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# Sex differences in secondary autism spectrum disorders: Differential risk by sex, neurologic load and ascertainment bias

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A R T I C L E I N F O

*Keywords:*
Autism
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A B S T R A C T

*Introduction:* Controversy persists as to whether there exists a bioendogenous and/or socioculturally determined male overprevalence and/or a predominantly female neurological protective effect against occurrence of autism but with greater brain morbidity (intellectual disability, epilepsy) in females who do have autism. Much research on idiopathic ASD has not fully resolved this conundrum.
*Method:* We assembled from case series in the literature 70 samples of male and female patients with specific bioetiologies (genetic, neurological, metabolic), all with complete documentation of sex, comorbid ASD, epilepsy and intellectual disability (total sample = 1264 cases).
*Results:* Male overprevalence in these patients with secondary ASD (sASD) was significant, but much lower than typically reported in idiopathic ASD (iASD). The males with sASD had significantly greater prevalence of intellectual disability than the females with sASD. The neuropathologies with highest risk of sASD presented the highest male overprevalence of sASD. "Ascertainment bias" emerged as a significant modulator of male overprevalence in sASD.
*Conclusion:* In secondary autism, male overprevalence is not as strong as in idiopathic autism, risk of female autism is modestly explained by risk of epilepsy, and male overprevalence is strongly explained by risk of intellectual disability and ascertainment bias.

## 1. Introduction

Autism spectrum disorder (ASD) occurs in a large set of heterogeneous neurodevelopmental disorders. ASD is defined behaviorally by significant deficits in social interaction, communication and repetitive, stereotyped behavior and interests. ASD has been reported as highly male prevalent since the initial case series (Asperger, 1944; Kanner, 1943). In the interim, much etiopathological research has been done and many gene variants have been found linked to ASD phenotypes. However, in most cases of ASD a bioetiology is not or still cannot be determined and the patient remains "non-syndromic" or "idiopathic". The male overprevalence in "syndromic" or "secondary" autism spectrum disorders (sASD), where a specific cause is known (be it teratologic, neurologic, genetic, chromosomic, autosomic, metabolic, infectious, etc.), remains to be investigated.

### 1.1. Idiopathic autism spectrum disorders (iASD)

#### 1.1.1. Comorbidities and male overprevalence

Epilepsy afflicts approximately 25% of patients with iASD (Viscidi et al., 2013) and intellectual disability characterizes about 50% of these patients (Matson & Shoemaker, 2009). Moreover, it is established that iASD is highly male overprevalent. With a vast epidemiological survey, Fombonne (2009) placed the sex bias of risk at a male to female (M:F) ratio of 4.2:1. More recently, Palmer et al. (2017) conducted the most extensive study, to date, of modulation by gender in iASD and found an overall population incidence of 1.25% and a M:F ratio of 4.10:1.

#### 1.1.2. Idiopathic ASD in the female sex

Claims of a "female protective effect" (see Robinson, Lichtenstein, Anckarsäter, Happé, & Ronald, 2013), or a "female overmorbidity" in iASD, suggest that a greater load of brain insults (genetic, metabolic, teratologic, etc.) are required for iASD to occur in girls and women.

Kirkovski, Enticott, and Fitzgerald, (2013) reported that de novo copy variation load was higher in female patients with iASD. Holtmann

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and colleagues (2007) found that female patients with iASD had ADI score levels and structures indistinguishable from male patients with iASD, but the female patients had significantly more pre/peri/or post-natal medical complications. Also, female patients with iASD appeared to have a more severe pattern of brain undergrowth following the overgrowth phase than their male counterpart (Ben-Itzchak, Ben-Shachar, & Zachor, 2013; Bloss & Courchesne, 2007). Gillberg, Cederlund, Lamberg, and Zeijlon, (2006) found that in iASD, the lower was the IQ, the higher was the female prevalence. Moreover, several studies found that the fewer neurological symptoms there were in iASD, the lower was the female prevalence (Ben-Itzchak, Ben Shachar & Zachor, 2013). Finally, autistic regression, which settles on average to a severe form of iASD, has been found to be more common in females than males (Ben-Itzchak, Ben Shachar & Zachor, 2013).

However, a few studies contradict what could seem to be a female “protective effect” or “overmorbidity” in iASD. For example, Supekar and Menon (2015) reported that males with iASD presented significantly more severe repetitive behaviors than the females, while language and social impairment were comparable. In addition, as noted by many commentators, both male “overprevalence” and the female protective effect in iASD could be artifacts of an “ascertainment bias”. Why then and how could the diagnosis of ASD be as culturally biased as it would have to be to explain discrepancies in risk that are consistently reported in epidemiological studies? Could it only be the behavioral rating scales themselves?

#### 1.1.3. Autism rating scales and sex bias

Murray et al. (2017) found that individual items did manifest some sex bias at the population level on the AQ-10 test (a brief screen for ASD conditions) favoring males or females, whereas taken altogether, the item-level biases cancelled out to give an unbiased overall score. Beggiato et al. (2017) found that several items of the Autism Diagnosis Interview-Revised (ADI-R) significantly differed in tally between males and females at the population level. The potential gender bias thus induced may participate in the underestimation of the prevalence of ASD in females. Loomes, Hull, and Mandy, (2017) report that among children meeting criteria for ASD, there appeared to be a diagnostic gender bias, meaning that girls who meet criteria for ASD were at disproportionate risk of not receiving a formal and legally binding clinical diagnosis. Dworzynski, Ronald, Bolton, and Happé, (2012) found that among individuals without co-morbid intellectual or behavioral problems, females were less likely than males to receive clinical diagnoses of ASD at equivalently high levels of autistic-like traits. However, when neuropathological load (IQ, epilepsy) was matched for male and female patients with ASD, there was in fact no difference in the repartition of autistic symptoms (aloofness, language, stereotypies) according to Harrop, Gulsrud, and Kasari, (2015). Cultural and bioendogenous factors do not have to be mutually incompatible, they can be additive. Thus, only further carefully and strategically planned research can unravel this conundrum.

### 1.2. Secondary autism spectrum disorders (sASD)

One way to get around this “ascertainment bias” versus “bioetiological” conundrum and to more deeply probe explanations of sex differences in iASD is to strategically quantitatively analyze cases with sASD. This approach could disentangle biological systems as well as sex-biased ascertainment bias, simultaneously. Afterall, when a specific severe neuropathology is isolated, and relatively well understood, and the case series published, ASD is often a secondary concern and is evaluated late in the investigation. When that is the case, the patients with sASD are not necessarily selected on the basis of parental bias in consultation favouring their children of one or the other sex, nor on the basis of strategic treatment-driven selection of a diagnosis. Rather, the search is often for a specific gene mutation or a specific morbid brain condition first and foremost. When that is the case, ASD, epilepsy, intellectual disability, are all progressively determined to be present or not later along the research agenda. The male and female prevalences in the whole series, and *a fortieri* in the ASD subsets, are then less likely to be biased by culturally perpetuated sex typing or treatment based diagnostic strategy that could be distorting research on iASD.

## 2. Purpose of the present study

Understanding sex differences in sASD is likely to contribute further elucidation of the pathoetiology of iASD. Given that iASD is male prevalent and that females with iASD are often found to be more neurologically, cognitively and genetically morbid (see introduction), the purpose of the present study was to shed light on sex differences in autism by investigating sASD instead of iASD. To this end, 70 published case series composed of distinct neuroetiologies of ASD were assembled and analyzed to simultaneously probe sexual segregation with regard to sex-specific brain morbidity and ascertainment bias in sASD.

## 3. Method

Using Google Scholar, an extensive literature search was conducted for articles presenting case series of patients afflicted by, and defined by, a specific neuroetiopathology comprising patients in which ASD, epilepsy, intellectual disability (ID) and sex were all screened and reported for each case. For a paper on a specific neuroetiopathology (and it’s given sample) to be included in our database, it had to present at least one male and one female autist (with or without epilepsy and/or ID), one male and one female non-autist (with or without epilepsy and/or ID), while other cases in the sample could present ASD, seizure/epilepsy, ID, or not, but the absence or presence of these variables had to be reported in addition to sex. In other words, failure to mention the presence or absence of any of these four variables (ASD, epilepsy, ID, sex) in the individual cases excluded a paper and its entire sample(s) from our database. The reader is thus cautioned that the sample to be reported here was assembled specifically to explore sex segregation in sASD. This sample of patients is not claimed to be representative of iASD as a whole, nor even sASD as a whole, and indeed it most certainly is not.

### 3.1. Variables of the present study

For each of the case series, the incidences of the four continuous variables of interest (ASD, epilepsy, ID and male overprevalence) were crosstabulated and tallied and converted to percentages. 70 reports of case-series covering 1264 cases altogether, categorizable on all the above dimensions, were found. For further modeling of sex segregation in sASD, codes were created for two other variables of interest, namely “transmission mode” and “ascertainment bias”.

### 3.2. Secondary autism Spectrum disorders (sASD)

Though the cases were identified as “autistic” or “ASD” or “autistic-like”, or not, using criteria that varied from one report to another, the criteria for any of these variables were uniform within any individual report. The 70 series were coded (in percentage) to account the overall prevalence of ASD in each cohort (case series) coming from a given published report. This continuous variable was named “Global prevalence of ASD” (weighted mean = 37%, range = 7%–91%, SD = 20%).

### 3.3. Epilepsy

Presence or absence of epilepsy or seizure(s) was tallied by us, regardless of and blind to the type, the age of onset, the duration of the seizure(s). The latter information was too rarely reported to be tabulated. In some reports, only occurrence of seizures were noted while in others it was a more demanding diagnosis of epilepsy that was tallied.

### 3.4. Intellectual disability

Intellectual disability (ID) was considered by us to have to involve an IQ of less than 70 and thus characterizations such as "developmental delay" did not qualify. The term had to be specifically mental or intellectual "disability", "retardation" or "deficiency". Intellectual disability was defined in some studies strictly by IQ and in other studies by a more demanding clinical diagnosis.

### 3.5. Male overprevalence

This code characterized the 70 series for male overprevalence in the syndrome or condition (weighted mean male to female proportion x 100 = 67%, range = 11%–95%, SD = 26%), a continuous variable which we named 'Global male overprevalence".

### 3.6. Transmission mode

The 70 series were coded for "transmission mode". This dichotomous code distinguished 14 series with the totality or the majority of cases (N = 178) with parental transmission (multiplex families) versus 56 series the totality or majority of cases (N = 1086) without parental transmission or in which no family member was reported to be afflicted.

### 3.7. Ascertainment bias

Thirty case series were also coded as including iASD as a first step inclusion criterion (N = 492), or not (40 series, 772 cases). This dichotomous factor was termed "ascertainment bias". The rationale for this was that iASD in the initial search pool would have been determined with maximum risk of sociocultural gender bias in the determination of the diagnosis of ASD. At the other extreme, risk of sociocultural gender bias appears minimal if a sample of cases with a specific genetic aberration has been assembled first and ASD ascertained later, by the research team itself.

The breakdown of variables described above, in each cases series, is provided in Supplementary materials #1. The bibliography of the 70 studies from which the case data were extracted is provided in Supplementary materials # 2.

## 4. Results

Qualitative inspection of segregation of the sexes as a function of the varying etiologies revealed that neuropathologies with moderate to high risk of ASD can manifest very high female prevalence as they can manifest very high male prevalence (see Chen, Van Horn, & GENDAAR Research Consortium, 2017, for a review). See Table 1.

### 4.1. Genetic liability in sASD

Genetic liability is indexed most credibly by recurrence rate, which is modulated by penetrance and expressivity of gene or epigene aberrations. This complexity is rarely fully characterized at the molecular, cellular or tissue levels in ASD, let alone the behavioral. The best way to quantify genetic liability for ASD is to study recurrence of the ASD phenotype in conjunction with gene or epigene aberrations in families. Few of the neurological syndromes and conditions (N = 7) of the current series have been studied systematically for recurrence (see Table 2).

### 4.2. Intellectual disability and sASD

We measured the association between risk of intellectual disability (which we consider a marker of brain damage) and risk of sASD with the curve estimate module of the SPSS regression package. The relation was significant and highly linearly positive, with the quadratic curve fit explaining no additional covariance ($R^2 = .12$, $F_{(1,\ 1262)} = 170.8$, $p = 1.1^{E-36}$). See Fig. 1.

### 4.3. Epilepsy and sASD

We also measured the association between risk of epilepsy and risk of sASD with the curve estimate module of the SPSSv24 regression package. The relation was significant and linearly positive ($F_{(1,\ 1262)} = 9.5$, $p = .007$), with the quadratic curve fit explaining no additional covariance. However, the effect size was negligible (see Fig. 2).

### 4.4. Genetic transmission

We were able to identify predominantly or exclusively familial genetic transmission in only 14 of the 70-case series reviewed here (N = 178 cases). In the 56 other case series, direct recurrence was absent or rare (N = 1086). Because recurrence was not systematically investigated, and the associated neurological disorders were wildly heterogenous, no further tests of assumptions or hypotheses about genetic liability were feasible.

### 4.5. Tests of male overprevalence and of greater female neurologic protective effect (female overmorbidity) in sASD

#### 4.5.1. sASD-specific male overprevalence

In the 70 neuroetiopathologies (N = 1264 cases altogether), the male overprevalence (N = 667, 52.75%) was statistically significant (test of intercept of ANOVA with series weighted by sample size: $F_{(1,\ 1263)} = 42.4$, $p = 8.9^{E-8}$). This represents a male to female ratio of 1.1:1. In the cases with sASD (N = 470), there was a higher male overprevalence (58.85%) and significantly so ($p < 1^{E\text{-}36}$) giving a M:F ratio 1.44:1. In the cases without sASD (n = 794), the male overprevalence was lower (52.22%) but was still significant (p = .002).

Given that there is an imbalanced sex segregation, significant in patients with as well as without ASD, and differing significantly between the two, to test the idea according to which there is a greater risk of sASD in males in this particular data set, it is only the interaction Presence/absence of sASD x Sex and modulations thereof that will be of interest in subsequent analyses. Sex segregation will thus be referred to as "sASD-specific male overprevalence".

#### 4.5.2. Female neurologic protective effect (morbidity)

ANOVA was used to compare our assembly of 1264 male and female patients with and without sASD regarding neurological morbidity (intellectual disability and epilepsy). There was no significant difference in risk of intellectual disability between the males and females without sASD ($t_{(1,\ 1263)} = .86$, $p = .39$). However, it was the males with sASD that presented the highest prevalence of intellectual disability, significantly more so than the females with ASD ($t_{(1,\ 1263)} = 4.4$, $p = .00001$).

There was no significant difference in risk of epilepsy between the males and females without sASD ($t_{(1,\ 1263)} = .18$, $p = .18$). However, the females with sASD presented the highest prevalence of epilepsy (44%), which was higher than the males (38%). This difference was significant ($t_{(1,\ 1263)} = 5.0$, $p = 7.1^{E-7}$). In short, in these neurological patients, epilepsy afflicted female patients with sASD more than male patients with sASD.

#### 4.5.3. Intellectual disability and sASD-specific male overprevalence

The male/female prevalence x sASD/non-sASD interaction was formatted as the % difference between the male prevalence of the patients with sASD versus of the patients without sASD. "Risk of intellectual disability" was coded as a continuous variable (% of cases in the series with intellectual disability). To more deeply and accurately

**Table 1**
title. Overview of all neuroetiopathologies included in this study, classified by male prevalence (in %).

| Neuroetiopathology | % male prevalence | % ASD | % DI | % EPI | Sample size | Reference (Author, year) |
|---|---|---|---|---|---|---|
| Late onset infantile spasms | 83 | 50 | 86 | 100 | 18 | Bednarek et al., 1998 |
| Tuberous sclerosis | 82 | 64 | 70 | 50 | 32 | Gillberg et al., 1994 |
| 2p15-p16.1 microdeletion syndrome2p15-p16.1 microdeletion syndrome | 80 | 50 | 100 | 17 | 6 | Félix et al., 2010 |
| Congenital cerebellar malformation without epilepsy | 76 | 33 | 48 | 0 | 27 | Tavano et al., 2007 |
| Prenatal exposure to misoprostol | 73 | 30 | 100 | 0 | 23 | Bandim et al., 2003 |
| Whole autosomal microarray of intellectually deficiency, developmental delay or autism | 72 | 17 | 31 | 14 | 29 | Shin et al., 2015 |
| Ring 20 chromosome syndrome | 71 | 38 | 25 | 88 | 8 | Giardino et al., 2010 |
| Deletion of the long arm of chromosome 4 | 71 | 16 | 22 | 13 | 16 | Strehle et al., 2007 |
| 15q11-q13 duplication | 70 | 57 | 37 | 40 | 30 | Al Ageeli et al., 2014 |
| WAGR syndrome (Wilms tumor, aniridia, genitourinary malformations and mental retardation) usually caused by deletion of 11p14-p12 | 70 | 55 | 97 | 10 | 31 | Xu et al., 2008 |
| Fetal alcohol syndrome | 70 | 43 | 93 | 7 | 14 | Nanson et al., 1992 |
| Microduplications of 3p26.3p26.2 containing CRBN gene in patients with intellectual disability and behavior abnormalities | 69 | 50 | 100 | 25 | 4 | Papuc et al., 2015 |
| Inv dup (15) marker chromosome | 67 | 64 | 100 | 64 | 11 | Webb et al., 1994 |
| Mutation of DEPDC5 at 22q12.3 – q12.3, mTORopathy anhd epilepsy | 67 | 50 | 50 | 100 | 6 | Carvill et al., 2015 |
| Mutation of EXOC6B gene at 2p13.2 | 67 | 33 | 50 | 80 | 6 | Evers et al., 2014 |
| Autosomal rearrangements of parental descent | 67 | 33 | 47 | 20 | 30 | Battaglia et al., 2013 |
| Dravet syndrome with SCN1A mutation at 2q24.3 | 66 | 71 | 100 | 94 | 17 | Catarino et al., 2011 |
| Novel missense variants in the GAMT gene at 19p13.3 | 64 | 30 | 40 | 70 | 21 | Mercimek-Mahmutoglu et al., 2014 |
| Heterozygous exonic deletions of NRXN1 at 2p16.3 | 63 | 65 | 91 | 43 | 25 | Bena et al., 2013 |
| Autosome-wide scan in high mutation risk patients | 63 | 26 | 56 | 11 | 27 | Iourov et al., 2012 |
| Adenylosuccinate lyase deficiency (usually involving mutation of the ADSL gene at 22q13.2) | 62.5 | 50 | 70 | 65 | 20 | Ciardo et al., 2001 |
| Aberrant supracallosal longitudinal bundle | 61 | 13 | 53 | 13 | 15 | Arrigoni et al., 2016 |
| Autosomal deletions | 60 | 29 | 100 | 14 | 7 | Mikhail et al., 2011 |
| Leukocyte Adhesion Deficiency Type II | 59 | 57 | 100 | 86 | 7 | Gazit et al., 2010 |
| SETBP1 LoF variants 18q12.3 | 58 | 33 | 42 | 42 | 13 | Coe et al., 2014 |
| 14q11.2 microdeletion, involving CHD8 gene | 58 | 26 | 89 | 11 | 19 | Prontera et al., 2014 |
| Deletion at the Neurexin3 Locus NRXN3 at 14q24.3-q31.1 | 57 | 56 | 33 | 11 | 9 | Vaags et al., 2012 |
| Deletions of NRXN1 | 57 | 40 | 25 | 42 | 12 | Ching et al., 2010 |
| Cornelia de Lange syndrome (about half the cases involve deletion of the CDLS1 gene at 5p13.2) | 57 | 29 | 100 | 29 | 14 | Borck et al., 2004 |
| Mutation (mostly rearrangements) of CHD8 gene at 14q11.2 | 55 | 87 | 60 | 20 | 15 | Bernier et al., 2014 |
| Distal 22q11.2 Microduplications | 55 | 31 | 57 | 25 | 16 | Wincent et al., 2010 |
| 1q44-qter trisomy | 53 | 42 | 92 | 0 | 13 | Lenzini et al., 2009 |
| Mutations in MEF2C from the 5q14.3q15 Microdeletion | 53 | 39 | 100 | 89 | 18 | Zweier et al., 2010 |
| Mutation of 2q21.1, including the brain-specific ARHGEF4 and GPR148 genes | 53 | 30 | 50 | 30 | 10 | Dharmadhikari et al., 2012 |
| 3q29 deletion | 53 | 29 | 29 | 0 | 7 | Biamino et al., 2016 |
| Jacobsen syndrome | 52 | 55 | 89 | 0 | 20 | Akshoomoff et al., 2015 |
| Subtelomeric rearrangements at any chromosome in unexplained mental retardation | 50 | 45 | 100 | 36 | 11 | Anderlid et al., 2002 |
| SYNGAP1 gene mutation at 6p21.32 | 50 | 36 | 92 | 64 | 12 | Berryer et al., 2013 |
| 16p11.2 deletion | 50 | 35 | 65 | 18 | 32 | Bijlsma et al., 2009 |
| Microdeletion 15q11.2 between breakpoints 1 and 2 | 50 | 33 | 60 | 22 | 10 | Doornbos et al., 2009 |
| Xp11.22-p11.23 duplication | 50 | 30 | 60 | 18 | 15 | El-Hattab et al., 2011 |
| Nicolaides-Baraitser syndrome which usually involves rearrangement of the SMARCA2 – SWI/SNF related gene at 9p24.3 | 50 | 28 | 100 | 70 | 15 | Sousa et al., 2009 |
| De novo SCN1A mutation at 2q24.3 | 50 | 22 | 44 | 100 | 9 | Usluer et al., 2016 |
| Angelman syndrome in patients suspected of autists | 47 | 91 | 83 | 68 | 23 | Bonati et al., 2007 |
| 18q deletion | 46 | 29 | 79 | 21 | 14 | Linnankivi et al., 2003 |
| KIAA2022 mutation at Xq13.2 | 45 | 58 | 91 | 65 | 43 | Webster et al., 2017 |
| Tuberous sclerosis | 45 | 33 | 75 | 33 | 12 | Baker et al., 1998 |
| CACNA1A haploinsufficiency at 19p13 | 45 | 19 | 31 | 13 | 16 | Damaj et al., 2015 |
| TRIP12 mutation at 2q36.3 | 44 | 73 | 64 | 36 | 11 | Bramswig et al., 2017 |
| 17p13.3 microduplication syndrome encompassing the region of the Miller-Dieker syndrome (MDS) | 44 | 50 | 79 | 0 | 14 | Bruno et al., 2010 |
| Hypomelanosis of Ito | 44 | 10 | 57 | 49 | 76 | Pascual-Castroviejoa et al., 1998 |
| Mobius sequence | 43 | 56 | 32 | 8 | 25 | Johansson et al., 2001 |
| Neurexin gene (NRXN1) deletion involving exons at 2p16.3 | 43 | 27 | 11 | 11 | 37 | Dabell et al., 2013 |
| Fragile-X syndrome (with confirmed mutation at Xq27.3) | 43 | 25 | 86 | 75 | 28 | Wisniewski et al., 1985 |
| B-ureidopropionase deficiency | 43 | 7 | 29 | 36 | 28 | Nakajima et al., 2014 |
| Recurrent distal 7q11.23 deletion Including HIP1 and YWHAG | 42 | 7 | 33 | 47 | 30 | Ramocki et al., 2010 |
| 7q11.23 duplication | 40 | 43 | 83 | 14 | 14 | Van der Aa et al., 2009 |
| X-chromosome general screen in genetics candidates | 40 | 22 | 67 | 22 | 9 | Bartnik et al., 2014 |
| SCN8A mutation at 12q13.13 and encephalopathy | 35 | 18 | 94 | 100 | 17 | Larsen et al., 2015 |
| Duplication Xp11.22-p14 | 34 | 15 | 65 | 50 | 26 | Evers et al., 2015 |
| Whole chromosome array on X-chromosome | 33 | 50 | 58 | 14 | 12 | Iourov et al., 2012 |
| SYNGAP1 mutation at 6p21.32 | 33 | 47 | 100 | 100 | 17 | Mignot et al., 2016 |
| DYRK1A-associated ID at 21q22.13 – 21q22.2 | 33 | 40 | 100 | 80 | 10 | Bronicki et al., 2015 |
| AUTS2 deletion at 7q11.22 | 29 | 56 | 100 | 33 | 12 | Jolley et al., 2013 |

(*continued on next page*)

**Table 1** (*continued*)

| Neuroetiopathology | % male prevalence | % ASD | % DI | % EPI | Sample size | Reference (Author, year) |
|---|---|---|---|---|---|---|
| Inverted duplication of chromosome 15 | 27 | 40 | 100 | 20 | 6 | Wandstrat et al., 1998 |
| Kleefstra syndrome (9q subtelomeric deletion syndrome (9qSTDS-15q)) | 27 | 9 | 100 | 32 | 22 | Kleefstra et al., 2009 |
| CREBBP mutations 16p13.3 in Individuals withoutRubinstein–Taybi Syndrome Phenotype | 25 | 67 | 89 | 27 | 11 | Menke et al., 2016 |
| 5q14.3q15 microdeletion syndrome (including MEF2C point mutation) | 25 | 47 | 100 | 89 | 17 | Zweier et al., 2010 |
| STAG1 mutation at 3q22.3 | 25 | 41 | 65 | 47 | 17 | Lehalle et al., 2017 |
| Congenital brain dysplasia with catastrophic epilepsy | 19 | 12 | 88 | 100 | 17 | Lettori et al., 2008 |

*In this table:* All neuroetiopathologies used in this study are shown above by decreasing male prevalence. The percentage of ASD, ID and epilepsy associated with every case series is also presented. *Note:* All additional data regarding these 70 cases series are in the Supplementary materials # 1. References are in Supplementary materials # 2. References are in Supplementary materials

**Table 2**
Recurrence rates of comorbid sASD in various neurodevelopmental syndromes.

| Syndrome or condition | Recurrence rate of ASD | Reference (Author, year) |
|---|---|---|
| Nicolaides-Baraitser syndrome | 0% | Sousa et al., 2014 |
| Cornelia de Lange syndrome | .9% | Jackson et al., 1993 |
| Tuberous sclerosis | 1.5% | Roach et al., 1998 |
| Mobius syndrome | 2% | MacDermot et al., 1991 |
| Dravet syndrome caused by SNC1 mutation | 11% | Depienne et al., 2010 |
| Fragile-X syndrome | 23% | Sherman et al., 1985 |
| Angelman syndrome caused by cis-acting IC deletion | 50% | Reis et al., 1994 |

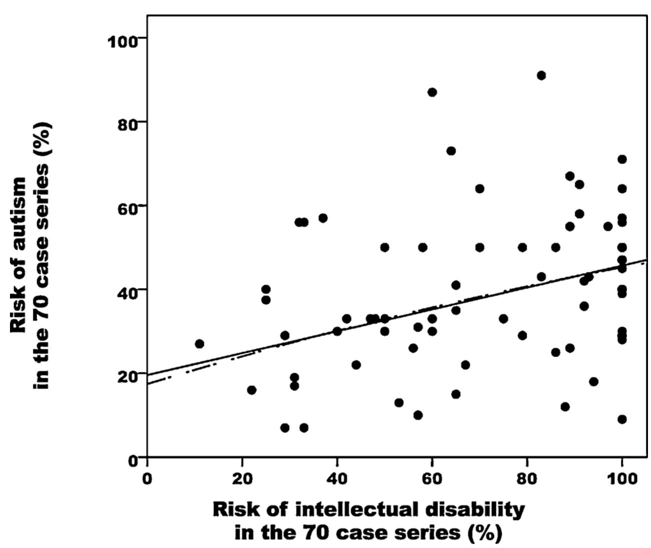


**Fig. 1.** Association between risk of sASD and risk of intellectual disability in patients with neurodevelopmental syndromes or conditions.
The continuous trait represents the significant linear relation ($R^2$ = .12, $F_{(1, 1262)}$ = 170.8, p = $1.1^{E-36}$) between the risk of intellectual disability (on the X-axis) and the risk of sASD (on the Y-axis). The dot/line trait curve represents the quadratic curve fit, but here, it explains no additional covariance. Linear (continuous trait) and quadratic (dot/line trait) curve fits of the association between risk of autism and risk of intellectual disability were generated by the "curb estimation" module of the Regression package of SPSSv24. The 70 data points are the case series, but note that the data are weighted for sample size (total N = 1264).

characterize the relation between sex and intellectual disability, we used the curve fit module of the SPSSv24 regression package which revealed that the linear trend was positive and significant ($R^2$ = .016, $F_{(1, 1262)}$ = 20.4, p = .000007). In addition, the dominant trend was neither linear nor cubic, it was radically quadratic ($R^2$ = .21, $F_{(1, 1261)}$ = 168.3, p = $1.7^{E-65}$) suggesting that one or several hidden variables are modulating the relation. In other words, things are more complex, at least in sASD, than a straightforward male overprevalence and refutation of the idea of a female overmorbidity (Robinson et al., 2013). To be clear, Robinson et al and the many other protagonists of the female ASD overmorbidity idea have all focussed on iASD. Here we do not contest that specific point of view. We merely state that we do not observe this phenomenon here in our cohort of *secondary* ASD, rather the contrary. See Fig. 3 for depiction of the linear and quadratic trends.

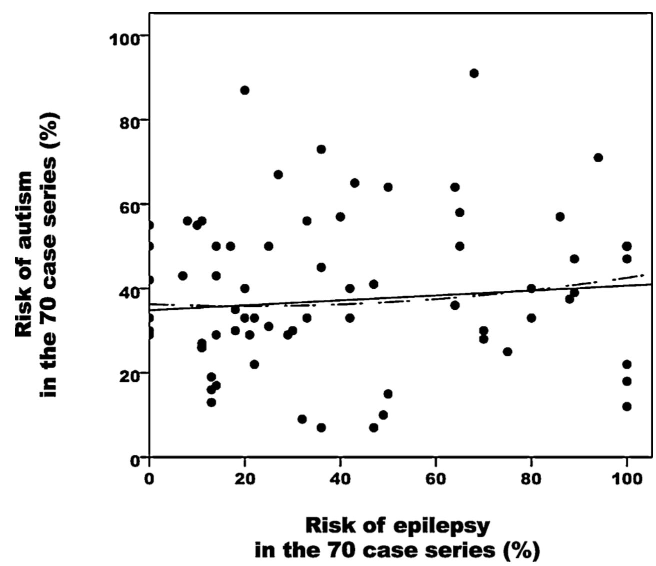


**Fig. 2.** Association between risk of sASD and risk of epilepsy in patients with neurodevelopmental syndromes or conditions.
The continuous trait represents the significant linear relation ($F_{(1, 1262)}$ = 9.5, p = .007) between the risk of epilepsy (on the X-axis) and the risk of sASD (on the Y-axis). The dot/line trait curve represents the quadratic curve fit, but here, it explains no additional covariance. Linear (continuous trait) and quadratic (dot/line trait) curve fits of the association between risk of epilepsy and risk of sASD were generated by the "curb estimation" module of the Regression package of SPSSv24. The 70 data points are the case series, but note that the data are weighted for sample size (total N = 1264).

#### 4.5.4. *Epilepsy and sASD-specific male overprevalence*

The male/female prevalence x sASD/non-sASD interaction was formatted as the % difference between the male prevalence of the patients with sASD versus the patients without sASD. "Risk of epilepsy" was coded as a continuous variable (% of cases in the series with epilepsy). In regression analysis of the relation between the two the linear trend was negative, but it was not statistically significant. The quadratic trend did however reach significance ($R^2$ = .08, $F_{(1, 1261)}$ = 53.2, p = $6.3^{E-23}$). The quadratic nature of the relation again suggests that

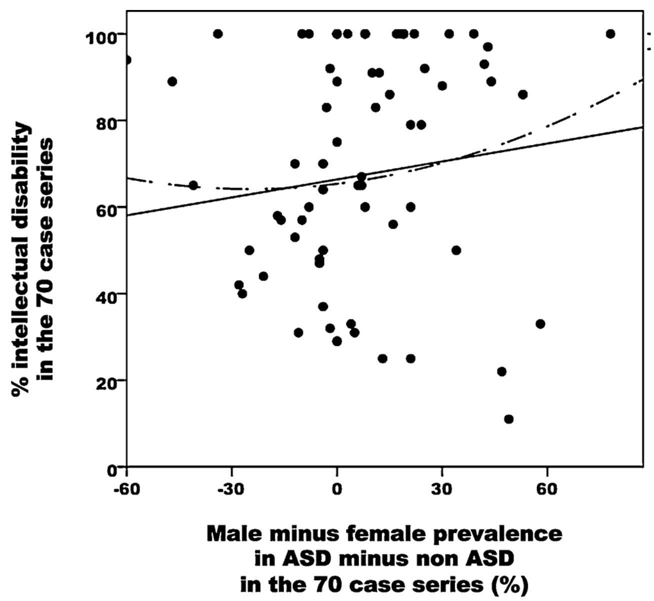


**Fig. 3.** Association between intellectual disability and sASD-specific male overprevalence.
The continuous trait represents the significant linear relation ($R^2$ = .016, $F_{(1, 1262)}$ = 20.4, p = .000007) between the % difference between the male prevalence of the patients with sASD versus of the patients without ASD. (on the X-axis) and the risk of intellectual disability (on the Y-axis). The dot/line trait curve represents the quadratic curve fit, which here is the dominant feature ($R^2$ = .21, $F_{(1, 1261)}$ = 168.3, p = $1.7^{E-65}$) and suggest that at least one hidden variable is modulating the relation. Linear (continuous trait) and quadratic (dot/line trait) curve fits of the association between sASD-specific male overprevalence and risk of intellectual disability were generated by the "curb estimation module of the Regression package of SPSSv24. The 70 data points are the case series, but note that the data are weighted for sample size (total N = 1264).

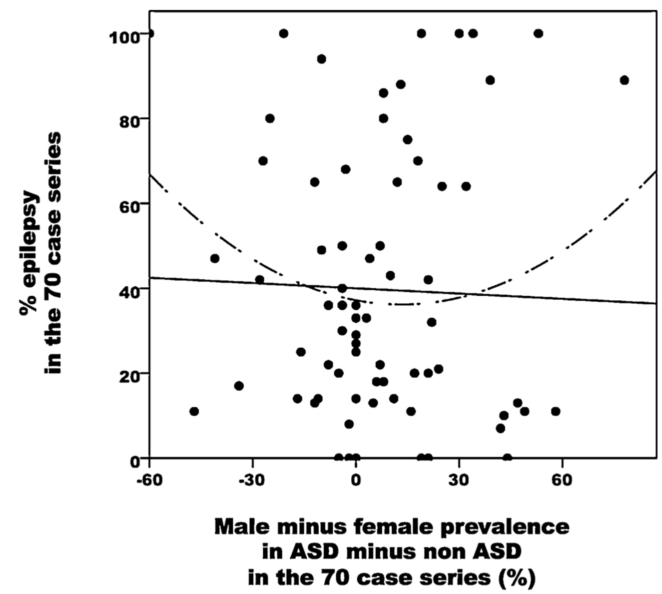


**Fig. 4.** Association between risk of epilepsy and sASD-specific male overprevalence.
The continuous trait represents the lightly negative and non-significant linear relation between the % difference between the male prevalence of the patients with sASD versus of the patients without ASD. (on the X-axis) and the risk of epilepsy (on the Y-axis). The dot/line trait curve represents the quadratic curve fit, which here is the most significant feature ($R^2$ = .08, $F_{(1, 1261)}$ = 53.2, p = $6.3^{E-23}$) and suggests that at least one hidden variable is modulating the relation. Linear (continuous trait) and quadratic (dot/line trait) curve fits of the association between sASD-specific male overprevalence and risk of epilepsy were generated by the "curb estimation" module of the Regression package of SPSSv24. The 70 data points are the case series, but note that the data are weighted for sample size (total N = 1264).

sex-specific morbidity in this sASD cohort is modulated by at least one important "unknown" variable. Altogether, the weak link between risk of autism and risk of epilepsy (Fig. 2) and the insignificance of the linear relation between sASD-specific male overprevalence and epilepsy (Fig. 4) inspire great caution in concluding that epilepsy explains sex segregation in sASD, in any unequivocal manner. See Fig. 4.

*4.5.5. Global risk of autism and sASD-specific male overprevalence*

The SPSSv24 curve fit module revealed that sASD-specific male overprevalence related positively and linearly to the proportion of patients with sASD overall ($R^2$ = .02, F = 28.0, p = $1.4^{E-7}$). In simpler terms, neurological conditions with high risk of autism comprised more males (See Fig. 5).

### *4.6. Global male overprevalence in the neurological conditions and sASD-specific male overprevalence*

A test with the SPSSv24 curve fit module indicated a trend to the effect that the sASD-specific male overprevalence was negatively linearly correlated with proportion of male patients in the overall dataset ($R^2$ = .003, F = 3.4, p = .064). However, the quadratic trend explained much more variance ($R^2$ = .07, F = 46.2, p = $4.2^{E-20}$). See Fig. 6. One or several unknown variables appear to be at play.

It is notable that in the curve fits depicted in Figs. 3 to 6, there are two outstandingly quadratic functions, and that the two are of opposite valence. sASD-specific male overprevalence holds a "valley-shaped" quadratic relation to epilepsy (Fig. 4) and male overprevalence holds a "mountain shaped" quadratic relation to global male overprevalence in the neurological condition of opposite valence (Fig. 6). Several genetic syndromes with very high or very low risk of epilepsy dramatically segregate by sex (see Tables 1 and 2) and these segregations fold over onto ASD in an equivocal manner. We recommend caution in interpreting interactions between sex, risk of epilepsy and risk of autism in sASD. With regard to intellectual disability, it clearly refutes the "female overmorbidity" idea as far as sASD is concerned and it does so unequivocally, i.e., linearly. However, the fact that the quadratic relation between risk of intellectual disability and sASD-specific male overprevalence (Fig. 3) is much more strongly quadratic also suggests that there is nothing simple in sex differences in our sASD cohort. The relations observed above obviously require further investigation.

### *4.7. Ascertainment bias and sASD-specific male overprevalence*

sASD-specific male overprevalence was significantly and positively associated with "ascertainment bias". sASD-specific male overprevalence was higher in the cases series in which ASD was one of the initial recruitment criteria, i.e. those case series most likely to be subject to ascertainment bias. There were 30 case series with this "higher" risk of ascertainment bias (N = 492). There were 40 case series without this risk (N = 772). In the first group, sASD-specific male overprevalence (weighted mean = 8.9%) was greater than in the second group (weighted mean = 3.7%). The Levene test revealed that the within variances of each two groups differed significantly. We tested the difference between the means with the SPSSv24 procedure adjusted for unequal within variances which indicated that the difference was significant ($t_{(1, 1235)}$ = 4.0, p = .00006, partial $eta^2$ = .01).

## 5. Discussion

The male sASD patients had a significantly higher prevalence of intellectual disability than the females with sASD. Though prevalence

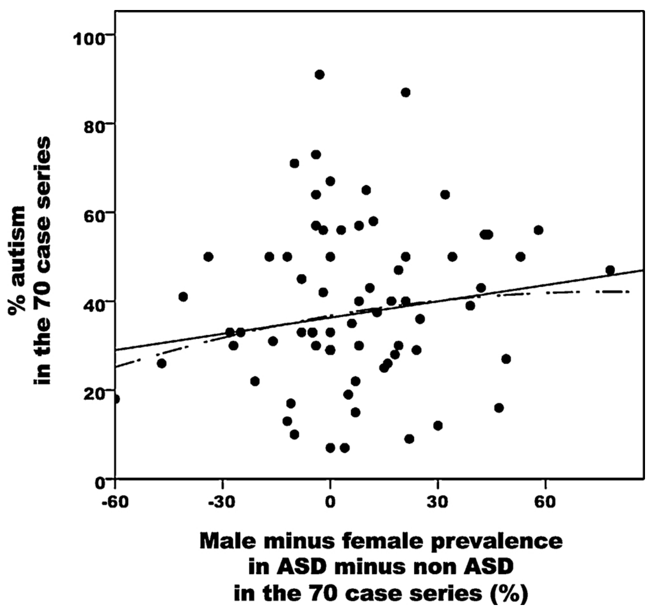


**Fig. 5.** Association between global risk of ASD and sASD-specific male overprevalence.
The continuous trait represents the lightly positive and-significant linear relation ($R^2$ = .02, F = 28.0, p = $1.4^{E-7}$) between the % difference between the male prevalence of the patients with sASD versus of the patients without ASD. (on the X-axis) and the risk of ASD (on the Y-axis). The dot/line trait curve represents the quadratic curve fit, but here, it explains no additional covariance. Linear (continuous trait) and quadratic (dot/line trait) curve fits of the association between sASD-specific male overprevalence and risk of ASD were generated by the "curb estimation" module of the Regression package of SPSSv24. The 70 data points are the case series, but note that the data are weighted for sample size (total N = 1264).

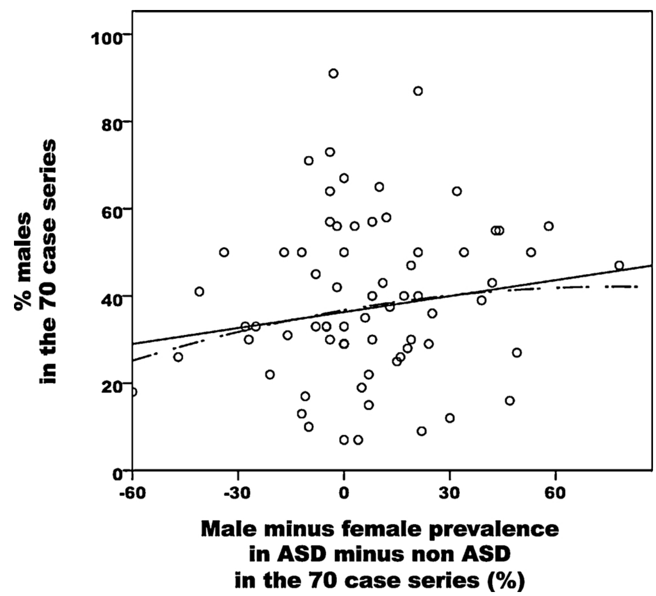


**Fig. 6.** Association between global male overprevalence and sASD-specific male overprevalence.
The continuous trait represents the lightly negative and-significant linear relation ($R^2$ = .003, F = 3.4, p = .064) between the sASD specific male overprevalence (on the X-axis) and the global male prevalence (on the Y-axis). The dot/line trait curve represents the quadratic curve fit, but here, it explains more variance ($R^2$ = .07, F = 46.2, p = $4.2^{E-20}$) and suggest that at least one hidden variable is modulating the relation. Linear (continuous trait) and quadratic (dot/line trait) curve fits of the association between sASD-specific male overprevalence and global male prevalence were generated by the "curb estimation" module of the Regression package of SPSSv24. The 70 data points are the case series, but note that the data are weighted for sample size (total N = 1264).

of epilepsy was higher in females with sASD than males with sASD, the series wide association between risk of epilepsy and risk of sASD was ambiguous, whereas with intellectual disability it was strong and relatively unequivocal (i.e., linear). As in the study of Polyak, Rosenfeld, and Girirajan, (2015), we find that there are more female-prevalent epilepsies carrying high risk of ASD than there are male-prevalent epilepsies carrying such high risk of ASD. This does not mean that epilepsy directly causes any sASD of itself. There is no evidence that it does (see Table 1, Fig. 2 and the Supplementary materials #1 for further evidence to that effect). However, our finding of higher risk of intellectual disability in male sASD than in female sASD argues against the so-called "female protective effect" so often seen in iASD.

Additionally, further challenging the idea of an endogenous "female protective effect" explaining fewer females with ASD, we found that ascertainment bias was a significant source of sASD-specific male overprevalence. We are not aware of any other research that has operationalized "ascertainment bias" as was done here. The ADI ascertainment scale is not particularly sex biased (see the introduction). We propose that when this scale is the only measure used to qualify a case as sASD or not sASD, there is probably little ascertainment bias. Accordingly, we propose that it is in the run of the mill settings (schools, psychiatric consultation, predominance of idiopathic cases, high functioning autism) that diagnoses are selected on all kinds of bases, beyond ADI or ADOS scores, including the benevolent intention of maximally helping the patient. We suspect that clinicians may be attracted to a diagnosis of emotional disorder in girls over a diagnosis of autism because the former is perceived as chemically treatable, not the latter. It is in these contexts, we think, that sex bias (ascertainment bias) probably operates most.

We feel compelled by our findings to alert the research community to the importance of 1) developing quantitative assessment tools that carefully exclude items which feed into cultural gender-stereotypes, 2) developing assessment tools for ASD that are normed separately for each sex, 3) not relying on diagnoses of ASD coming from other settings for neurological investigation, i.e., neurologists and geneticists should use quantitative measures of ASD in their own research, 4) becoming more mindful of and concerned about the potentially unfair current risk of depriving one of the sexes of proper clinical care and management of ASD.

### *5.1. Strengths and weaknesses of the methodology of the current investigation*

The large sample of individual cases assembled for this study is its greatest strength. It gave us the power to test competing accounts. The reader is however cautioned that there are limitations and shortcomings of the methodology adopted here and of the sample described. Many published case series of various central nervous system pathologies would have been added to our sample had we not required at least one autistic and one non-autistic female in each case series (i.e., in each published report). Indeed, many such case series of sASD report quite small samples, and these often do not include one or the other type of female patient required here (autistic and non-autistic). This selection criterion alone was most likely an important cause of the reduced male to female ratio observed in the present study. However, our sampling of sASD limited to cases screened for ID and epilepsy further deeply whittled down and displaced sampling as well. We remind the reader that the sample analyzed here is not representative of sASD in general.

When there was a small proportion of missing information concerning one of the obligatory variables (presence/absence of ASD, epilepsy, ID, and sex) in a given case, we did not exclude the case, we adjusted the percentages of the case series in order to maintain maximum sampling. When weighting these series and converting them into percentages, there were of course no missing data. Our weighting procedure thus could have slightly distorted sample size. It also reduced within group variance, thereby inflating results of inference tests. The reader is thus advised to read our results more with a "comparative" or "valence" mindset than a focus on the absolute significance of any particular inference test.

### 5.2. In conclusion

Finally, we extend the finding of Polyak et al. (2015) that there is a moderate male overprevalence in sASD. Also, though we found that sASD-specific male overprevalence was associated with ascertainment bias, we also found that biological factors are equally robustly associated. In this, we agree with the vast majority of current researchers on this topic that male overprevalence of ASD certainly exists, that it is overarching (present in sASD as well as in iASD), and that there are important bioendogenous factors involved. This leads us to speculate about why more male than female patients with neurodevelopmental syndromes and conditions receive a diagnosis of sASD. It seems there are two reasons, a bioendogenous susceptibility of male development to ASD and ascertainment bias.

This suggests that male overprevalence in idiopathic ASD is caused by one or several early subtle universal modulators of the development of brain organization. The perinatal brain/hormone interface would appear as a good candidate. This modulator or set of modulators is probably released or activated in some of the severe neurodevelopmental disorders but is probably not a dominant factor pressing for autism in most. More precisely, this hormonal modulating variable is probably more causative of autism in iASD than in sASD where other brain stressors related to intellectual disability are more at play.

Note. This research was done without funding nor honoraria. There is no conflict of interest. No humans or animals were used. The case reports used as data were all processed confidentially and anonymously.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.npbr.2019.03.003.

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