected to affect ASD liability in both females and males, with no sex-specific effects or gene-by-sex interaction. It should be noted that a male but not a female with the same variant could be affected in the absence of interaction if the variant carried enough liability to exceed the male, but not the female, threshold. (2) The DT-LTM assumes that the population liability in both sexes follows a normal distribution and that the variance is identical between sexes. Differences in variance across sexes could generate prevalence differences above a threshold but that would violate the LTM (Traglia et al., 2017). (3) Liability from additive risk factors can either be large and sufficient to exceed the threshold alone (e.g., *de novo* variants) or small and only exceed the threshold in combination with other genetic variants (e.g., single-nucleotide polymorphisms [SNPs]; Box 1). For this reason, the LTM is also known as a multifactorial

A

B

C

Prerequisites of the DT-LTM
1. Multifactorial & Additive Risk
2. Male and Female Genetic Risk Factors Must Be Identical
3. Equal Variance Across Sexes

Figure 1. The liability threshold model
(A) This normal curve illustrates ASD liability in a population. Individuals exceeding a liability threshold would be diagnosed with ASD (shaded areas). The 4:1 ratio of males to females under the DT-LTM can be observed with the female threshold being shifted to the right.
(B) A variation of this model conceptualizes that a single ASD threshold exists for the population. In this version, both males and females would have normal distributions of ASD liability, but the male mean would be shifted slightly toward ASD, such that more males cross the single diagnostic threshold (black line) to yield a 4:1 ratio.
(C) The LTM has three explicit prerequisites: risk must be multifactorial and additive, the genetic risk factors and their relative impact must be identical between sexes (perfect genetic correlation), and the variance in liability must be the same for both sexes.

ters of affected fathers have the highest recurrence, and siblings of affected males have significantly higher recurrence rates than siblings of affected females (Kruse et al., 2008). Finally, similar to ASD, Hirschsprung's disease has high heritability (>80%; Tilghman et al., 2019), a multifactorial contribution of rare and common genetic variants, and a 4:1 sex bias. Hirschsprung's disease shows the predicted increased recurrence rates in relatives of female probands (Box 1; Badner et al., 1990) and exhibits a good fit to the DT-LTM (Emison et al., 2010).

The FPE and DT-LTM in prior ASD literature
The DT-LTM has frequently been referenced, explicitly or implicitly, to explain the observed sex differences in ASD. The term FPE was initially used in the ASD literature to refer to differences in the sex ratio of ASD with concurrent intellectual disability (ID) (~2:1) relative to ASD without ID (~4:1), suggesting that females require a greater genetic "insult" to develop ASD (Tsai and Beisler, 1983; Wing, 1981). Recent references to the FPE consider rare variant studies (De Rubeis et al., 2014; Iossifov et al., 2012; Jacquemont et al., 2014; Levy et al., 2011; Lim et al., 2013; Neale et al., 2012; Sanders et al., 2011; Satterstrom et al., 2020) and conclude that ASD-affected females have proportionally more highly penetrant de novo mutations. Liability thresholds are specifically mentioned in a subset of these papers (De Rubeis et al., 2014; Girirajan et al., 2012; Neale et al., 2012; Ye et al., 2017) or the FPE is explicitly explained as a LTM (Satterstrom et al., 2020). Likewise, the literature describing quantitative autistic traits (QATs; Box 1) in the general population refers to the differing mean version of the DT-LTM (Figure 1B); this corresponds to lower QAT levels in females in the general population (Constantino and Gruber, 2012;

threshold model (Reich et al., 1975), reflecting additive liabilities from multiple factors.

The DT-LTM fits several complex disorders reasonably well. For example, young-onset venous thromboembolism (VTE) often reflects strong family history and/or highly penetrant risk variants, since youth is protective for VTE (Zöller et al., 2016). The same observations extend to sex differences in the manifestation of several diseases. Females, for example, have a higher prevalence of Hashimoto's thyroiditis, whereas males have greater heritability (Skov et al., 2021). In another female-biased condition (scoliosis), the DT-LTM predicts that sons of affected mothers have the lowest recurrence among first-degree relatives, daugh-

Constantino and Todd, 2003). Some reviews directly equate the FPE to the DT-LTM (Werling, 2016), whereas others mention the DT-LTM or similar models as a possible explanation for "female protection" (Ferri et al., 2018; Robinson et al., 2013; Szatmari et al., 2012; Tsai and Beisler, 1983). However, the FPE is also sometimes mentioned without reference to a DT-LTM, and one must infer the underlying model (Doan et al., 2019; Gockley et al., 2015; Iossifov et al., 2014; Jacquemont et al., 2014; Sanders et al., 2015). Nevertheless, the data used to support the FPE in these papers are aligned with expectations of the DT-LTM, but not with other models of "female protection" (see discussion). For these reasons, we assume that the predominant testable model underlying the "FPE" in ASD is the DT-LTM. However, the FPE/DT-LTM is often referenced in studies examining a single variable, such as *de novo* mutation rates, and thus has not been comprehensively evaluated. Herein, we describe and evaluate assumptions about how liability is related to measurable variables, delineate testable hypotheses derived from the DT-LTM, and review the evidence as to whether sex differences in ASD fit the DT-LTM.

Testable predictions and assumptions of the DT-LTM and outline of the review

Liability is an unmeasurable entity, which theoretically sums all risk factors and assumes that they are normally distributed in a population. Here, since ASD is highly heritable (Box 1), we exclusively focus on genetic liability and include *de novo* mutation as a nonheritable genetic source of liability. We consider sex (and sex hormones, diagnostic bias, or other sex-correlated variables) as potentially modifying the threshold for ASD (Figure 1A). *The resulting DT-LTM hypothesis predicts that females have a higher threshold than males, and thus females with ASD will, on average, carry more genetic risk than males with ASD.* Measurable categories of genetic risk include heritable polygenic risk summed over the genome and highly penetrant (often *de novo*) individual risk variants.

Indicators of higher genetic risk examined:

- heritability estimates (heritability)
- recurrence rates in relatives (recurrence rates [the Carter effect]), and
- penetrance (Box 1) measures for specific risk variants/genes (correlation between penetrance for ASD and sex ratio).

Beyond direct genetic tests of the DT-LTM, other features of how ASD manifests across sexes *might* support the DT-LTM. However, their utility depends on testable secondary assumptions:

Testable secondary assumptions examined:

- QATs in the population reflect ASD genetic liability (population traits),
- specific clinical features (e.g., ID or core symptom severity) increase with greater liability (clinical features and sex ratio), and
- recent increases in ASD prevalence result from inclusion of individuals with less genetic liability (sex ratio versus population prevalence).

If these secondary assumptions hold, respectively, then, the DT-LTM could also be evaluated using:

- sex differences in QATs in the general population (population traits),
- sex differences in co-morbidities/severity (clinical features and sex ratio), and
- the relationship between changes in ASD prevalence and changes in ASD sex ratios over time (sex ratio versus population prevalence).

Importantly, the LTM itself has three key prerequisites (prerequisites underlying the LTM):

- risk is multifactorial and additive,
- variance in liability is the same between sexes, and
- risk factors are identical between sexes.

Finally, we discuss alternative models to the DT-LTM and their derivative predictions (toward alternate models to explain the sex difference in ASD).

HERITABILITY

Under the DT-LTM, the heritability (Box 1) of ASD is expected to be higher for females. However, there could be opposing consequences of different categories of genetic risk (i.e., less polygenic effects versus more highly penetrant *de novo* variants), and the latter would not contribute to heritability estimates under some study designs. Therefore, we evaluate sex-specific heritability estimates from two complementary data sources: (1) twin study estimates, including polygenic/common variants, rare variants, and *de novo* mutations and (2) SNP-based estimates assessing common variant risk. Used together, these sources should enable evaluation of predictions of the DT-LTM. For example, if common-variant risk is insufficient to push females over their higher threshold, females with ASD would be predicted to have lower SNP-based heritability (Box 1) for ASD than males. In contrast, higher female ASD heritability would be predicted in twin studies, where *de novo* mutation contributes to heritability estimates.

One limitation for most studies is that although ID often co-occurs with ASD, ID represents a distinct diagnostic entity (DSM-5 Task Force, 2013). This is problematic because epidemiological work suggests lower heritability (Box 1) for a diagnosis of "ASD with ID" (33%) than a diagnosis of "ASD without ID" (65%) (Xie et al., 2020). ID accompanies ASD in ~20%–35% of all currently diagnosed cases but is more likely to be attributed to a single highly deleterious (often *de novo*) mutation (Maenner et al., 2021; Myers et al., 2020). As such, a failure to consider ID can confound the interpretation of sex differences in heritability. Similar observations have been made for ID without ASD, where mild ID shows high heritability and siblings with decreased mean IQs, whereas severe ID shows little heritability and siblings with typical IQs (Reichenberg et al., 2016). Because of the limited availability of IQ/ID information in many ASD studies, we will assess the fit of the DT-LTM within each category of genetic risk separately (rare/*de novo* versus common/polygenic).

Twin-based estimates of heritability

Twin studies capture both heritable and *de novo* variations, as monozygotic twins share early *de novo* mutations. The largest sample with sex-specific heritability based on categorical diagnosis includes over 17,000 twins in Sweden (Anckarsäter et al., 2011). Using a categorical threshold based on parental report on the autism-tics, AD/HD and other comorbidities inventory (A-TAC), higher additive heritability (Box 1) was identified in males than in females, contrary to the prediction of the DT-LTM. Another study, which identified 192 twin pairs from the California Department of Developmental Services (Hallmayer et al., 2011) found equivalent additive heritability in males relative to females.

Beyond categorical classification, QATs (Box 1) have also been used to estimate ASD heritability. QATs capture continuous variation in traits that correlate with ASD across individuals both with and without ASD, allowing for larger sample sizes and increased power. The majority of twin studies that evaluate QATs, which collectively have applied several QAT metrics (Constantino and Todd, 2003; Hoekstra et al., 2007; Ronald et al., 2006; Taniai et al., 2008; Taylor et al., 2020; de Zeeuw et al., 2017), similarly find no evidence for sex differences in additive genetic contributions or for sex-specific genetic influences. Of the few studies showing sex differences, all found greater heritability in males, contrary to the prediction of the DT-LTM (Holmboe et al., 2014; Stilp et al., 2010; Taylor et al., 2020). Taken together, twin studies of ASD, by either diagnosis or QATs, do not support the DT-LTM.

SNP-based heritability and polygenic risk

Current evidence suggests that polygenic risk contributes to ASD in both sexes; however, observations of sex differences in SNP-based heritability (Box 1) or polygenic risk scores (PRSs, Box 1) have been inconsistent. The DT-LTM predicts heritability estimates to be higher in females than in males. Although it is straightforward to generate sex-specific estimates for SNP-based heritability and polygenic risk, power is limited by the size of the female subset, requiring careful matching of cases. The study by Mitra et al. (2016) used several approaches to overcome the greater power of the male subset, concluding that males and females carry similar polygenic risk. In their estimates, neither a first hypothesis that female cases would have higher polygenic liability than males nor an alternate hypothesis that females could have lower polygenic risk (e.g., if polygenic risk is insufficient to reach a higher female-specific threshold, such that females would instead require high-liability events such as *de novo* causal mutations) was supported. However, these studies were underpowered to detect subtle sex differences in heritability or polygenic risk. A recent study (Warrier et al., 2022) examined SNP-based heritability using combined Simons simplex collection (SSC) & Simons Foundation Powering Autism Research (SPARK) data. In contrast to the findings above, they observed higher SNP heritability in males; however, the female estimate was in between estimates for ASD with ID or ASD mutation carriers and ASD without ID, as could reflect the known sex differences in proportion of ID and mutation carrier rates. Thus, their heritability observations failed to support a DT-LTM.

If PRS contributes substantially to genetic liability, the DT-LTM predicts that female cases will have a higher mean PRS than male cases. Prior calculations of PRS (Mitra et al., 2016; Weiner et al., 2017) did not show a sex difference: the PRS mean was elevated in cases versus controls as expected but not between well-matched males and females in either group. We have repeated this PRS analysis using the same dataset (Mitra et al., 2016) but with the PRS score defined using recent, more powerful data (Grove et al., 2019). We still find case-control differences in each sex, but no sex differences [unpublished]. A recent study using PRS from the iPsych cohort applied to SSC and SPARK data also failed to show differences in PRS between male and female ASD probands (Wigdor et al., 2021), similar to a prior study on SPARK samples (Matoba et al., 2020). Notably, a separate study of 11,313 ASD families also including SSC, SPARK, and additional REACH samples found significantly increased PRS in female versus male probands (Antaki et al., 2022) using family-based data and a "composite" PRS score aggregated from PRSs for ASD, schizophrenia, and educational attainment. It is unclear whether the use of a composite score, an increased sample size, or different selection criteria contribute to the differences observed between these studies. A separate analysis of largely overlapping datasets reported an increased PRS in females compared with males with ASD and no ID (Warrier et al., 2022). However, it is not clear whether the PRS sex difference observed in Antaki et al. could account for a 4:1 M:F ratio, as PRS captures only approximately 2% of ASD variance (Antaki et al., 2022; Grove et al., 2019; Figure S1). In studies designed to address sex differences in SNP-based heritability and PRS, it may be possible to use well-matched, unrelated case-control data, as related controls that are not matched for sex can confound data interpretation.

RECURRENCE RATES (THE CARTER EFFECT)

The most direct prediction of the DT-LTM is a difference in familial recurrence based on the sex of the proband, termed the Carter effect (Box 1; Carter, 1961; Falconer, 1965). This can be assessed for both the ASD diagnosis and QATs. Although familial recurrence is related to heritability, the *proportion* of trait variation that results from genetic factors, this proportion could differ between sexes—even with similar genetic liability—if non-genetic contributions to variation differ by sex. In contrast, recurrence in relatives examines the proportion of affected relatives by sex of proband and can hold the sex of both the relatives and non-genetic contributions constant. The DT-LTM predicts that rates of ASD will be higher in relatives of female probands compared with relatives of male probands. Overall, recurrence rates would be expected to be highest among male relatives of female probands and lowest for female relatives of male probands (Box 1). However, unlike many common heritable disorders, ASD has a large impact on fecundity, which is larger in males than in females (Power et al., 2013). For this reason, we cannot consider parental data and can only assess sibling or extended relative data.

Evidence for the Carter effect has been mixed in two studies using data from the multiplex-focused autism genetic resource exchange (AGRE). An initial study examining 405 sibling

relationships in AGRE found no difference in the recurrence of ASD among relatives of ASD-affected males and females, including when the analysis was restricted to individuals without ID (Goin-Kochel et al., 2007). However, another sampling from AGRE (120 multiplex nuclear families with 305 twin pairs) exhibited a 2.25-fold higher ASD recurrence in siblings of female relative to male probands (Werling and Geschwind, 2015). In an additional study using data from the online Interactive Autism Network, proband sex had a negligible contribution to the level of QATs in siblings (Constantino et al., 2010).

Prospective examination of ASD diagnosis using infant sibling studies have provided an additional opportunity to explore recurrence. Using this study design, younger siblings of a proband with ASD are assessed from infancy with a standardized protocol to establish ASD diagnosis, thereby removing clinical ascertainment bias and concerns of sex bias in ASD ascertainment as children age. Leveraging the Baby Siblings Research Consortium, which contains pooled recurrence data across over 1,000 unique families (Messinger et al., 2015; Ozonoff et al., 2011), a male sex bias in affected siblings (approximately 3:1) was reported, but there were no differences in recurrence by proband sex.

Epidemiologic approaches using population databases have the advantage of minimizing bias from clinically referred samples and can provide comparisons with population rates of ASD. Earlier, smaller studies (N < 300 families) appeared consistent with the Carter effect (Ritvo et al., 1989; Sumi et al., 2006). Likewise, a QAT study using dizygotic twins (N > 9,000) found that females scoring in the $90^{th}/95^{th}$ percentile (i.e., with the highest level of QATs) had more impaired relatives than males with similar scores, consistent with a Carter effect if liability correlates with QATs (Robinson et al., 2013). The most substantial support derives from a retrospective study using insurance records from >37,000 families with at least one diagnosis of ASD (Palmer et al., 2017). For male probands, ASD was diagnosed in 4.2% of sisters and 12.9% of brothers, whereas for female probands, ASD was diagnosed in 7.6% of sisters and 16.7% of brothers. The increase in all siblings of female, compared with male, probands is consistent with a Carter effect.

Epidemiologic registries employ data analysis from tens of thousands of ASD-affected families over several decades; however, these larger datasets fail to support a Carter effect. One study using the Swedish Registry found no differences in recurrence for cousins, aunts, and uncles, based on proband sex (Sandin et al., 2014), and failed to find differences in recurrence in nieces and nephews of ASD probands (Bai et al., 2020). Another study using multinational registries (ASD N > 60,000) reported the highest recurrence risk with an older female sibling. However, unlike the Carter effect, which predicts that ASD recurrence in male siblings of female probands would be highest and recurrence in female siblings of male probands would be lowest, this study found that younger female siblings of female probands were at highest risk, whereas the lowest recurrence involved male-male sibling pairs (Hansen et al., 2019). In a study using the population-based Danish Registry (Grønborg et al., 2013), which contains 7,284 cases of ASD among 1.5 million children, there were no statistically significant sex differences in recurrence when considering sibling sex, and counter to the

DT-LTM predictions, recurrence risk in male siblings of female probands was lowest (5.7; 95% CI 3.4–9.5). Finally, one unpublished study in the Danish iPsych cohort found that siblings of female probands (ASD without ID) demonstrated higher recurrence (N_f = 1,707; odds ratio [OR] 7.2) than those of male probands (N_m = 6,270; OR 3.8; Wigdor et al., 2021). However, it should be noted that these sibling recurrence rates were both substantially lower than those seen in prior studies (Grønborg et al., 2013; Ozonoff et al., 2011), despite recurrence typically increasing for ASD without ID.

In summary, recurrence by proband sex has been examined using a wide range of study designs, and several of the larger studies do not support a Carter effect. In contrast, studies of other heritable disorders with a similar sex bias (pyloric stenosis, multiple sclerosis, and clubfoot) (Carter, 1961; Kantarci et al., 2006; Kruse et al., 2008) provide stronger evidence for a Carter effect, which, given the very large ASD sample sizes, implies sufficient power to detect effects. In the future, more consistent subtyping of ASD cases with and without ID could be informative. Implementing both categorical and QAT measures could confer additional sensitivity and minimize ascertainment bias related to diagnosis by sex.

CORRELATION BETWEEN PENETRANCE FOR ASD AND SEX RATIO

One prediction of the DT-LTM is that the liability of a specific gene or variant, which is measured by penetrance or OR (Box 1), should be correlated with the sex ratio of those affected. Specifically, the greater the penetrance, the closer to 1 the sex ratio should be because highly penetrant variants would increase risk enough that both males and females cross the diagnostic threshold, whereas less penetrant mutations could fall between the male and female thresholds. Overall, for ASD cases carrying rare, highly penetrant (usually *de novo*) variants, the sex ratio is close to 1:1 (Turner et al., 2019). Thus, proportionally, females with ASD carry more highly penetrant *de novo* mutations (Iossifov et al., 2014; Jacquemont et al., 2014; Sanders et al., 2015; Turner et al., 2019). However, within the class of highly penetrant *de novo* mutations, there has not been any investigation of the relationship between sex ratio and ASD liability. For this reason, we examined the correlation between penetrance and sex ratio for ASD in several paradigms: (1) rare variants ascertained in non-syndromic genome-wide sequencing studies for ASD, (2) Mendelian disorders, and (3) quantiles of polygenic risk corresponding to varying ORs.

First, we compared a recent analysis of sex ratios by gene for rare variants (Turner et al., 2019) with published penetrance data, focusing on 10 genes from the Simons Foundation Autism Research Initiative (SFARI) curated high-confidence ASD gene list (Abrahams et al., 2013) with the most publications and N > 10 cases observed in Turner et al., and thus better estimates of sex ratio. Contrary to the prediction of the DT-LTM, we did not find a negative relationship between penetrance and sex ratio (Figure 2A). However, one caveat is that penetrance data are from different sources with potential differences in ascertainment and diagnostic biases. Furthermore, due to the low rate of loss-of-function variation in high-confidence ASD genes in
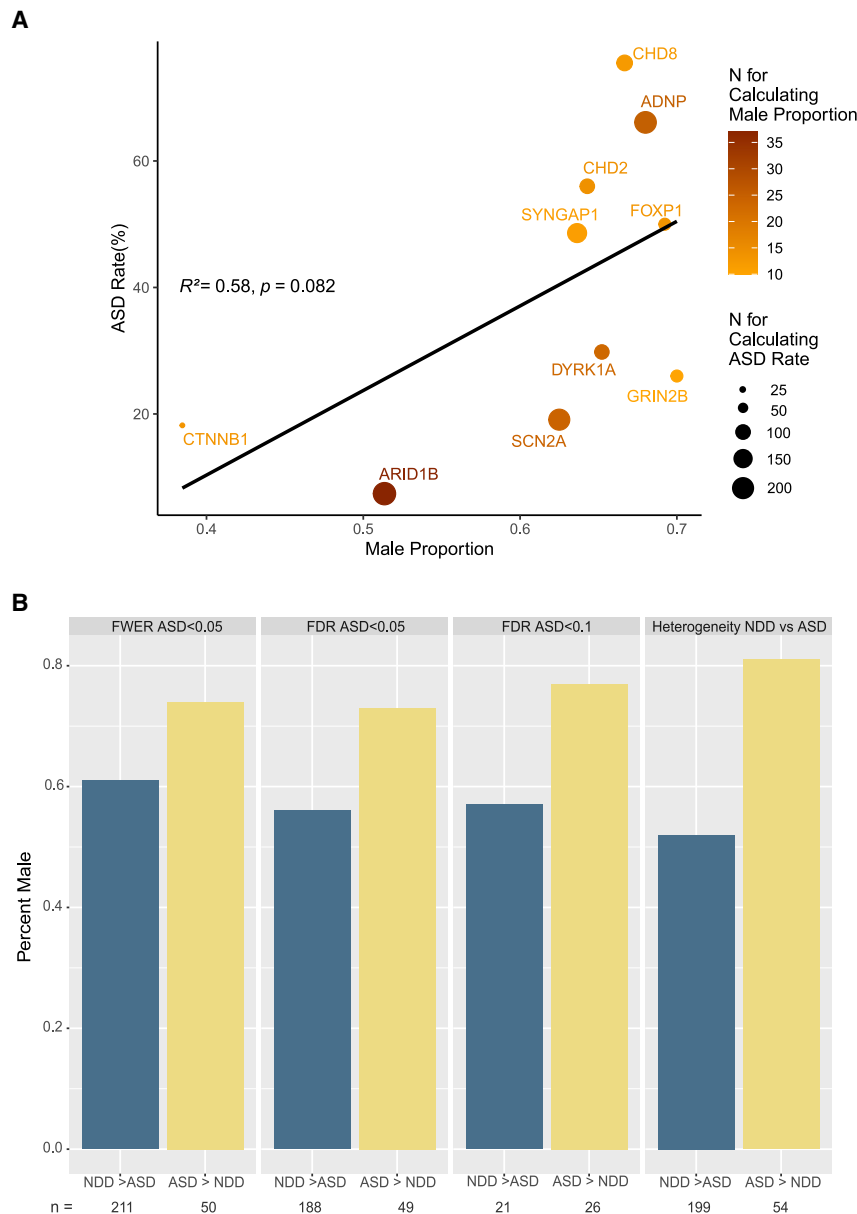
A

B

ASD penetrance in this high-liability category of mutation. Under the DT-LTM, higher ASD-specific liability genes should lead to ASD in females and in males equally (each gene having a threshold-crossing effect on liability, regardless of sex). Meanwhile, lower ASD-specificity genes (that also cause NDD) would show increased M:F sex ratios, as the ASD-specific liability they contribute may not be sufficient to cross the greater female threshold. However, examining gene categories from Satterstrom et al. with SNV data by sex from Turner et al., genes with increased ASD specificity did not show the decreased sex ratios predicted using the DT-LTM. Instead, the proportion of males was higher with more ASD-specific genes. Taken together, the observations based on currently available data are inconsistent with the DT-LTM (Figures 2B and 2C).

One limitation of both analyses is that they are based on loss-of-function variation in highly constrained genes. The data most useful for this kind of comparison may be intermediate penetrance mutations (e.g., damaging missense variants or strong regulatory variants), which might occur with sufficient frequency in controls to measure ASD ORs with confidence. Additional data may make these analyses possible in the future.

We next considered Mendelian syndromes that have moderate ASD penetrance but that were less likely to be ascertained due to ASD symptomatology alone and contained larger sample numbers to improve penetrance estimates. A 2008 review summarized syndromes with ASD penetrance ranging from

the general population, it is difficult to precisely estimate ORs or penetrance without ascertainment by mutation. However, Satterstrom et al. also evaluated categories of genes based on their evolutionary constraint (Box 1) in the human population, with variants of more constrained genes being expected to confer greater liability. Although they found the expected relationship of elevated mutation rates in constrained genes in cases over controls, they did not find sex differences within ASD for the high or intermediate constraint genes (Satterstrom et al., 2020).

We also examined the ASD specificity (rather than neurodevelopmental disorders [NDDs]) of a given gene as another framework for penetrance or ASD-specific liability. Since the prevalence of highly constrained gene mutations is nearly zero in unaffected controls, we compared NDD (without ASD) with

20%–90%; however, despite variations in penetrance, nearly all autosomal conditions showed equal sex ratios in ASD-affected probands (Abrahams and Geschwind, 2008). In several RASopathies with modest ASD penetrance, we did not find the DT-LTM predicted relationship between sex ratio and penetrance (Adviento et al., 2014). In our large study of Neurofibromatosis type 1 (NF1), 13% of NF1-affected subjects scored in the top centile of the population for ASD traits, and the sex ratio was 1.6:1 in this subset (Morris et al., 2016). Another example is tuberous sclerosis complex (TSC), which also exhibits moderate penetrance for ASD (25%–50%) (Specchio et al., 2020). In the largest study, no sex differences were found for ASD diagnosis within TSC, although another study reported a male bias of 1.6:1 (Mitchell et al., 2021; de Vries et al., 2007). Similarly, *PTEN* mutations (with intermediate penetrance for ASD) have sex ratios for ASD close to 1 (Hansen-Kiss et al., 2017). The DT-LTM would predict that mutations carrying modest penetrance for ASD (i.e., *NF1*, *TSC1/TSC2*, and *PTEN*) would have more male-skewed sex ratios than highly penetrant syndromes or genes, such as *CHD8* (Figure 2A, sex ratio 3:1; Hanly et al., 2021), but they do not.

Finally, we compared polygenic risk (binned into quantiles) with the sex ratio. When applying Grove et al. ASD PRS to proband data from Mitra et al., we found no difference in sex ratios between the lowest and highest quantiles, contrary to the predictions of the DT-LTM (Grove et al., 2019; Mitra et al., 2016). Likewise, we examined individual datasets with different sex ratios, as well as performed a meta-analysis, and found no evidence of differing sex ratios. Although the highest categories of PRS may still have lower ORs than monogenic causes of ASD, data across both categories of genetic variation fail to support a relationship between ASD penetrance and sex ratio.

POPULATION TRAITS

QATs are sometimes considered a proxy for ASD genetic liability. QATs usually derive from caregiver- or self-report questionnaires that quantify behaviors related to core ASD domains of social communication and restricted, repetitive behaviors, comprising a continuum across typical and affected populations. We first evaluated the secondary assumption that measured QATs are closely related to genetic liability for ASD, so that we could determine whether the data collected for QATs fit the DT-LTM. Specifically, the DT-LTM predictions show that (1) the distribution of QATs in the general population has a lower (further from impairment) mean in females compared with males, but similar variance, and (2) that the mean difference between sexes can account for prevalence differences in diagnosis if the same "diagnostic" threshold is applied to both sexes (Figure 1B).

There are two lines of evidence demonstrating that some QATs may be reasonable proxies for liability. First, like ASD itself, QATs are highly heritable. Across twin studies, QAT heritability ranges from 0.4 to 0.8 for several instruments, including the social responsiveness scale (SRS) (Constantino and Todd, 2003), autism spectrum quotient (ASQ) (Baron-Cohen et al., 2006; Hoekstra et al., 2007), and childhood autism spectrum test (CAST) (Ronald et al., 2006). Heritability is also supported by

family studies finding parent-offspring correlations in QATs, including in the general population (Klusek et al., 2014; Lyall et al., 2014; Page et al., 2016). Second, QATs share heritability with ASD based on both behavioral genetic approaches and genetic association studies. Specifically, family studies (Lundström et al., 2012; Robinson et al., 2011) have identified overlap between heritable variation related to ASD and QATs, both at levels typical of the general populations and extremes consistent with ASD. In addition, human genetic studies identified common genetic signals for ASD and QATs through linkage (Coon et al., 2010; Duvall et al., 2007; Liu et al., 2008), genome-wide association studies (GWASs) (Bralten et al., 2018; Robinson et al., 2016; St Pourcain et al., 2010, 2018), and methylome-wide association studies (MWASs) (Massrali et al., 2019). Finally, ASD PRS also shows associations with QATs (Nayar et al., 2021; Takahashi et al., 2020). One study using several large ASD consortium and population-based resources (total n > 38,000) found that estimated genetic correlations between ASD and social communication traits ranged from $r_g = 0.27-0.30$ (Robinson et al., 2016). These findings support the secondary assumption that QATs reflect genetic liability for ASD, thus allowing us to use them to examine the DT-LTM.

Examining the predictions of the DT-LTM, QAT scores for females in the general population are shifted farther away from the ASD threshold than scores for males. For example, in the standardization sample for the SRS-2 (Constantino and Gruber, 2012), males show higher mean raw SRS scores (mean = 33) than females (mean = 28), indicating greater population-wide QAT burden in males (Figure 3). Higher population-wide QATs in males have also been observed in other quantitative measures, including the ASQ (Baron-Cohen et al., 2006; Greenberg et al., 2018; Hoekstra et al., 2007) and A-TAC (Lundström et al., 2019). Cross-cultural adaptations of these measures have also shown greater population-wide QATs in males (Cheon et al., 2016; Wakabayashi et al., 2006). This implies a fundamental sex difference in QATs across distinct cultures.

Although the higher population-wide QAT means in males conform to the predictions of the DT-LTM (Figure 1), other observations do not. For example, modeling the observed mean difference by sex in the SRS cannot create a large enough sex bias in the tail of the distribution (representing ASD) (Figure 3). Applying the same threshold (Figure 3A) to both males and females produces a ~2-fold difference in the number of males to females above threshold, rather than the expected 4:1 ratio. In addition, when applying the SRS-2 to the general population (Constantino and Gruber, 2012), higher variance was observed for males relative to females ($SD_{male} = 25.2$, $SD_{female} = 23.7$). A difference in variance explicitly violates the prerequisites of the DT-LTM. Taken together, although evidence supports overlapping genetic factors and a male sex bias for both ASD and QATs, properties of the population-wide distributions of QATs in males and females and variance in scores for each sex do not conform to prerequisites and predictions under the DT-LTM.

CLINICAL FEATURES AND SEX RATIO

There are additional testable predictions of the DT-LTM if we can accept specific assumptions about clinical features. One
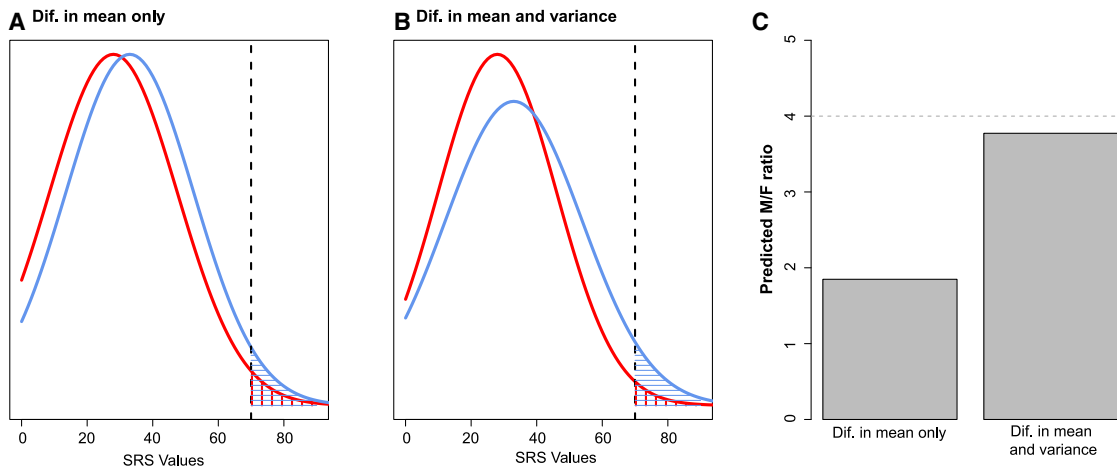
Figure 3. The difference in the means of a QAT does not account for the difference in the extremes when constrained by the "equal variance" prerequisite of the DT-LTM

Population SRS scores show a male bias in the ASD-impaired direction.

(A) Parameterizing two normal curves with these population means, but the same variance, as expected under the LTM, will result in a <2:1 ratio of males (blue shading) and females (red shading) exceeding a diagnostic threshold (dashed line) of 70.

(B and C) (B) Parameterizing with the same means, but with the observed SRS male and female standard deviations increases the ratio to nearly 4. (C) Predicted M/F ratios in individuals exceeding the thresholds in (A) and (B). A cutoff of 70 was selected here for illustration, but similar results are seen with a variety of diagnostic thresholds.

common assumption is that clinical features correlate with genetic liability. This is true for Hirschsprung's disease, where, as the severity of the phenotype increases (longer affected segment of the colon), the sex ratio decreases (Badner et al., 1990). However, for scoliosis, which exhibits a Carter effect, recurrence does not seem to vary with the severity of scoliosis (Rudnick et al., 2018). For ASD, a relationship between severity and liability would be supported by the correlation of specific clinical features with measures of high genetic liability, such as a family history of ASD, ORs of known variants, or PRSs. For this reason, we reviewed the evidence for a common co-morbid feature, ID, as well as for core features of ASD. If we find a correlation between any clinical features and genetic liability, we could then test the DT-LTM prediction that females with ASD have more severe clinical features than males.

Clinical co-morbidities

Existing data strongly indicate that those ASD cases with highly penetrant rare genetic variants (both syndromic conditions and *de novo* mutations) are more likely to have co-morbid ID. However, it is not clear whether this principle holds for specific categories of rare variants. For example, our study of RASopathies showed that the level of average intellectual impairment did not predict the penetrance of ASD (Adviento et al., 2014). To expand this analysis to other well-studied, highly penetrant mutations causing ASD, we extracted the rates of ID and ASD from the 22 SFARI high-confidence genes with the most publications. These data did not support a positive relationship between penetrance for ID and ASD across genes (Figure 4). However, these results should be interpreted cautiously: a relatively small subset of ASD genes is currently available, rates were not obtained by using a uniform process or from a single study (thus ascertainment biases across studies cannot be ruled out), and

conditional probabilities (e.g., ASD with ID) cannot be derived from these reported data.

Finally, contrary to expectations if ID correlates with ASD liability, polygenic risk for ASD is *positively* associated with IQ or educational attainment. Specifically, estimation of genetic correlation revealed that years of education was positively correlated with polygenic ASD risk, and this was not explained by oversampling from more highly educated parents (Bulik-Sullivan et al., 2015). Furthermore, polygenic risk for ASD calculated from GWAS studies demonstrated a positive correlation with general cognitive ability and IQ (Clarke et al., 2016). Overall, there is not a consistent pattern of association between genetic liability and IQ for either rare or common variation.

Core features

We also did not find consistent evidence to indicate that severity of core ASD symptoms/features are associated with genetic liability. Although individuals with "high-functioning" ASD have fewer highly penetrant pathogenic variants (Jensen et al., 2020), the definition of "high functioning" is tied to IQ/ID, rather than to core ASD features. Regardless, for rare variants, the relationship to severity of symptomology is not a clear one: for example, autism diagnostic observation schedule (ADOS) scores, which index ASD symptom severity, did not track with rare variants in a recent study (Balicza et al., 2019), and CNV carriers do not have a more severe symptom profile than those with idiopathic ASD (Chawner et al., 2021). Of two recent studies (Antaki et al., 2022; Warrier et al., 2022) examining core features of ASD, only one (Antaki et al., 2022) showed *de novo* variant associations with one measure of core features (social communication questionnaire [SCQ], although not SRS). In both studies, ASD polygenic liability showed a small, but significant, association with core features measured by SCQ (but not SRS or
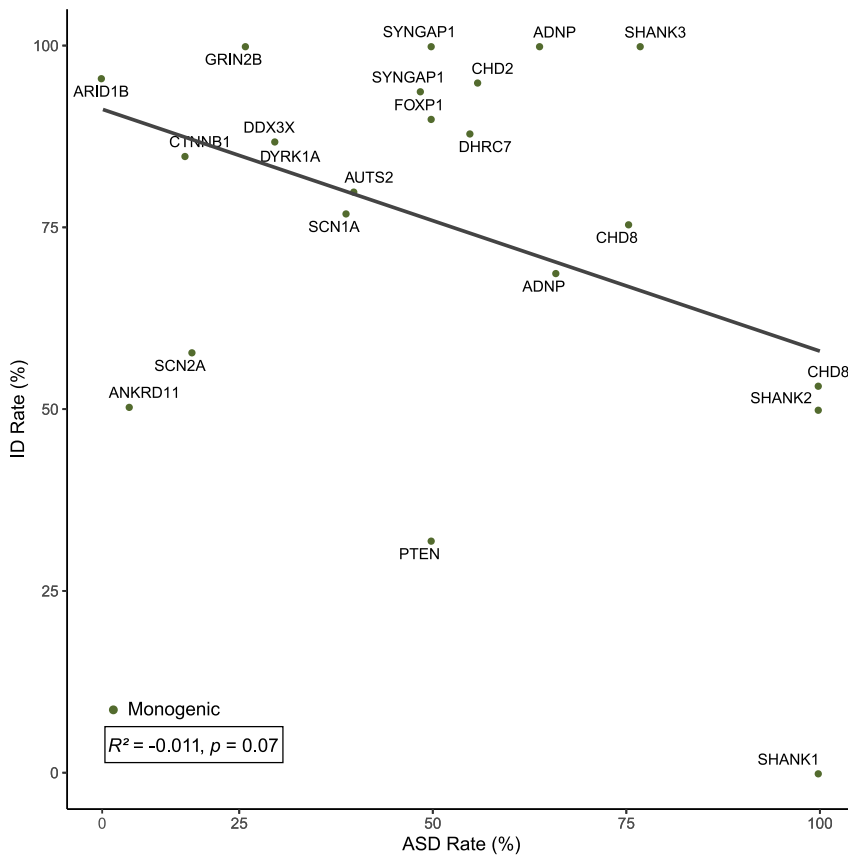
Figure 4. Rate of ID does not positively correlate with rate of ASD across monogenic causes of ASD
Literature review of most well-studied ASD-associated genes does not indicate there is a positive correlation between rate of ASD diagnosis and rate of ID diagnosis (r = −0.011, p = 0.07). This suggests a given gene's prevalence of ID (when mutated) cannot be used as a proxy for that gene's contribution to genetic liability for ASD.

repetitive behavior scale [RBS]) (Antaki et al., 2022), or SCQ and RBS (Warrier et al., 2022). Together, the overall evidence that genetic liability scales with severity is weak, as it would be expected for all sources of genetic liability (i.e., *de novo* and PRS). As there is no clear relationship between clinical features and genetic liability, any observed sex differences in clinical features would not support a DT-LTM. In addition, the sex differences observed have tended to be small and inconsistent, and new approaches to assessment are likely to be needed (Van Wijngaarden-Cremers et al., 2014).

SEX RATIO VERSUS POPULATION PREVALENCE

Over the past several decades, there has been considerable debate regarding the increased prevalence of ASD. Several factors related to ascertainment have been proposed to contribute, including increased detection of less impaired individuals, younger age at diagnosis, changes in practice around dual diagnoses of ID/ASD, service availability for ASD versus other diagnoses, and changes in ASD diagnostic criteria. If prevalence is linked to genetic liability, we can make predictions about sex ratios under the DT-LTM. For example, there are known relationships between genetic risk and "high-functioning" ASD, as well as "low-functioning" ASD co-morbid with ID. The former is expected to be driven by inherited or polygenic risk and, therefore, associated with individuals closer to the threshold (and thus more male-biased under the DT-LTM), whereas the latter is predicted to be associated with highly penetrant genetic variants (less male-biased). Most studies estimate that the increased dual diagnosis of ID and ASD accounts for a small proportion of the increased prevalence, with the majority accounted for by broadening criteria to include "higher-functioning" individuals (King and Bearman, 2009; Lundström et al., 2015; Nassar et al., 2009). When we examined a comprehensive CDC review of prevalence estimates (Centers for Disease Control, 2020), we observed a decrease in ID co-morbidity with ASD over time (correlation between year and percent of ASD with IQ < 70, r = −0.477, p < 0.00162). Under the DT-LTM, we expect M:F sex ratios to *decrease* as prevalence increases if we are moving the threshold to include a greater proportion of lower-liability individuals (Figures 5A–5D). However, although the prevalence estimate doubled between 2008 and 2016 in the United States, the sex ratio remained stable.

In order to examine changes in prevalence, it is possible to study the international classification of disease codes (versions 9 and 10) (ICD-9/ICD-10) diagnosis at age 15 as a function of birth year in Sweden. Considering birth years from 1987 to 2002, the sex ratio remains stable as well, with a differing sex ratio ~2. Likewise, a comprehensive examination of all prevalence studies reveals that although prevalence increases (Figure 5E), the sex ratio does not change (Figure 5F).

We acknowledge that extant data have limitations when correlating specific changes in ascertainment to likely sources of sex bias in ascertainment. For this reason, we rely on the assumption that changes in diagnosis in the population would intersect with sources of sex bias in diagnosis (e.g., diagnosis of lower-functioning individuals would be less influenced by clinician sex bias than that of "higher-functioning" individuals). However, given this assumption, the data do not support a DT-LTM.

PREREQUISITES UNDERLYING THE LTM

The underlying assumptions of the DT-LTM are that (1) ASD has multifactorial risk factors in both sexes, (2) the shape and variance of the liability distribution is identical across sexes, and (3) risk factors are identical for males and females.
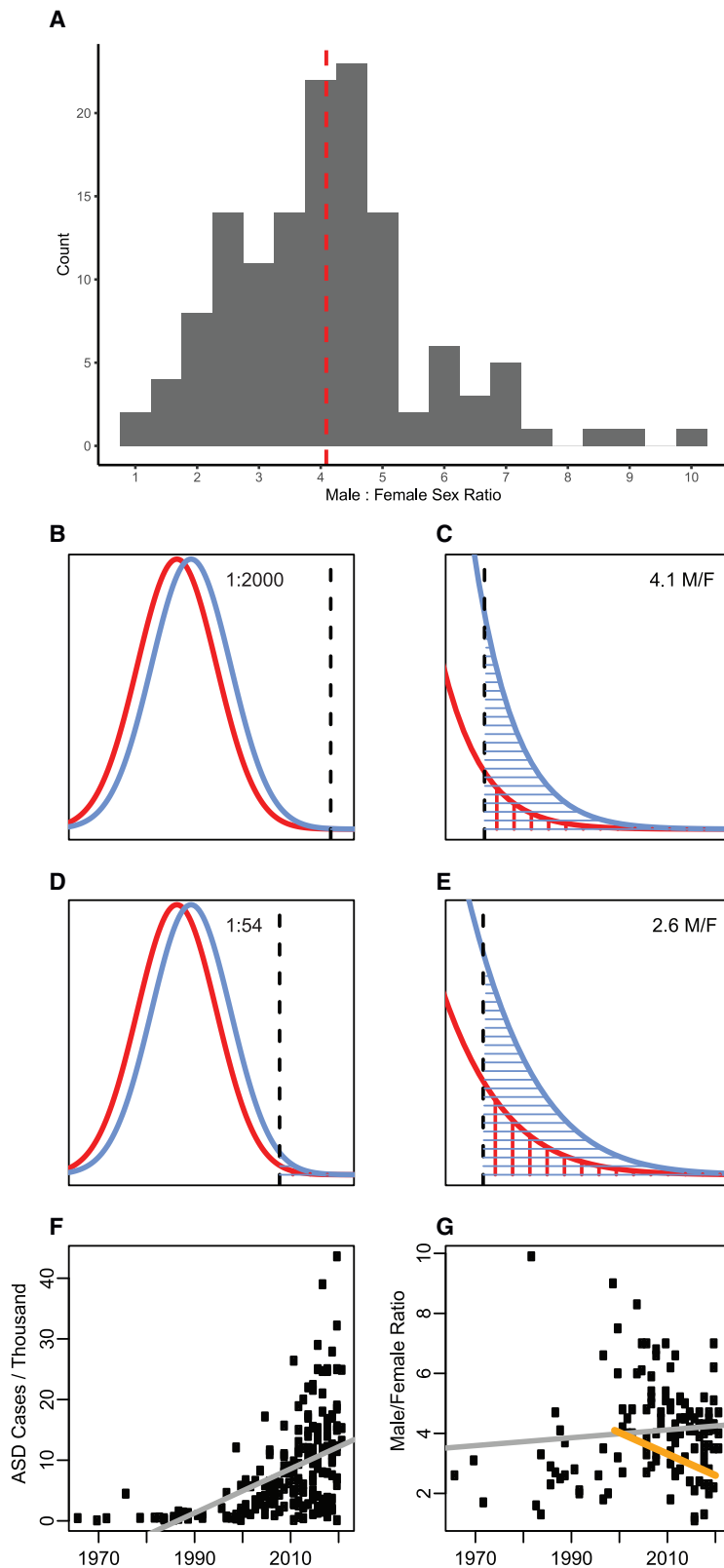
Figure 5. ASD prevalence changed over time although M:F ratio did not

A histogram (A) of M:F ratios from a comprehensive review of 200 ASD prevalence studies from 1966 onward (Centers for Disease Control, 2020) indicates a mean M:F ratio of 4.1. The DT-LTM predicts that if the agreed threshold to diagnose ASD is shifting over time to be more lenient (e.g., to go from ~1:2,000 in 1999, illustrated in (B), to 1:54 in 2020, as illustrated in (D)), it should have also altered the M:F ratio exceeding that new diagnostic thresholds from 4.1 to 2.6:1. ((C) and (E) are zoomed views of (B) and (D), respectively). Examining the dates of each of the 200 studies shows that although (F) prevalence rates (in cases per 1,000) of ASD have gone up with time (Pearson's r > 0.5), (G) sex ratios (M:F) have remained flat or slightly increased (Pearson's r > 0.1). The DT-LTM predicts they should have gone down (gray line: observed linear fit to data, orange line: expected line from 4.1 to 2.6).

However, close examination suggests that these criteria might be violated.

Multifactorial risk

There is clear evidence for multifactorial risk in both sexes. As reviewed above, PRS consistently shows case-control differences in both males and females, and rare *de novo* mutations contribute to ASD in both sexes. Moreover, inheritance patterns are complex in both sexes, with simple dominant, recessive, and X-linked patterns of inheritance violated for males and for females. Taken together, the multifactorial liability prerequisite is well supported.

Equal variance

When examining QAT data, in addition to the difference between male and female population means, variance is greater in males in the general population (e.g., SRS; Constantino and Gruber, 2012). Provided that QATs reflect genetic liability, the equal variance prerequisite is then violated. Adding this greater variance to our modeling better approximates the 4:1 M:F ratio (Figures 2B and 2C). Increased male variance can explain sex differences in diagnosis without creating large differences in heritability, as has been previously described (Traglia et al., 2017). Therefore, variance differences could also be consistent with lack of heritability sex differences observed in most ASD studies.

Same risk factors and perfect genetic correlation

The DT-LTM requires that risk factors are identical between sexes, but this prerequisite is violated by risk factors on the X chromosome, although the magnitude is debatable. First, two well-known Mendelian conditions associated with ASD risk are X-linked, *FMR1* in Fragile X syndrome (male-biased; Verkerk et al., 1991) and *MECP2* in Rett syndrome (female-biased; Amir et al., 1999). Furthermore, one study in ∼1,500 probands and ∼5,000 controls suggested that mutations in the genes on the X chromosome that have no Y-equivalent in males contribute to ∼2% of genetic liability for ASD (Lim et al., 2013). Likewise, in common variant data (Box 1), we observed nominal enrichment of association signal on the X chromosome in males and significant sex heterogeneity (Box 1) on the X chromosome in contrast to autosomes (Mitra et al., 2016). We have also shown female (but not male) enrichment for rare variants in X chromosome genes (Turner et al., 2019).

As the X chromosome is less well studied than the autosomes, there remain open questions about whether the X chromosome could explain sex differences in prevalence. If there were a large class of rare X variation that was lethal in males (i.e., more genes like *MECP2* and *DDX3X*) and led to ASD in females (suggested by Turner et al. data), this would not result in increased male prevalence. Other work also identified genes with excess mutations on the X chromosome (Piton et al., 2011; Niranjan et al., 2015; Martin et al., 2021); however, the burden of protein-coding variation on the X chromosome was not sufficient to explain the sex bias (Martin et al., 2021). Likewise, substantial X chromosome (or other sex-limited) risk leading to increased male prevalence should also lead male probands to have more male-affected siblings than female probands, which has not been observed (Messinger et al., 2015; Ozonoff et al., 2011). Overall, if one were to exclude the X chromosome, it remains an open question as to whether the remaining autosomal risk factors would be identical between males and females. As sample sizes increase, this should be addressable. It should be noted that a substantially lower bar is required for X-linked liability to violate the DT-LTM. The X chromosome accounts for ∼5% of the genome; hence, even a slightly disproportionate role in ASD liability would be sufficient to violate this DT-LTM prerequisite.

SUMMARY

We have systematically delineated specific prerequisites and predictions of the DT-LTM, often referred to as a FPE in ASD. We assessed evidence in: (1) heritability, (2) recurrence, (3) correlation between sex ratio and penetrance, (4) population traits related to ASD, (5) clinical features reflecting liability, and (6) changes in sex ratio with prevalence and (7) examined the prerequisites of the DT-LTM (Figure 6). We failed to find consistent support for DT-LTM when considering predictions for rare and common variant liability (*de novo* or inherited). Our conclusions from the published literature similarly argue against other simplistic models to explain sex bias (or "female protection"), such as sex chromosome contributions (inconsistent with recurrence patterns) or ascertainment bias (inconsistent with the stable ratio over time). Finally, population-level sex differences in ASD-related QATs cannot account for the 4:1 prevalence difference (Figure 4). Although there are some data indicating the true sex ratio may be somewhat lower (e.g., 3.2:1) due to likely diagnostic bias (Loomes et al., 2017), the only category where we specified this ratio (QATs) also demonstrated differing variance by sex, which could alone violate the DT-LTM. However, a reduced "true" sex ratio might also reduce the power to detect differences in heritability or recurrence consistent with the DT-LTM, if missed cases are independent of their liability.

There are inherent limitations to our review. Our hypothesis testing was limited to existing data, primarily from studies not specifically designed to test the DT-LTM. For example, co-occurring ID has not been consistently reported and could refine many analyses. Future investigation of categories of genetic variation with intermediate ASD liability, such as missense or regulatory mutations, may also help bridge observations about genetic variation. In some cases, we have combined data from studies with different ascertainment, designs, strengths, and limitations to assess domains with limited investigation, and we recognize that some of these data were not ideal for testing our hypothesis. Additionally, our modeling was performed with assumptions and parameters that may not perfectly fit real-world data. A final limitation is that our analyses (and largely the field) examine sex differences based on binary chromosomal sex only, as self-reported gender has historically not been collected for most large studies (and may be difficult to reliably ascertain in non-verbal individuals and young children). Thus, individuals not fitting binary sex categories or with reported gender not matching chromosomal sex may have been excluded from genetic datasets but could add important information to the future study of sex differences. These limitations notwithstanding, the strength of this investigation is that we utilized a variety of independent data sources, study designs, and domains to be as comprehensive
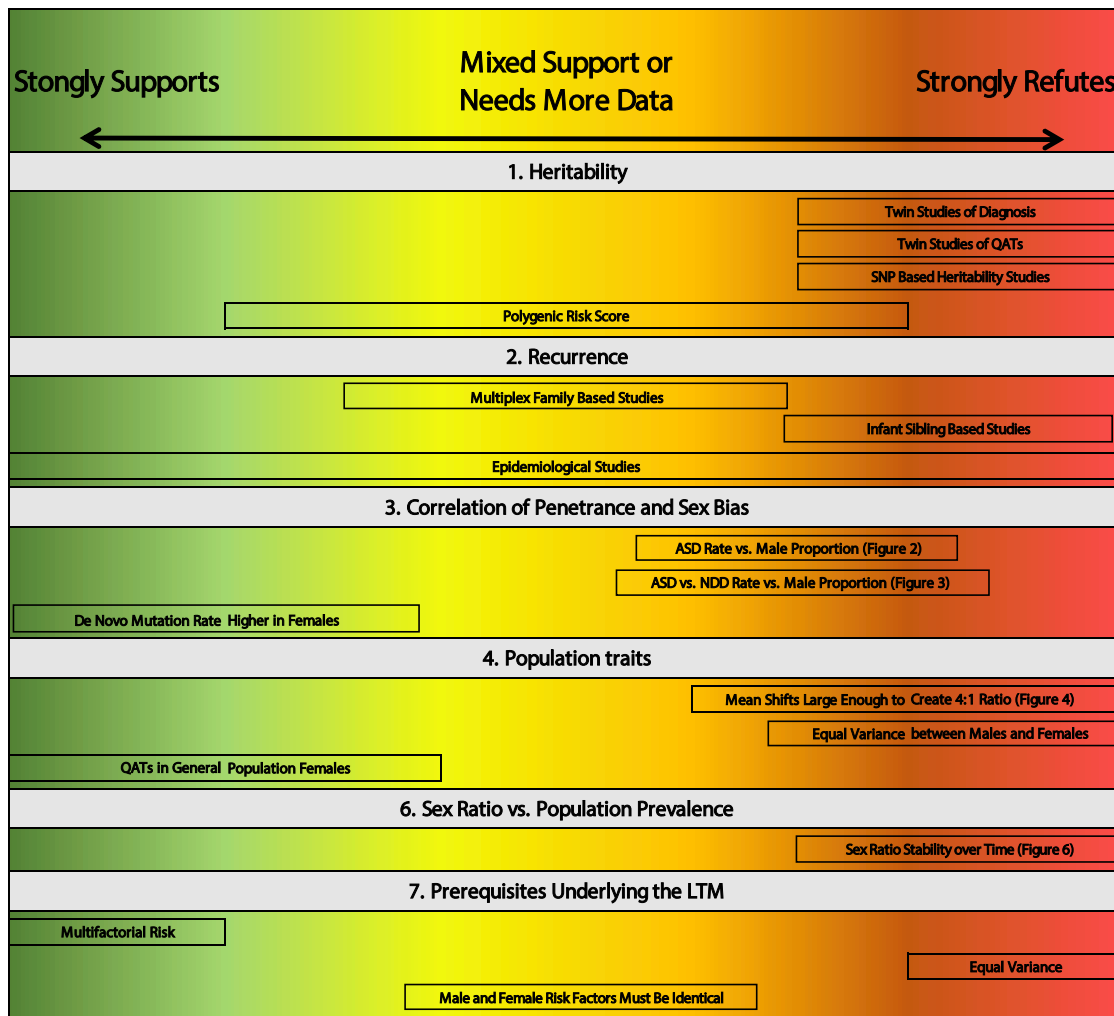
Figure 6. Summary of evidence regarding the LTM in ASD suggests the model should now be rejected
A theory can be disproven by a single counterexample. Although no single study is perfect, across our 7 domains of review and inquiry, we identified multiple lines of evidence that did not support the FPE/DT-LTM (red) or had evidence from several large studies that disagreed in their conclusions (e.g., epidemiology). Although some observations remain consistent with some aspects of the DT-LTM (green), the number of counterexamples here indicates new models are needed that can better fit the entirety of the observations.

as possible. Taken together, we believe that alternatives to the DT-LTM are needed to better fit the entirety of the observations.

We also propose that "female protection" is not sufficiently well defined to be a useful concept for ASD, unless restricted to referring to the observation of fewer females with diagnoses. Distinct mechanisms of female protection from ASD unrelated to a liability threshold may exist. These etiologies, such as a second X chromosome, male-specific autosomal risk loci, different early hormonal exposure, or cultural socialization could individually generate testable predictions to be examined.

TOWARD ALTERNATE MODELS TO EXPLAIN THE SEX DIFFERENCE IN ASD

With better power in the future, some of the analyses described herein might determine whether some aspects of differing liability thresholds by sex exist for ASD. However, we believe that the preponderance of evidence argues for alternatives to the DT-LTM. A number of robust observations may provide clues for proposing a different model better fitting all available evidence. (1) Heritability appears to be equivalent in males and females, and male bias is retained similarly in affected siblings of male and female probands (Messinger et al., 2015; Ozonoff et al., 2011). (2) The sex bias is reduced when considering only rare, highly penetrant (frequently *de novo*) individual variants. (3) QATs in the general population are biased toward males in mean values but may also differ in variance by sex. (4) Sex ratios have remained exceptionally stable, despite huge increases in prevalence over time. These observations can help refine alternative models most likely to fit existing data. Below, we consider alternatives to both aspects of the LTM, specifically considering possibilities for threshold-less models and models with sex differences in liability distributions. We can then speculate about what neurobiological mechanisms could cause different liability

distributions by sex, and which neurobiological measures could be used to explore alternative genetic models explaining sex differences. Finally, we propose potential patterns in genetic data like those examined above that might be consistent with alternative models.

Non-threshold model: Canalization

To our knowledge, few alternatives to a threshold concept have been investigated. In developmental biology, one alternative model is canalization. Canalization is the suppression of genetic and environmental variation allowing most of the population to maintain an evolutionarily beneficial phenotype through inherently stochastic developmental processes (Waddington, 1942). Several observations are in keeping with a model whereby ASD occurs following loss of developmental buffering under canalization. For example, above the clinical threshold of ASD, QATs appear more strongly influenced by stochastic, random environmental variation (Castelbaum et al., 2020). Prospective infant sibling studies have consistently observed pre-diagnostic developmental deviations across multiple domains prior to ASD, including those related to core ASD features (e.g., early communication skills) as well as domains less specifically associated with ASD (e.g., motor function) (Estes et al., 2015; Miller et al., 2017). Robustness of typical developmental outcomes might explain why it is difficult to find mutations with >50% penetrance for ASD, either in syndromic conditions or highly penetrant mutations (Figure 2A). We also did not find any evidence for correlation between core feature severity and genetic liability, consistent with suppression of the effects of genetic variation and influence of stochastic developmental processes. However, the full range of phenotypic variation observed in QATs and their high heritability and correlation with ASD does not seem to fit a canalization model. Although the large role of stochastic variation in the canalization framework also does not fit with high ASD heritability estimates for ASD, these estimates have been obtained *after* ASD occurs. To test the potential impact of canalization, heritability of domains contributing to the development of ASD, including by sex, warrants characterization prior to the age of ASD diagnosis.

Differing liability distribution models

What would models for sex differences in the liability distribution look like? We envision several scenarios extrapolated from single locus models. First, we might see differing variance in the liability distribution by sex, with a similar or slightly different mean (e.g., Figure 3B). Second, we could see skewing from normality for one or both sexes (which could also impact variance). Third, we could see differing underlying contributions (e.g., different genes contributing for each sex or different effect sizes by sex for each gene), which could occur with or without differences in variance.

One key observation to support sex differences in liability distributions is that the difference in mean population QATs between males and females is insufficient to result in a 4:1 difference at the extremes (Figure 3), *unless males have higher variance*. The idea of "greater male variation" (variance) was initially thought to drive sex differences in 1972 (Ounsted and Taylor, 1972) and was proposed more specifically for ASD since

the 1980s (Ferri et al., 2018; Lai et al., 2015; Tsai and Beisler, 1983). In prior work, we have shown that variance differences do not need to create differing heritability to generate large prevalence differences (Traglia et al., 2017), consistent with similar ASD heritability by sex. Increased variance in liability could also be consistent with different sex ratios in different categories of genetic risk (e.g., *de novo*, highly penetrant versus heritable, polygenic variation). It would be interesting to test whether such increased liability variance in males could also be consistent with very stable sex ratios, despite increasing population prevalence (Figure 5), if the DT-LTM is not applied.

Several plausible neurogenetic mechanisms might lead to greater variance in genetic liability in males than females. First, the sex chromosomes could be driving greater male variance directly or indirectly. Because of their hemizygous status, X and Y loci may show greater variance in males than in females in gene expression in the presence of any genetic variation influencing expression on the sex chromosomes. This could be important for risk loci on the sex chromosomes and also for autosomal risk loci downstream of sex-linked chromatin-modifying genes (e.g., sex-linked lysine demethylases, *KDM5D*, *KDM5C*, and *KDM5A*). Second, epigenetic states influenced by sex may lead to greater male variation in expression. Third, sex differences in placental biology could also lead to differences in the robustness or variance of early brain development. Fourth, male hormonal surges during the prenatal or neonatal stages may establish inter-individual variation. Some of these mechanisms are being explored in mammalian model systems, but differences in X chromosome activation and brain development across species might make more research in primates and using human tissues (placenta and fetal brain) necessary to understand the relevance of these phenomena for human NDDs.

Model predictions

Predictions of a model with different liability distributions by sex could be assessed using behavioral measures, as one example. Any measure correlated with ASD liability would also be expected to show greater variance under this model and can thus be used to explore the likelihood of this alternative model. Currently, quantitative behavioral scales such as SRS and broad autism phenotype questionnaire (BAP-Q) are robustly correlated with genetic liability, i.e., close relatives of ASD probands who are themselves unaffected show values intermediate between ASD and population controls, and genetic correlation exists between these measures and ASD (Constantino and Todd, 2005; Constantino et al., 2010; Maxwell et al., 2013). By toddlerhood, language scores have also shown intermediate values for unaffected siblings of ASD probands in comparison with ASD-affected siblings and controls without a family history of ASD (Marrus et al., 2018). Recent behavioral data also appear promising with respect to behavioral flexibility and response inhibition (Cheng et al., 2021).

Several neurobiological measures may also be correlated with ASD liability and useful for testing alternative models to the DT-LTM. In idiopathic ASD, both increased rates of macrocephaly and increased head circumference (HC) have been robustly found, with high heritability for HC in the general population and ASD probands (Lai et al., 2014; Kanner, 1968; Chaste and

Leboyer, 2012). Unaffected siblings have an intermediate distribution of HC between controls and ASD (Constantino et al., 2010). HC is correlated with core ASD features, like social functioning and delayed language, where variance in ASD is increased compared with unrelated controls (Green et al., 2019; Stevenson et al., 1997; Sacco et al., 2007; Chawarska et al., 2011; Lainhart et al., 2006; Rommelse et al., 2011; Nordahl et al., 2011). Blood serotonin levels are another consistent ASD biomarker that correlates with genetic liability to ASD. Hyperserotonemia (>95th centile) is relatively common in ASD (25%) and correlated in unaffected first-degree relatives of ASD probands, and serotonin levels are highly heritable in the general population (Muller et al., 2016). Sex differences in serotonin have been widely reported, with female levels typically increased, but no differences in variance have been reported. Our genetic study of serotonin levels in a founder population identified differences in heritability estimates by sex, including greater contribution of dominance/interaction in males, and differences in genetic loci associated with serotonin levels, both potentially leading to differences in liability distributions by sex (Weiss et al., 2005). Further studies, including X chromosome modeling, estimates significant X-linked heritability (with dosage-compensation) for serotonin in this population (Pan et al., 2007). There have also been some functional MRI measures elevated in female relatives of ASD probands (e.g., altered response to biological motion), but replication data are less clear (Eggebrecht et al., 2020; Kaiser et al., 2010). Other potential biomarkers to investigate for their correlation with ASD liability might be increased variance in gene expression in specific gene regulatory networks or developmental time points or involving particular brain structural elements, immune molecules, or functional connectivity measures.

Genetic mechanisms

What genetic mechanisms might produce such increased variance? Falconer recognized the importance of differences in variance in liability explicitly, indicating that one manner whereby such increased variance could occur is "that some genes affect the liability in one sex but not in the other" (Falconer, 1965). This phenomenon would be consistent with an epistatic or interaction model, or "genetic heterogeneity"—that the genetic factors driving ASD may be different in males and females or have differing effects (Szatmari et al., 2012). This sex heterogeneity can produce differing genetic variance without differing thresholds (or means), which could lead to the observed 4:1 ratio in the absence of generalized female protection. If sex differences in ASD were driven by risk loci primarily affecting males (or protective loci primarily affecting females), including those on the X/Y chromosome, we would expect some specific patterns in families. If some male-specific risk loci were highly penetrant, we should observe unaffected mothers transmitting risk variants to affected sons. This has been observed in some limited examples (Antaki et al., 2022; Krumm et al., 2015; Yu et al., 2013) but is not yet supported as a widespread pattern. Similarly, we might see some *de novo* gene mutations accumulating disproportionately in males (>4:1). In general, this does not occur but may exist at specific loci (e.g., *CHD8*). Alternatively, if sex-specific risk were mostly restricted to the polygenic component of ASD, it would be consistent with the observed reduced sex ratios for

highly penetrant *de novo* mutations and would explain why we have not yet widely seen the inheritance pattern above. However, such transmission of polygenic risk should be observable with increased GWAS power to detect sex differences and generate sex-specific PRS. It should also manifest as higher heritability for male-only and female-only estimates compared with combined-sex estimates, which is typically performed only for twin studies (but is limited in power). In multiplex families, sex-specific loci would manifest as increased ratios of same-sex affected pairs (i.e., families with female probands would have more even sex ratios and families with male probands would have increased sex ratios). To date, there is scant support for this at current sample sizes; however, further studies and modeling may uncover support.

Molecular experimental approaches

Support for specific genetic models could suggest further molecular experimental exploration of underlying mechanisms. Here, we will use a sex-by-genetics epistasis model as an illustrative example. If sex heterogeneity is ultimately observed in ASD genetic data, plausible biological mechanisms (e.g., variants altering sex hormone binding sites, Figure S2) could be measured in a number of ways. First, functional genomics assays (e.g., ATAC-seq, high-throughput reporter assays) could be applied to postmortem brain samples of typically developing individuals during critical developmental time points and in ASD-related structures for sex differences in gene expression. In at least one prior study, it was shown that genes expressed at higher levels in males are over-represented among those upregulated in post-mortem brain tissue of individuals with ASD (Werling, 2016). Second, molecular genetic approaches can be used to explore epistatic effects of sex on polygenic risk by determining whether regulatory elements that are influenced by common variants associated with ASD tend to show differential expression in males versus females. A prediction might be that in general, such sex-interacting variants occur more frequently in ASD-associated loci than across the genome. Regarding rare variants, those that are shown to be preferentially associated with ASD in one sex could provide key insights into general mechanisms of sex epistasis; to the best of our knowledge, the only autosomal gene associated with ASD that exhibits a robust sex ratio is NF1 (Morris et al., 2016), for which animal and circuit studies are in progress. Additional well-powered studies to identify and functionally assess other genes could be conducted.

Mixed models

Finally, it is also worth noting that some kind of mixed model could exist, such that some cases fit a DT-LTM, whereas others adhere to a different model. This is difficult to either support or refute without an *a priori* hypothesis about a defined subset of ASD that will follow the DT-LTM and an alternative model that the remainder might fit. The strongest supporting data for a DT-LTM are the reduced sex ratio in carriers of highly penetrant variants, increased rate of ID in females, and the shifted mean of QATs such as the SRS; however, in each case, much of the available evidence fails to fit the predicted expectations, such as the lack of expected correlation between penetrance of ASD and sex ratio

or ID and increased SRS variance in males. Thus, mixed models may be hard to prove or disprove without further specification.

As male sex remains the known risk factor explaining the greatest amount of trait variance, it is critical to understand the model underlying sex bias in ASD. Improved understanding of the interactions between sex and genetic liability could lead to identification of specific categories of genetic variation sensitive to sex (e.g., based on molecular function, cellular expression, or genomic classification), which could, in turn, result in both new neurobiological insights into the pathophysiology of ASD, as well as improved sex-personalized diagnostics, treatment approaches, and genetic counseling.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.neuron.2022.06.020.

DECLARATION OF INTERESTS

J.N.C. receives royalties from Western Psychological Services for the commercial distribution of the social responsiveness scale, a quantitative measure of autistic traits implemented in some of the studies cited in this article.

SUPPORTING CITATIONS

The following reference appears in the supplemental information: McCarthy and Arnold, 2011.

REFERENCES

Abrahams, B.S., Arking, D.E., Campbell, D.B., Mefford, H.C., Morrow, E.M., Weiss, L.A., Menashe, I., Wadkins, T., Banerjee-Basu, S., and Packer, A. (2013). SFARI Gene 2.0: a community-driven KnowledgeBase for the autism spectrum disorders (ASDs). Mol. Autism 4, 36. https://doi.org/10.1186/2040-2392-4-36.

Abrahams, B.S., and Geschwind, D.H. (2008). Advances in autism genetics: on the threshold of a new neurobiology. Nat. Rev. Genet. 9, 341–355. https://doi.org/10.1038/nrg2346.

Adviento, B., Corbin, I.L., Widjaja, F., Desachy, G., Enrique, N., Rosser, T., Risi, S., Marco, E.J., Hendren, R.L., Bearden, C.E., et al. (2014). Autism traits in the RASopathies. J. Med. Genet. 51, 10–20. https://doi.org/10.1136/jmedgenet-2013-101951.

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature genetics 23, 185–188. https://doi.org/10.1038/13810.

Anckarsäter, H., Lundström, S., Kollberg, L., Kerekes, N., Palm, C., Carlström, E., Långström, N., Magnusson, P.K.E., Halldner, L., Bölte, S., et al. (2011). The child and adolescent twin study in Sweden (CATSS). Twin Res. Hum. Genet. 14, 495–508. https://doi.org/10.1375/twin.14.6.495.

Antaki, D., Guevara, J., Maihofer, A.X., Klein, M., Gujral, M., Grove, J., Carey, C.E., Hong, O., Arranz, M.J., Hervas, A., et al. (2022). A phenotypic spectrum of autism is attributable to the combined effects of rare variants, polygenic risk and sex. Nat. Genet. https://doi.org/10.1038/s41588-022-01064-5.

Badner, J.A., Sieber, W.K., Garver, K.L., and Chakravarti, A. (1990). A genetic study of Hirschsprung disease. Am. J. Hum. Genet. 46, 568–580.

Bai, D., Marrus, N., Yip, B.H.K., Reichenberg, A., Constantino, J.N., and Sandin, S. (2020). Inherited risk for autism Through maternal and paternal lineage. Biol. Psychiatry 88, 480–487. https://doi.org/10.1016/j.biopsych.2020.03.013.

Balicza, P., Varga, N.Á., Bolgár, B., Pentelényi, K., Bencsik, R., Gál, A., Gézsi, A., Prekop, C., Molnár, V., and Molnár, M.J. (2019). Comprehensive analysis of rare variants of 101 autism-linked genes in a Hungarian cohort of autism spectrum disorder patients. Front. Genet. 10, 434. https://doi.org/10.3389/fgene.2019.00434.

Baron-Cohen, S., Hoekstra, R.A., Knickmeyer, R., and Wheelwright, S. (2006). The Autism-Spectrum Quotient (AQ)–adolescent version. J. Autism Dev. Disord. 36, 343–350. https://doi.org/10.1007/s10803-006-0073-6.

Bralten, J., van Hulzen, K.J., Martens, M.B., Galesloot, T.E., Arias Vasquez, A., Kiemeney, L.A., Buitelaar, J.K., Muntjewerff, J.W., Franke, B., and Poelmans, G. (2018). Autism spectrum disorders and autistic traits share genetics and biology. Mol. Psychiatry 23, 1205–1212. https://doi.org/10.1038/mp.2017.98.

Bulik-Sullivan, B., Finucane, H.K., Anttila, V., Gusev, A., Day, F.R., Loh, P.R., ReproGen Consortium, Psychiatric Genomics Consortium, Genetic Consortium for Anorexia Nervosa of the Wellcome Trust Case Control Consortium 3, Duncan, L., et al. (2015). An atlas of genetic correlations across human diseases and traits. Nat. Genet. 47, 1236–1241. https://doi.org/10.1038/ng.3406.

Carter, C.O. (1961). The inheritance of congenital pyloric stenosis. Br. Med. Bull. 17, 251–254. https://doi.org/10.1093/oxfordjournals.bmb.a069918.

Castelbaum, L., Sylvester, C.M., Zhang, Y., Yu, Q., and Constantino, J.N. (2020). On the Nature of Monozygotic Twin Concordance and Discordance for Autistic Trait Severity: A Quantitative Analysis. Behav Genet. 50, 263–272. https://doi.org/10.1007/s10519-019-09987-2.

Chawarska, K., Campbell, D., Chen, L., Shic, F., Klin, A., and Chang, J. (2011). Early generalized overgrowth in boys with autism. Arch Gen Psychiatry 68, 1021–1031. https://doi.org/10.1001/archgenpsychiatry.2011.106.

Chawner, S.J.R.A., Doherty, J.L., Anney, R.J.L., Antshel, K.M., Bearden, C.E., Bernier, R., Chung, W.K., Clements, C.C., Curran, S.R., Cuturilo, G., et al. (2021). A genetics-first approach to dissecting the heterogeneity of autism: phenotypic comparison of autism risk copy number variants. Am. J. Psychiatry 178, 77–86. https://doi.org/10.1176/appi.ajp.2020.20010015.

Chaste, P., and Leboyer, M. (2012). Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 14, 281–292. https://doi.org/10.31887/DCNS.2012.14.3/pchaste.

Cheng, X., Li, Y., Cui, X., Cheng, H., Li, C., Fu, L., Jiang, J., Hu, Z., and Ke, X. (2021). Atypical neural responses of cognitive flexibility in parents of children With autism spectrum disorder. Front. Neurosci. 15, 747273.

Cheon, K.A., Park, J.I., Koh, Y.J., Song, J., Hong, H.J., Kim, Y.K., Lim, E.C., Kwon, H., Ha, M., Lim, M.H., et al. (2016). The social responsiveness scale in relation to DSM IV and DSM5 ASD in Korean children. Autism Res. 9, 970–980. https://doi.org/10.1002/aur.1671.

Clarke, T.K., Lupton, M.K., Fernandez-Pujals, A.M., Starr, J., Davies, G., Cox, S., Pattie, A., Liewald, D.C., Hall, L.S., MacIntyre, D.J., et al. (2016). Common polygenic risk for autism spectrum disorder (ASD) is associated with cognitive ability in the general population. Mol. Psychiatry 21, 419–425. https://doi.org/10.1038/mp.2015.12.

Centers for Disease Control. Autism prevalence studies data table. https://www.cdc.gov/ncbddd/autism/data.html.

Constantino, J.N., and Gruber, C.P. (2012). Social responsiveness scale (SRS-2).

Constantino, J.N., Majmudar, P., Bottini, A., Arvin, M., Virkud, Y., Simons, P., and Spitznagel, E. (2010). Infant head growth in male siblings of children with and without autism spectrum disorders. J Neurodev Disord 2, 39–46. https://doi.org/10.1007/s11689-009-9036-5.

Constantino, J.N., and Todd, R.D. (2003). Autistic traits in the general population: A twin study. Arch. Gen. Psychiatry 60, 524–530. https://doi.org/10.1001/archpsyc.60.5.524.

Constantino, J.N., and Todd, R.D. (2005). Intergenerational transmission of subthreshold autistic traits in the general population. Biol. Psychiatry 57, 655–660. https://doi.org/10.1016/j.biopsych.2004.12.014.

Constantino, J.N., Zhang, Y., Frazier, T., Abbacchi, A.M., and Law, P. (2010). Sibling recurrence and the genetic epidemiology of autism. Am. J. Psychiatry 167, 1349–1356. https://doi.org/10.1176/appi.ajp.2010.09101470.

Coon, H., Villalobos, M.E., Robison, R.J., Camp, N.J., Cannon, D.S., Allen-Brady, K., Miller, J.S., and McMahon, W.M. (2010). Genome-wide linkage using the Social Responsiveness Scale in Utah autism pedigrees. Mol. Autism 1, 8. https://doi.org/10.1186/2040-2392-1-8.

De Rubeis, S., He, X., Goldberg, A.P., Poultney, C.S., Samocha, K., Cicek, A.E., Kou, Y., Liu, L., Fromer, M., Walker, S., et al. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515, 209–215. https://doi.org/10.1038/nature13772.

de Vries, P.J., Hunt, A., and Bolton, P.F. (2007). The psychopathologies of children and adolescents with tuberous sclerosis complex (TSC): a postal survey of UK families. Eur. Child Adolesc. Psychiatry 16, 16–24. https://doi.org/10.1007/s00787-006-0570-3.

de Zeeuw, E.L., van Beijsterveldt, C.E.M., Hoekstra, R.A., Bartels, M., and Boomsma, D.I. (2017). The etiology of autistic traits in preschoolers: a population-based twin study. J. Child Psychol. Psychiatry 58, 893–901. https://doi.org/10.1111/jcpp.12741.

Doan, R.N., Lim, E.T., De Rubeis, S., Betancur, C., Cutler, D.J., Chiocchetti, A.G., Overman, L.M., Soucy, A., Goetze, S., et al.; Autism Sequencing Consortium (2019). Recessive gene disruptions in autism spectrum disorder. Nat. Genet. 51, 1092–1098. https://doi.org/10.1038/s41588-019-0433-8.

Task Force, D.S.M.-5 (2013). Diagnostic And Statistical Manual Of Mental Disorders, Fifth Edition (American Psychiatric Association).

Duvall, J.A., Lu, A., Cantor, R.M., Todd, R.D., Constantino, J.N., and Geschwind, D.H. (2007). A quantitative trait locus analysis of social responsiveness in multiplex autism families. Am. J. Psychiatry 164, 656–662. https://doi.org/10.1176/ajp.2007.164.4.656.

Eggebrecht, A.T., Dworetsky, A., Hawks, Z., Coalson, R., Adeyemo, B., Davis, S., Gray, D., McMichael, A., Petersen, S.E., Constantino, J.N., et al. (2020). Brain function distinguishes female carriers and non-carriers of familial risk for autism. Mol. Autism 11, 82. https://doi.org/10.1186/s13229-020-00381-y.

Emison, E.S., Garcia-Barcelo, M., Grice, E.A., Lantieri, F., Amiel, J., Burzynski, G., Fernandez, R.M., Hao, L., Kashuk, C., West, K., et al. (2010). Differential contributions of rare and common, coding and noncoding Ret mutations to multifactorial Hirschsprung disease liability. Am. J. Hum. Genet. 87, 60–74. https://doi.org/10.1016/j.ajhg.2010.06.007.

Estes, A., Zwaigenbaum, L., Gu, H., St John, T., Paterson, S., Elison, J.T., Hazlett, H., Botteron, K., Dager, S.R., Schultz, R.T., et al. (2015). Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. J. Neurodev. Disord. 7, 24. https://doi.org/10.1186/s11689-015-9117-6.

Falconer, D.S. (1965). The inheritance of liability to certain diseases, estimated from the incidence among relatives. Ann. Hum. Genet. 29, 51–76. https://doi.org/10.1111/j.1469-1809.1965.tb00500.x.

Ferri, S.L., Abel, T., and Brodkin, E.S. (2018). Sex differences in autism spectrum disorder: a review. Curr. Psychiatry Rep. 20, 9. https://doi.org/10.1007/s11920-018-0874-2.

Girirajan, S., Rosenfeld, J.A., Coe, B.P., Parikh, S., Friedman, N., Goldstein, A., Filipink, R.A., McConnell, J.S., Angle, B., Meschino, W.S., et al. (2012). Phenotypic heterogeneity of genomic disorders and rare copy-number variants. N. Engl. J. Med. 367, 1321–1331. https://doi.org/10.1056/NEJMoa1200395.

Gockley, J., Willsey, A.J., Dong, S., Dougherty, J.D., Constantino, J.N., and Sanders, S.J. (2015). The female protective effect in autism spectrum disorder is not mediated by a single genetic locus. Mol. Autism 6, 25. https://doi.org/10.1186/s13229-015-0014-3.

Goin-Kochel, R.P., Abbacchi, A., and Constantino, J.N.; Autism Genetic Resource Exchange Consortium (2007). Lack of evidence for increased genetic loading for autism among families of affected females: a replication from family history data in two large samples. Autism 11, 279–286. https://doi.org/10.1177/1362361307076857.

Green, R.R., Bigler, E.D., Froehlich, A., Prigge, M.B.D., Zielinski, B.A., Travers, B.G., Anderson, J.S., Alexander, A., Lange, N., and Lainhart, J.E. (2019). Beery VMI and Brain Volumetric Relations in Autism Spectrum Disorder. J Pediatr Neuropsychol. 5, 77–84. https://doi.org/10.1007/s40817-019-00069-z.

Greenberg, D.M., Warrier, V., Allison, C., and Baron-Cohen, S. (2018). Testing the Empathizing–Systemizing theory of sex differences and the Extreme Male Brain theory of autism in half a million people. Proc. Natl. Acad. Sci. USA 115, 12152–12157. https://doi.org/10.1073/pnas.1811032115.

Grønborg, T.K., Schendel, D.E., and Parner, E.T. (2013). Recurrence of autism spectrum disorders in full- and half-siblings and trends over time: a population-based cohort study. JAMA Pediatr. 167, 947–953. https://doi.org/10.1001/jamapediatrics.2013.2259.

Grove, J., Ripke, S., Als, T.D., Mattheisen, M., Walters, R.K., Won, H., Pallesen, J., Agerbo, E., Andreassen, O.A., Anney, R., et al. (2019). Identification of common genetic risk variants for autism spectrum disorder. Nat. Genet. 51, 431–444. https://doi.org/10.1038/s41588-019-0344-8.

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., Miller, J., Fedele, A., Collins, J., Smith, K., et al. (2011). Genetic heritability and shared environmental factors Among twin pairs With autism. Arch. Gen. Psychiatry 68, 1095–1102. https://doi.org/10.1001/archgenpsychiatry.2011.76.

Hanly, C., Shah, H., Au, P.Y.B., and Murias, K. (2021). Description of neurodevelopmental phenotypes associated with 10 genetic neurodevelopmental disorders: a scoping review. Clin. Genet. 99, 335–346. https://doi.org/10.1111/cge.13882.

Hansen, S.N., Schendel, D.E., Francis, R.W., Windham, G.C., Bresnahan, M., Levine, S.Z., Reichenberg, A., Gissler, M., Kodesh, A., Bai, D., et al. (2019). Recurrence risk of autism in siblings and cousins: a multinational, population-based study. J. Am. Acad. Child Adolesc. Psychiatry 58, 866–875. https://doi.org/10.1016/j.jaac.2018.11.017.

Hansen-Kiss, E., Beinkampen, S., Adler, B., Frazier, T., Prior, T., Erdman, S., Eng, C., and Herman, G. (2017). A retrospective chart review of the features of PTEN hamartoma tumour syndrome in children. J. Med. Genet. 54, 471–478. https://doi.org/10.1136/jmedgenet-2016-104484.

Hoekstra, R.A., Bartels, M., Verweij, C.J.H., and Boomsma, D.I. (2007). Heritability of autistic traits in the general population. Arch. Pediatr. Adolesc. Med. 161, 372–377. https://doi.org/10.1001/archpedi.161.4.372.

Holmboe, K., Rijsdijk, F.V., Hallett, V., Happé, F., Plomin, R., and Ronald, A. (2014). Strong genetic influences on the stability of autistic traits in childhood. J. Am. Acad. Child Adolesc. Psychiatry 53, 221–230. https://doi.org/10.1016/j.jaac.2013.11.001.

Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., Levy, D., Stessman, H.A., Witherspoon, K.T., Vives, L., Patterson, K.E., et al. (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature 515, 216–221. https://doi.org/10.1038/nature13908.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., et al. (2012). De novo gene disruptions in children on the autistic spectrum. Neuron 74, 285–299. https://doi.org/10.1016/j.neuron.2012.04.009.

Jacquemont, S., Coe, B.P., Hersch, M., Duyzend, M.H., Krumm, N., Bergmann, S., Beckmann, J.S., Rosenfeld, J.A., and Eichler, E.E. (2014). A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. Am. J. Hum. Genet. 94, 415–425. https://doi.org/10.1016/j.ajhg.2014.02.001.

Jensen, M., Smolen, C., and Girirajan, S. (2020). Gene discoveries in autism are biased towards comorbidity with intellectual disability. J Med Genet. 57, 647–652. https://doi.org/10.1136/jmedgenet-2019-106476.

Kaiser, M.D., Hudac, C.M., Shultz, S., Lee, S.M., Cheung, C., Berken, A.M., Deen, B., Pitskel, N.B., Sugrue, D.R., Voos, A.C., et al. (2010). Neural signatures of autism. Proc. Natl. Acad. Sci. USA 107, 21223–21228. https://doi.org/10.1073/pnas.1010412107.

Kanner, L. (1943). Autistic disturbances of affective contact. Nerv. Child 2, 217–250.

Kanner, L. (1968). Autistic disturbances of affective contact. Acta Paedopsychiatr 35, 100–136.

Kantarci, O.H., Barcellos, L.F., Atkinson, E.J., Ramsay, P.P., Lincoln, R., Achenbach, S.J., De Andrade, M., Hauser, S.L., and Weinshenker, B.G. (2006). Men transmit MS more often to their children vs women: the Carter effect. Neurology 67, 305–310. https://doi.org/10.1212/01.wnl.0000225070.13682.11.

King, M., and Bearman, P. (2009). Diagnostic change and the increased prevalence of autism. Int. J. Epidemiol. 38, 1224–1234. https://doi.org/10.1093/ije/dyp261.

Klusek, J., Losh, M., and Martin, G.E. (2014). Sex differences and within-family associations in the broad autism phenotype. Autism 18, 106–116. https://doi.org/10.1177/1362361312464529.

Krumm, N., Turner, T.N., Baker, C., Vives, L., Mohajeri, K., Witherspoon, K., Raja, A., Coe, B.P., Stessman, H.A., He, Z.X., et al. (2015). Excess of rare, inherited truncating mutations in autism. Nat. Genet. 47, 582–588. https://doi.org/10.1038/ng.3303.

Kruse, L.M., Dobbs, M.B., and Gurnett, C.A. (2008). Polygenic threshold model with sex dimorphism in clubfoot inheritance: the Carter effect. J. Bone Joint Surg. Am. 90, 2688–2694. https://doi.org/10.2106/JBJS.G.01346.

Lai, M.C., Lombardo, M.V., Auyeung, B., Chakrabarti, B., and Baron-Cohen, S. (2015). Sex/gender differences and autism: setting the scene for future research. J. Am. Acad. Child Adolesc. Psychiatry 54, 11–24. https://doi.org/10.1016/j.jaac.2014.10.003.

Lai, M.C., Lombardo, M.V., and Baron-Cohen, S. (2014). Autism. Lancet. 383, 896–910. https://doi.org/10.1016/S0140-6736(13)61539-1.

Lainhart, J.E., Bigler, E.D., Bocian, M., Coon, H., Dinh, E., Dawson, G., Deutsch, C.K., Dunn, M., Estes, A., Tager-Flusberg, H., et al. (2006). Head circumference and height in autism: a study by the Collaborative Program of Excellence in Autism. Am J Med Genet A 140, 2257–2274. https://doi.org/10.1002/ajmg.a.31465.

Levy, D., Ronemus, M., Yamrom, B., Lee, Y.H., Leotta, A., Kendall, J., Marks, S., Lakshmi, B., Pai, D., Ye, K., et al. (2011). Rare de novo and transmitted copy-number variation in autistic spectrum disorders. Neuron 70, 886–897. https://doi.org/10.1016/j.neuron.2011.05.015.

Lim, E.T., Raychaudhuri, S., Sanders, S.J., Stevens, C., Sabo, A., MacArthur, D.G., Neale, B.M., Kirby, A., Ruderfer, D.M., Fromer, M., et al. (2013). Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders. Neuron 77, 235–242. https://doi.org/10.1016/j.neuron.2012.12.029.

Liu, X.Q., Paterson, A.D., and Szatmari, P.; Autism Genome Project Consortium (2008). Genome-wide linkage analyses of quantitative and categorical autism subphenotypes. Biol. Psychiatry 64, 561–570. https://doi.org/10.1016/j.biopsych.2008.05.023.

Loomes, R., Hull, L., and Mandy, W.P.L. (2017). What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. J. Am. Acad. Child Adolesc. Psychiatry 56, 466–474. https://doi.org/10.1016/j.jaac.2017.03.013.

Lundström, S., Chang, Z., Råstam, M., Gillberg, C., Larsson, H., Anckarsäter, H., and Lichtenstein, P. (2012). Autism spectrum disorders and autisticlike traits: similar etiology in the extreme end and the normal variation. Arch. Gen. Psychiatry 69, 46–52. https://doi.org/10.1001/archgenpsychiatry.2011.144.

Lundström, S., Mårland, C., Kuja-Halkola, R., Anckarsäter, H., Lichtenstein, P., Gillberg, C., and Nilsson, T. (2019). Assessing autism in females: the importance of a sex-specific comparison. Psychiatry Res. 282, 112566. https://doi.org/10.1016/j.psychres.2019.112566.

Lundström, S., Reichenberg, A., Anckarsäter, H., Lichtenstein, P., and Gillberg, C. (2015). Autism phenotype versus registered diagnosis in Swedish children: prevalence trends over 10 years in general population samples. BMJ 350, h1961. https://doi.org/10.1136/bmj.h1961.

Lyall, K., Constantino, J.N., Weisskopf, M.G., Roberts, A.L., Ascherio, A., and Santangelo, S.L. (2014). Parental social responsiveness and risk of autism spectrum disorder in offspring. JAMA Psychiatry 71, 936–942. https://doi.org/10.1001/jamapsychiatry.2014.476.

Maenner, M.J., Shaw, K.A., Bakian, A.V., Bilder, D.A., Durkin, M.S., Esler, A., Furnier, S.M., Hallas, L., Hall-Lande, J., Hudson, A., et al. (2021). Prevalence and characteristics of autism spectrum disorder Among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, United States, 2018. MMWR Surveill. Summ. 70, 1–16. https://doi.org/10.15585/mmwr.ss7011a1.

Marrus, N., Hall, L.P., Paterson, S.J., Elison, J.T., Wolff, J.J., Swanson, M.R., Parish-Morris, J., Eggebrecht, A.T., Pruett, J.R., Hazlett, H.C., et al. (2018). Language delay aggregates in toddler siblings of children with autism spectrum disorder. J. Neurodev. Disord. 10, 29. https://doi.org/10.1186/s11689-018-9247-8.

Martin, J., Khramtsova, E.A., Goleva, S.B., Blokland, G., Traglia, M., Walters, R.K., Hübel, C., Coleman, J.R., Breen, G., Børglum, A.D., et al.; Sex Differences Cross-Disorder Analysis Group of the Psychiatric Genomics Consortium (2021). Examining Sex-Differentiated Genetic Effects Across Neuropsychiatric and Behavioral Traits. Biological psychiatry 89, 1127–1137. https://doi.org/10.1016/j.biopsych.2020.12.024.

Massrali, A., Brunel, H., Hannon, E., Wong, C., iPSYCH-MINERvA Epigenetics Group, Baron-Cohen, S., and Warrier, V. (2019). Integrated genetic and methylomic analyses identify shared biology between autism and autistic traits. Mol. Autism 10, 31. https://doi.org/10.1186/s13229-019-0279-z.

Matoba, N., Liang, D., Sun, H., Aygün, N., McAfee, J.C., Davis, J.E., Raffield, L.M., Qian, H., Piven, J., Li, Y., et al. (2020). Common genetic risk variants identified in the SPARK cohort support DDHD2 as a candidate risk gene for autism. Transl. Psychiatry 10, 265. https://doi.org/10.1038/s41398-020-00953-9.

Maxwell, C.R., Parish-Morris, J., Hsin, O., Bush, J.C., and Schultz, R.T. (2013). The broad autism phenotype predicts child functioning in autism spectrum disorders. J. Neurodev. Disord. 5, 25. https://doi.org/10.1186/1866-1955-5-25.

McCarthy, M.M., and Arnold, A.P. (2011). Reframing sexual differentiation of the brain. Nat. Neurosci. 14, 677–683. https://doi.org/10.1038/nn.2834.

Messinger, D.S., Young, G.S., Webb, S.J., Ozonoff, S., Bryson, S.E., Carter, A., Carver, L., Charman, T., Chawarska, K., Curtin, S., et al. (2015). Early sex differences are not autism-specific: A Baby Siblings Research Consortium (BSRC) study. Mol. Autism 6, 32. https://doi.org/10.1186/s13229-015-0027-y.

Miller, M., Iosif, A.M., Hill, M., Young, G.S., Schwichtenberg, A.J., and Ozonoff, S. (2017). Response to name in infants developing autism spectrum disorder: A prospective study. J. Pediatr. 183, 141–146.e1. https://doi.org/10.1016/j.jpeds.2016.12.071.

Mitchell, R.A., Barton, S.M., Harvey, A.S., Ure, A.M., and Williams, K. (2021). Factors associated with autism spectrum disorder in children with tuberous sclerosis complex: a systematic review and meta-analysis. Dev. Med. Child Neurol. 63, 791–801. https://doi.org/10.1111/dmcn.14787.

Mitra, I., Tsang, K., Ladd-Acosta, C., Croen, L.A., Aldinger, K.A., Hendren, R.L., Traglia, M., Lavillaureix, A., Zaitlen, N., Oldham, M.C., et al. (2016). Pleiotropic mechanisms indicated for sex differences in autism. PLoS Genet 12, e1006425. https://doi.org/10.1371/journal.pgen.1006425.

Morris, S.M., Acosta, M.T., Garg, S., Green, J., Huson, S., Legius, E., North, K.N., Payne, J.M., Plasschaert, E., Frazier, T.W., et al. (2016). Disease burden and symptom structure of autism in neurofibromatosis Type 1: A study of the international NF1-ASD consortium team (INFACT). JAMA Psychiatry 73, 1276–1284. https://doi.org/10.1001/jamapsychiatry.2016.2600.

Muller, C.L., Anacker, A., and Veenstra-VanderWeele, J. (2016). The serotonin system in autism spectrum disorder: From biomarker to animal models. Neuroscience 321, 24–41. https://doi.org/10.1016/j.neuroscience.2015.11.010.

Myers, S.M., Challman, T.D., Bernier, R., Bourgeron, T., Chung, W.K., Constantino, J.N., Eichler, E.E., Jacquemont, S., Miller, D.T., Mitchell, K.J., et al. (2020). Insufficient evidence for "autism-specific" genes. Am. J. Hum. Genet. 106, 587–595. https://doi.org/10.1016/j.ajhg.2020.04.004.

Nassar, N., Dixon, G., Bourke, J., Bower, C., Glasson, E., de Klerk, N., and Leonard, H. (2009). Autism spectrum disorders in young children: effect of

changes in diagnostic practices. Int. J. Epidemiol. *38*, 1245–1254. https://doi.org/10.1093/ije/dyp260.

Nayar, K., Sealock, J.M., Maltman, N., Bush, L., Cook, E.H., Davis, L.K., and Losh, M. (2021). Elevated polygenic burden for autism spectrum disorder is associated With the broad autism phenotype in mothers of individuals With autism spectrum disorder. Biol. Psychiatry *89*, 476–485. https://doi.org/10.1016/j.biopsych.2020.08.029.

Neale, B.M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K.E., Sabo, A., Lin, C.F., Stevens, C., Wang, L.S., Makarov, V., et al. (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature *485*, 242–245. https://doi.org/10.1038/nature11011.

Niranjan, T.S., Skinner, C., May, M., Turner, T., Rose, R., Stevenson, R., Schwartz, C.E., and Wang, T. (2015). Affected kindred analysis of human X chromosome exomes to identify novel X-linked intellectual disability genes. PLoS One *10*, e0116454. https://doi.org/10.1371/journal.pone.0116454.

Nordahl, C.W., Lange, N., Li, D.D., Barnett, L.A., Lee, A., Buonocore, M.H., Simon, T.J., Rogers, S., Ozonoff, S., and Amaral, D.G. (2011). Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. Proc Natl Acad Sci USA *108*, 20195–20200. https://doi.org/10.1073/pnas.1107560108.

Ounsted, C., and Taylor, D.C. (1972). Gender Differences: their Ontogeny and Significance (Churchill Livingstone).

Ozonoff, S., Young, G.S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., Bryson, S., Carver, L.J., Constantino, J.N., Dobkins, K., et al. (2011). Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. Pediatrics *128*, e488–e495. https://doi.org/10.1542/peds.2010-2825.

Page, J., Constantino, J.N., Zambrana, K., Martin, E., Tunc, I., Zhang, Y., Abbacchi, A., and Messinger, D. (2016). Quantitative autistic trait measurements index background genetic risk for ASD in Hispanic families. Mol. Autism *7*, 39. https://doi.org/10.1186/s13229-016-0100-1.

Palmer, N., Beam, A., Agniel, D., Eran, A., Manrai, A., Spettell, C., Steinberg, G., Mandl, K., Fox, K., Nelson, S.F., et al. (2017). Association of sex With recurrence of autism spectrum disorder among siblings. JAMA Pediatr. *171*, 1107–1112. https://doi.org/10.1001/jamapediatrics.2017.2832.

Pan, L., Ober, C., and Abney, M. (2007). Heritability estimation of sex-specific effects on human quantitative traits. Genet. Epidemiol. *31*, 338–347. https://doi.org/10.1002/gepi.20214.

Piton, A., Gauthier, J., Hamdan, F.F., Lafrenière, R.G., Yang, Y., Henrion, E., Laurent, S., Noreau, A., Thibodeau, P., Karemera, L., et al. (2011). Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. Molecular psychiatry *16*, 867–880. https://doi.org/10.1038/mp.2010.54.

Power, R.A., Kyaga, S., Uher, R., MacCabe, J.H., Långström, N., Landen, M., McGuffin, P., Lewis, C.M., Lichtenstein, P., and Svensson, A.C. (2013). Fecundity of patients With schizophrenia, autism, bipolar disorder, depression, anorexia nervosa, or substance abuse vs their unaffected siblings. JAMA Psychiatry *70*, 22–30. https://doi.org/10.1001/jamapsychiatry.2013.268.

Reich, R., Cloninger, C.R., and Guze, S.B. (1975). The multifactorial model of disease transmission: I. Description of the model and its use in psychiatry. Br. J. Psychiatry *127*, 1–10. https://doi.org/10.1192/bjp.127.1.1.

Reichenberg, A., Cederlöf, M., McMillan, A., Trzaskowski, M., Kapra, O., Fruchter, E., Ginat, K., Davidson, M., Weiser, M., Larsson, H., et al. (2016). Discontinuity in the genetic and environmental causes of the intellectual disability spectrum. Proc. Natl. Acad. Sci. USA *113*, 1098–1103. https://doi.org/10.1073/pnas.1508093112.

Ritvo, E.R., Jorde, L.B., Mason-Brothers, A., Freeman, B.J., Pingree, C., Jones, M.B., McMahon, W.M., Petersen, P.B., Jenson, W.R., and Mo, A. (1989). The UCLA-University of Utah epidemiologic survey of autism: recurrence risk estimates and genetic counseling. Am. J. Psychiatry *146*, 1032–1036. https://doi.org/10.1176/ajp.146.8.1032.

Robinson, E.B., Koenen, K.C., McCormick, M.C., Munir, K., Hallett, V., Happé, F., Plomin, R., and Ronald, A. (2011). Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%). Arch. Gen. Psychiatry *68*, 1113–1121. https://doi.org/10.1001/archgenpsychiatry.2011.119.

Robinson, E.B., Lichtenstein, P., Anckarsäter, H., Happé, F., and Ronald, A. (2013). Examining and interpreting the female protective effect against autistic behavior. Proc. Natl. Acad. Sci. USA *110*, 5258–5262. https://doi.org/10.1073/pnas.1211070110.

Robinson, E.B., St Pourcain, B., Anttila, V., Kosmicki, J.A., Bulik-Sullivan, B., Grove, J., Maller, J., Samocha, K.E., Sanders, S.J., Ripke, S., et al. (2016). Genetic risk for autism spectrum disorders and neuropsychiatric variation in the general population. Nat. Genet. *48*, 552–555. https://doi.org/10.1038/ng.3529.

Rommelse, N.N., Peters, C.T., Oosterling, I.J., Visser, J.C., Bons, D., van Steijn, D.J., Draaisma, J., van der Gaag, R.J., and Buitelaar, J.K. (2011). A pilot study of abnormal growth in autism spectrum disorders and other childhood psychiatric disorders. J Autism Dev Disord *41*, 44–54. https://doi.org/10.1007/s10803-010-1026-7.

Ronald, A., Happé, F., Bolton, P., Butcher, L.M., Price, T.S., Wheelwright, S., Baron-Cohen, S., and Plomin, R. (2006). Genetic heterogeneity between the three components of the autism spectrum: a twin study. J. Am. Acad. Child Adolesc. Psychiatry *45*, 691–699. https://doi.org/10.1097/01.chi.0000215325.13058.9d.

Rudnick, S.B., Zabriskie, H., Ho, J., Gurnett, C.A., and Dobbs, M.B. (2018). Scoliosis severity does not impact the risk of scoliosis in family members. J. Pediatr. Orthop. B *27*, 147–151. https://doi.org/10.1097/BPB.0000000000000473.

Sacco, R., Militerni, R., Frolli, A., Bravaccio, C., Gritti, A., Elia, M., Curatolo, P., Manzi, B., Trillo, S., Lenti, C., et al. (2007). Clinical, morphological, and biochemical correlates of head circumference in autism. Biol Psychiatry *62*, 1038–1047. https://doi.org/10.1016/j.biopsych.2007.04.039.

Sanders, S.J., Ercan-Sencicek, A.G., Hus, V., Luo, R., Murtha, M.T., Moreno-De-Luca, D., Chu, S.H., Moreau, M.P., Gupta, A.R., Thomson, S.A., et al. (2011). Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron *70*, 863–885. https://doi.org/10.1016/j.neuron.2011.05.002.

Sanders, S.J., He, X., Willsey, A.J., Ercan-Sencicek, A.G., Samocha, K.E., Cicek, A.E., Murtha, M.T., Bal, V.H., Bishop, S.L., Dong, S., et al. (2015). Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. Neuron *87*, 1215–1233. https://doi.org/10.1016/j.neuron.2015.09.016.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C.M., and Reichenberg, A. (2014). The familial risk of autism. JAMA *311*, 1770–1777. https://doi.org/10.1001/jama.2014.4144.

Satterstrom, F.K., Kosmicki, J.A., Wang, J., Breen, M.S., De Rubeis, S.D., An, J.Y., Peng, M., Collins, R., Grove, J., Klei, L., et al. (2020). Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. Cell *180*, 568–584.e23. https://doi.org/10.1016/j.cell.2019.12.036.

Skov, J., Calissendorff, J., Eriksson, D., Magnusson, P., Kämpe, O., Bensing, S., and Kuja-Halkola, R. (2021). Limited genetic overlap Between overt Hashimoto's thyroiditis and Graves' disease in twins: a population-based study. J. Clin. Endocrinol. Metab. *106*, 1101–1110. https://doi.org/10.1210/clinem/dgaa956.

Stevenson, R.E., Schroer, R.J., Skinner, C., Fender, D., and Simensen, R.J. (1997). Autism and macrocephaly. Lancet *349*, 1744–1745. https://doi.org/10.1016/S0140-6736(05)62956-X.

Specchio, N., Pietrafusa, N., Trivisano, M., Moavero, R., De Palma, L., Ferretti, A., Vigevano, F., and Curatolo, P. (2020). Autism and epilepsy in patients With tuberous sclerosis complex. Front. Neurol. *11*, 639. https://doi.org/10.3389/fneur.2020.00639.

St Pourcain, B., Robinson, E.B., Anttila, V., Sullivan, B.B., Maller, J., Golding, J., Skuse, D., Ring, S., Evans, D.M., Zammit, S., et al. (2018). ASD and schizophrenia show distinct developmental profiles in common genetic overlap with population-based social communication difficulties. Mol. Psychiatry *23*, 263–270. https://doi.org/10.1038/mp.2016.198.

St Pourcain, B., Wang, K., Glessner, J.T., Golding, J., Steer, C., Ring, S.M., Skuse, D.H., Grant, S.F.A., Hakonarson, H., and Davey Smith, G. (2010). Association Between a high-risk autism locus on 5p14 and social communication spectrum phenotypes in the general population. Am. J. Psychiatry *167*, 1364–1372. https://doi.org/10.1176/appi.ajp.2010.09121789.

Stilp, R.L.H., Gernsbacher, M.A., Schweigert, E.K., Arneson, C.L., and Goldsmith, H.H. (2010). Genetic variance for autism screening items in an unselected sample of toddler-age twins. J. Am. Acad. Child Adolesc. Psychiatry *49*, 267–276. https://doi.org/10.1016/j.jaac.2009.11.012.

Sumi, S., Taniai, H., Miyachi, T., and Tanemura, M. (2006). Sibling risk of pervasive developmental disorder estimated by means of an epidemiologic survey in Nagoya, Japan. J. Hum. Genet. *51*, 518–522. https://doi.org/10.1007/s10038-006-0392-7.

Szatmari, P., Liu, X.Q., Goldberg, J., Zwaigenbaum, L., Paterson, A.D., Woodbury-Smith, M., Georgiades, S., Duku, E., and Thompson, A. (2012). Sex differences in repetitive stereotyped behaviors in autism: implications for genetic liability. Am. J. Med. Genet. B Neuropsychiatr. Genet. *159B*, 5–12. https://doi.org/10.1002/ajmg.b.31238.

Takahashi, N., Harada, T., Nishimura, T., Okumura, A., Choi, D., Iwabuchi, T., Kuwabara, H., Takagai, S., Nomura, Y., Takei, N., et al. (2020). Association of genetic risks With autism spectrum disorder and early neurodevelopmental delays Among children Without intellectual disability. JAMA Netw. Open *3*, e1921644. https://doi.org/10.1001/jamanetworkopen.2019.21644.

Taniai, H., Nishiyama, T., Miyachi, T., Imaeda, M., and Sumi, S. (2008). Genetic influences on the broad spectrum of autism: study of proband-ascertained twins. Am. J. Med. Genet. B Neuropsychiatr. Genet. *147B*, 844–849. https://doi.org/10.1002/ajmg.b.30740.

Taylor, M.J., Rosenqvist, M.A., Larsson, H., Gillberg, C., D'Onofrio, B.M., Lichtenstein, P., and Lundström, S. (2020). Etiology of autism spectrum disorders and autistic traits Over time. JAMA Psychiatry *77*, 936–943. https://doi.org/10.1001/jamapsychiatry.2020.0680.

Tilghman, J.M., Ling, A.Y., Turner, T.N., Sosa, M.X., Krumm, N., Chatterjee, S., Kapoor, A., Coe, B.P., Nguyen, K.-D.H., Gupta, N., et al. (2019). Molecular genetic anatomy and risk profile of Hirschsprung's disease. N. Engl. J. Med. *380*, 1421–1432. https://doi.org/10.1056/NEJMoa1706594.

Traglia, M., Croen, L.A., Lyall, K., Windham, G.C., Kharrazi, M., DeLorenze, G.N., Torres, A.R., and Weiss, L.A. (2017). Independent maternal and fetal genetic effects on midgestational circulating levels of environmental pollutants. G3 (Bethesda) *7*, 1287–1299. https://doi.org/10.1534/g3.117.039784.

Tsai, L.Y., and Beisler, J.M. (1983). The development of sex differences in infantile autism. Br. J. Psychiatry *142*, 373–378. https://doi.org/10.1192/bjp.142.4.373.

Turner, T.N., Wilfert, A.B., Bakken, T.E., Bernier, R.A., Pepper, M.R., Zhang, Z., Torene, R.I., Retterer, K., and Eichler, E.E. (2019). Sex-based analysis of de novo variants in neurodevelopmental disorders. Am. J. Hum. Genet. *105*, 1274–1285. https://doi.org/10.1016/j.ajhg.2019.11.003.

Van Wijngaarden-Cremers, P.J.M., van Eeten, E., Groen, W.B., Van Deurzen, P.A., Oosterling, I.J., and Van der Gaag, R.J. (2014). Gender and age differences in the core triad of impairments in autism spectrum disorders: a systematic review and meta-analysis. J. Autism Dev. Disord. *44*, 627–635. https://doi.org/10.1007/s10803-013-1913-9.

Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M.F., and Zhang, F.P. (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell *65*, 905–914. https://doi.org/10.1016/0092-8674(91)90397-h.

Waddington, C.H. (1942). Canalization of development and the inheritance of acquired characters. Nature *150*, 563–565. https://doi.org/10.1038/150563a0.

Wakabayashi, A., Baron-Cohen, S., Wheelwright, S., and Tojo, Y. (2006). The Autism-Spectrum Quotient (AQ) in Japan: a cross-cultural comparison. J. Autism Dev. Disord. *36*, 263–270. https://doi.org/10.1007/s10803-005-0061-2.

Warrier, V., Zhang, X., Reed, P., Havdahl, A., Moore, T.M., Cliquet, F., Leblond, C.S., Rolland, T., Rosengren, A., et al.; EU-AIMS LEAP (2022). Genetic correlates of phenotypic heterogeneity in autism. Nat. Genet. 1–12. https://doi.org/10.1038/s41588-022-01072-5.

Weiner, D.J., Wigdor, E.M., Ripke, S., Walters, R.K., Kosmicki, J.A., Grove, J., Samocha, K.E., Goldstein, J.I., Okbay, A., Bybjerg-Grauholm, J., et al. (2017). Polygenic transmission disequilibrium confirms that common and rare variation act additively to create risk for autism spectrum disorders. Nat. Genet. *49*, 978–985. https://doi.org/10.1038/ng.3863.

Weiss, L.A., Abney, M., Cook, E.H., Jr., and Ober, C. (2005). Sex-specific genetic architecture of whole blood serotonin levels. American journal of human genetics *76*, 33–41. https://doi.org/10.1086/426697.

Werling, D.M. (2016). The role of sex-differential biology in risk for autism spectrum disorder. Biol. Sex Differ. *7*, 58. https://doi.org/10.1186/s13293-016-0112-8.

Werling, D.M., and Geschwind, D.H. (2015). Recurrence rates provide evidence for sex-differential, familial genetic liability for autism spectrum disorders in multiplex families and twins. Mol. Autism *6*, 27. https://doi.org/10.1186/s13229-015-0004-5.

Wigdor, E.M., Weiner, D.J., Grove, J., Fu, J.M., Thompson, W.K., Carey, C.E., Baya, N., Merwe, C. van der, Walters, R.K., Satterstrom, F.K., et al. (2021). The female protective effect against autism spectrum disorder. Preprint at medRxiv. https://doi.org/10.1101/2021.03.29.21253866.

Wing, L. (1981). Sex ratios in early childhood autism and related conditions. Psychiatry Res. *5*, 129–137. https://doi.org/10.1016/0165-1781(81)90043-3.

Xie, S., Karlsson, H., Dalman, C., Widman, L., Rai, D., Gardner, R.M., Magnusson, C., Sandin, S., Tabb, L.P., Newschaffer, C.J., et al. (2020). The familial risk of autism spectrum disorder with and without intellectual disability. Autism Res. *13*, 2242–2250. https://doi.org/10.1002/aur.2417.

Ye, K., Iossifov, I., Levy, D., Yamrom, B., Buja, A., Krieger, A.M., and Wigler, M. (2017). Measuring shared variants in cohorts of discordant siblings with applications to autism. Proc. Natl. Acad. Sci. USA *114*, 7073–7076. https://doi.org/10.1073/pnas.1700439114.

Yu, T.W., Chahrour, M.H., Coulter, M.E., Jiralerspong, S., Okamura-Ikeda, K., Ataman, B., Schmitz-Abe, K., Harmin, D.A., Adli, M., Malik, A.N., et al. (2013). Using whole-exome sequencing to identify inherited causes of autism. Neuron *77*, 259–273. https://doi.org/10.1016/j.neuron.2012.11.002.

Zöller, B., Li, X., Ohlsson, H., Ji, J., Memon, A.A., Svensson, P.J., Palmér, K., Dahlbäck, B., Sundquist, J., and Sundquist, K. (2016). Epidemiology of familial aggregation of venous thromboembolism. Semin. Thromb. Hemost. *42*, 821–832. https://doi.org/10.1055/s-0036-1593543.