

No increase in autism-associated genetic events in children conceived by assisted reproduction

Sean Ackerman, M.D.,^a Julia Wenegrat, M.A.,^b David Rettew, M.D.,^a Robert Althoff, M.D., Ph.D.,^a and Raphael Bernier, Ph.D.^b

^a Department of Psychiatry, School of Medicine, University of Vermont, Burlington, Vermont; and ^b Department of Psychiatry and Behavioral Sciences, School of Medicine, University of Washington, Seattle, Washington

Objective: To understand the rate of genetic events in patients with autism spectrum disorder (ASD) who were exposed to assisted reproduction.

Design: Case control study using genetics data.

Setting: Twelve collaborating data collection sites across North America as part of the Simons Simplex Collection.

Patient(s): 2,760 children with ASD, for whom 1,994 had published copy number variation data and 424 had published gene mutation status available.

Intervention(s): None.

Main Outcome Measure(s): Rates of autism-associated genetic events in children with ASD conceived with assisted reproduction versus those conceived naturally.

Result(s): No statistically significant differences in copy number variations or autism-associated gene-disrupting events were found when comparing ASD patients exposed to assisted reproduction with those not exposed to assisted reproduction.

Conclusion(s): This is the first large genetic association to concurrently examine the genotype of individuals with ASD in relation to their exposure to ART versus natural conception, and it adds reassuring evidence to the argument that ART does not increase the risk of ASD. (Fertil Steril® 2014;102:388–93. ©2014 by American Society for Reproductive Medicine.)

Key Words: Assisted reproduction, assisted reproductive technology, autism, copy number variation

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Autism spectrum disorder (ASD) is a neuropsychiatric condition that includes impairments in social interaction and communication as well as restricted or stereotyped behavior (1). The prevalence of ASD in U.S. children aged 6 to 17 years is estimated to be as high as 1 in 50 from recent data (2). The etiology of ASD is unknown; however, there is strong evidence of significant genetic influence,

with hundreds of genes implicated, each of which generally conveys a slight increase in risk (3, 4). Autism risk is also elevated after environmental insult including in utero exposure to infections, toxins, and birth complications among others (5–7). More recently, epigenetic factors have been proposed as playing a significant role in the etiology of ASD, highlighting the contribution of

both genetic and environmental factors (8, 9). Given the building evidence for the interplay of genetics and environment in the etiology of ASD, it is important to consider whether certain medical interventions may also play a role.

Assisted reproduction is a broad term referring to various interventions primarily used to treat infertility, including but not limited to fertility medications, in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and artificial insemination. Assisted reproductive technology (ART) as defined by the Centers for Disease Control and Prevention, is a more specific term, only including IVF, GIFT, and ZIFT. These three treatments alone

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Reprint requests: Sean Ackerman, M.D., University of Vermont, Fletcher Allen Health Care, 111 Colchester Avenue, MS #341BA1, Burlington, Vermont 05401 (E-mail: sean.ackerman@vtmednet.org).

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accounted for 163,038 ART cycles resulting in 47,090 live births in 2011 (10).

As ART has become increasingly common, there have been concomitant concerns regarding the developmental outcomes of these pregnancies. Fortunately, major reviews and individual studies of ART procedures have been overall reassuring (11, 12). In general, neurodevelopmental outcomes of children conceived by ART seem to be comparable to outcomes in spontaneously conceived children (13). A number of studies have demonstrated that neither intracytoplasmic sperm injection (ICSI) or IVF are associated with cognitive disorders, developmental delays, or psychological/behavioral issues (14–21). Other studies have disputed the possibility of an association between IVF and a higher rate of karyotype abnormalities (22, 23).

Despite the positive outlook from these broad outcome measures, a number of specific differences have been observed between the outcomes of pregnancies conceived spontaneously versus those with ART. There have been suggestions that ART may affect genetic events that occur during ovulation or fertilization, such as with imprinted genes. Of the nine known imprinting syndromes, ART has been associated with two: Beckwith-Wiedemann syndrome and Silver-Russell syndrome (24). Moreover, it has been reported that the total risk of congenital malformations may be increased by about one-third through ART, with approximately a twofold increased risk of nervous systems defects (25). Pregnancies from IVF are also at higher risk for low birth weight and preterm birth compared with pregnancies conceived spontaneously (26–28). There are also other differences between IVF pregnancies and spontaneously conceived pregnancies, the most well known of which has been the increased multiple gestation rates (29). Specifically in regards to ICSI, there has been some recent evidence that ICSI is associated with mental retardation (30).

Various studies, primarily epidemiologic, have looked at the association between ASD and ART (30–35). Overall, the available data have not found a clear association between ASD and ART, but the results have not been entirely uniform. A 2009 meta-analysis looked at eight studies that examined possible relations between ART and ASD (35). The investigators noted methodological problems in the available studies as well as inconsistent results, and concluded that “possible associations with ASD need assessment in larger studies.” One recently published study concluded that ART was a risk factor for ASD (33), but another study claimed the opposite (34). However, the investigators in the latter study noted that the apparent increase in risk was due to confounding factors such as maternal age, educational level, parity, smoking, birth weight, and multiplicity. Another recent epidemiologic study documented similar findings, concluding that the risk between ASD and IVF disappeared once the analyses had been adjusted (31). It may be particularly important to adjust for parental characteristics because users of ART are more likely than fertile individuals both to be of increased age and to have chromosomal abnormalities (20). Furthermore, recent studies have shown that older paternal age is associated with autism-associated *de novo* copy number variation (CNV) (36, 37).

Perhaps most notably, a recent systematic review of many of the observational studies regarding ASD and ART concluded that there is no evidence that ART is significantly associated with ASD (38). Importantly, it should be pointed out that the findings tying ART to imprinting disorders seem particularly relevant in the context of ASD because imprinting disorders involve aberrant methylation (an epigenetic change in the quantity of methyl groups attached at certain sites in the genome) and aberrant methylation has been implicated in ASD (39). Finally, although there has been specific attention to ASD and ART, the broader question of whether assisted reproduction in general is associated with ASD has also been raised (32).

Although these studies have proven informative, no study to date has concurrently examined the genotype of individuals with ASD in relation to their exposure to ART versus natural conception. Given the concerns regarding the effect of ART on pregnancy outcomes, including genetic abnormalities, and in the context of the identified genetic contributions to ASD risk, it is important to look more specifically at the autism-associated genetic anomalies in ASD patients exposed to assisted reproduction. Therefore, we examined the relation between assisted reproduction and genetic mutations in children with ASD.

MATERIALS AND METHODS

Participants were 2,760 4- to 18-year-old children with ASD and their families who previously had participated in the Simons Simplex Collection (SSC). The SSC, a project funded by the Simons Foundation Autism Research Initiative to identify *de novo* genetic variants related to ASD, includes 12 collaborating data collection sites across North America (for a description of the ascertainment, data collection, and validation procedures for the sample, see <http://sfari.org>) (40). Approval was obtained from each local institutional review board. All participants completed informed consent before participation in the study. As part of SSC study participation, experienced clinicians confirmed the ASD diagnosis using the Autism Diagnostic Interview, Autism Diagnostic Observation Schedule, and expert clinical judgment according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria (41).

Information regarding assisted reproduction was collected through a clinician-administered, semistructured caregiver interview designed to gather data regarding early medical history for children and families participating in the SSC. Clinicians administering the interview were primarily specialists in mental health, not obstetrics. Specific to this study, caregivers were asked whether assisted reproduction had been used to initiate the pregnancy of the proband or siblings. If the caregiver responded yes, data regarding what type of assisted reproduction was collected. The options were oral fertility medications, injected fertility medications, artificial insemination, IVF, assisted hatching, frozen embryo, frozen eggs, sperm donor, egg donor, ICSI, GIFT, ZIFT, surrogacy, unknown, and other. Additionally, the maternal and paternal age at the time of conception was gathered.

Genotypic data regarding each participating family were collated from previously published studies identifying autism-associated CNV and putative causal ASD gene mutations (4, 42–44). For the purposes of this study, two categories of genetic data were examined, autism-associated CNV and autism-associated gene-disrupting events. Autism-associated gene-disrupting events were defined as gene-truncating loss-of-function mutations identified through exome sequencing.

In the sample of 2,760 SSC families with ASD, information regarding assisted reproduction history was available for 2,418 (87.6%) of the children with ASD. Of this subsample, 1,994 had published CNV information available, and 424 had published gene mutation status yielded through exome sequencing. Analyses were conducted with the subset of individuals with CNV and gene mutation data separately.

Genetic data was not available for all patients primarily due to the limits of the cost of the genetic analysis. The participants were selected for genetic analysis out of the larger pool by authors who had previously published their results with the SSC data set. These authors used a variety of criteria to select the patients they believed would most likely provide valuable genetic information. The criteria included low nonverbal intelligence, multiple unaffected siblings, and known multigenic CNV (43). Some patients were selected randomly as well.

A chi-square goodness of fit test was used to compare the rates of autism-associated genetic events in children with ASD conceived via assisted reproduction versus those conceived spontaneously. Analysis of variance (ANOVA) was used to examine relations between assisted reproduction use and genetic events (either CNV or gene mutation). Logistic regression was used to examine relations between genetic events and parental age.

RESULTS

As shown in Table 1, in the sample of 1,994 individuals with ASD, 122 were the product of assisted reproduction. Demographics are not listed in the table but are as follows: 264 female, 1,730 male; 1,567 white, 84 Asian, 76 African American, 5 Native American, 157 more than one race, 90 other, 15 not specified. The percentage of autism-associated CNVs in ASD patients exposed to ART was 9.8%, compared with 10.3% in ASD patients not exposed to assisted reproduction, a nonsignificant difference [chi-square (1, $N = 1994$) = .02, $P < .88$]. Of the 424 patients with available gene mutation status, 24 were the result of assisted reproduction. The percentage of autism-associated gene-disrupting events in ASD patients exposed to assisted reproduction was 58.3%, compared with 51.3% in ASD patients not exposed to assisted reproduction, here again indicating no statistically significant differences in mutation rate [chi-square (1, $N = 219$) = .45, $P < .50$].

Despite the statistically nonsignificant results, given the 7% difference of autism-associated gene-disrupting events in ASD patients exposed to assisted reproduction versus ASD patients not exposed to assisted reproduction, a power analysis was run. Using G*Power 3.13 for a 1 degree of freedom test and assuming an effect size of $w = 0.14$ (as

TABLE 1

Numbers and rates of genetic events.

	With event	Without event	Rate (%)
Autism associated CNVs			
Without assisted reproduction	192	1,680	10.3
With assisted reproduction	12	110	9.8
IVF	2	24	7.7
GIFT	0	0	NA
ZIFT	0	0	NA
ICSI	0	4	NA
Artificial insemination	2	22	8.3
Fertility medication only	8	60	11.8
Autism associated gene disrupting events			
Without assisted reproduction	205	195	51.3
With assisted reproduction	14	10	58.3
IVF	3	1	75.0
GIFT	0	0	NA
ZIFT	0	0	NA
ICSI	0	0	NA
Artificial insemination	3	1	75.0
Fertility medication only	8	8	50.0

Note: CNV = copy number variation; IVF = in vitro fertilization; GIFT = gamete intrafallopian transfer; NA = not applicable; ZIFT = zygote intrafallopian transfer; ICSI = intracytoplasmic sperm injection.

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determined by the 7% difference in the observed sample) with $\alpha = 0.05$ an observed power of 0.83 was calculated to detect this difference using the chi-square analysis. Thus, there was adequate power to have detected a significant difference had there been one.

Importantly, because assisted reproduction is a broad category of interventions, Table 1 also presents numbers of autism-associated CNVs and gene-disrupting events according to the following types of assisted reproduction: IVF (including with cryopreservation), GIFT, ZIFT, ICSI, artificial insemination, and fertility medication only. These numbers did not in general reach sufficient power for statistical analysis, but the genetic mutations appeared to be evenly distributed throughout the types of assisted reproduction.

Tables 2 and 3 present detailed information regarding all autism-associated genetic events identified in the ASD group exposed to assisted reproduction, along with descriptors such as parental ages, pregnancy number, and infertility reason. In Table 2, out of 12 patients with identified CNVs, 16 different regions were affected—none more than once—across 11 different chromosomes. In Table 3, out of 14 patients with genetic events, 23 genes were affected—none more than once—across 13 chromosomes.

Results from the ANOVA in the subsample of individuals with CNV status indicated that assisted reproduction usage was associated with increased paternal ($P < .000017$) and maternal ($P < .000014$) age. Results from the ANOVA in the subsample of individuals with gene mutation status indicated a somewhat different pattern. No relation between assisted reproduction usage and paternal age was observed ($P = .13$) in this subsample, but a trend was observed suggesting that mothers of children with autism-associated gene-disrupting events using assisted reproduction were older than mothers not using assisted reproduction ($P = .06$).

TABLE 2

Specific copy number variation and subject descriptors.

Subject ID ^a	Event	Region ^b	Inheritance	Paternal age (mo)	Maternal age (mo)	Pregnancy number	Multiple birth	Infertility reason
12130.p1	Dup	5q22.2	Paternal	550	492	2	Yes	Unexplained
13335.p1	Del	8p22	Maternal	533	493	2	Yes	Unexplained
	Dup	16p11.2	De novo					
11121.p1	Dup	3q23	Paternal	569	534	2	No	Absent ovulation
	Del	17p13.3	Paternal					
11154.p1	Dup	7p22.1	Paternal	372	388	2	No	Endometriosis
11415.p1	Dup	7q11.23	De novo	388	466	2	No	Blocked fallopian tubes
13784.p1	Dup	3p21.31	Paternal	522	419	1	No	Unexplained
13494.p1	Dup	12p11.23	De novo	372	345	1	No	Absent ovulation
	Del	10q22.2	Maternal					
11660.p1	Del	18q23	Paternal	546	472	3	No	Unexplained
	Del	2q13	Maternal					
11184.p1	Del	16q23.2	Paternal	295	267	1	No	Absent ovulation
14074.p1	Del	15q13.1	Paternal	395	366	1	No	Blocked fallopian tubes
12132.p1	Del	15q24.1	Paternal	395	350	1	No	Absent ovulation
13215.p1	Del	16p13.11	Paternal	370	316	2	No	Polycystic ovary syndrome

^a Simons Simplex Collection (SSC) subject ID number.

^b These data are from previously published data (42).

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Results from the logistic regression indicated that CNV status was not associated with paternal ($P=.97$) or maternal age ($P=.73$). However, results from the logistic regression in the subsample of individuals with gene mutation status indicated a different pattern. Although paternal age again did not reach statistical significance ($P=.56$), mothers of children with autism-associated gene-disrupting events were older than mothers of children without autism-associated gene-disrupting events ($P=.03$). This association is illustrated in Figure 1, with use of assisted reproduction included in the figure so as to demonstrate the impact of maternal age regardless of assisted reproduction.

DISCUSSION

Our study is the first to examine the genotype of patients with ASD who were conceived by assisted reproduction versus a control group in a relatively large sample. The purpose of this study was to examine the relations between assisted reproduction and genetic abnormalities in patients with ASD. Our results did not show an increased rate of autism-associated CNVs or autism-associated gene-disrupting events in children with ASD who were conceived by assisted reproduction versus children with ASD who had no contact with assisted reproduction. Further, autism-associated CNVs and

TABLE 3

Specific genetic events and subject descriptors.

Subject ID ^a	Chromosome	Gene ^{b,c}	Paternal age (mo) ^d	Maternal age (mo) ^d	Pregnancy number	Multiple birth	Infertility reason
11184.p1	19	HDGFRP2	431	324	1	No	Absent ovulation
11246.p1	2	ARMC9	421	375	1	No	Male factor
11610.p1	2	HDLBP DNAH5	371	332	4	No	Unexplained
	5						
11660.p1	1, 19	NTNG1, CACNA1A	546	472	3	No	Unexplained
11828.p1	7	TRRAP	381	371	1	Yes	Unexplained
12130.p1	20	ADNP	550	492	2	Yes	Unexplained
12603.p1	1, 1, 12	DDI2 L1TD1 C12orf72	448	453	2	Yes	Unexplained
13335.p1	11, 19	ACP2 ZNF420	533	493	2	Yes	Unexplained
13494.p1	10	NOLC1	372	345	1	No	Absent ovulation
13742.p1	12, 17	CELA1 MYH10	348	344	2	No	Absent ovulation
12375.p1	9	C9orf144B	566	388	4	No	Endometriosis
13000.p1	6	SLC17A3	358	332	2	No	Unexplained
13739.p1	11, 11	CD151	458	455	4	Yes	Low hormone level
		MARK2					
13392.p1	15, 17, 17	ITGA11 SLFN5 TUBG1	362	366	2	No	Unexplained

^a Simons Simplex Collection (SSC) subject ID number.

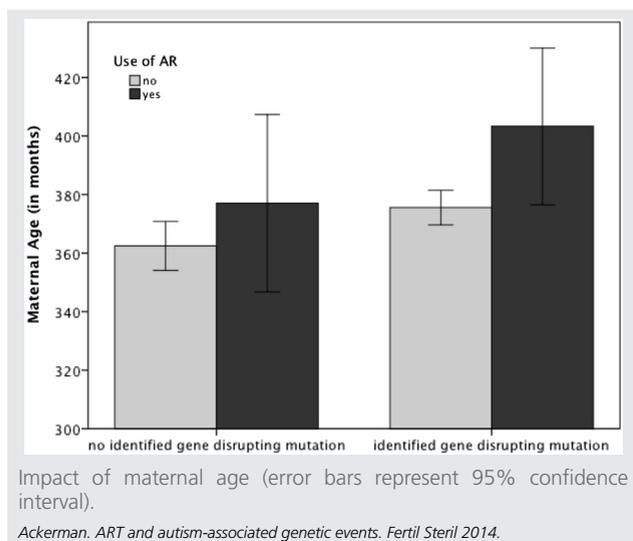
^b All gene mutations are de novo.

^c These events are from previously published data (4, 36, 43).

^d Paternal and maternal ages (in months) at time of conception were estimated based on month-year birth information assuming a 9-month pregnancy.

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FIGURE 1



autism-associated gene-disrupting events did not appear overrepresented within any particular subtype of assisted reproduction. Additionally, CNVs and gene mutations were seen across a wide variety of genomic loci.

If assisted reproduction was a significant independent risk factor for autism-associated genetic events, it would be expected that there would be a higher rate of these events in the group of children with ASD who were exposed to assisted reproduction. Additionally, it might be expected that if assisted reproduction leads to genetic events, there would be a specific type or pattern of genetic event that was seen. Neither was the case.

Increased paternal and maternal age was statistically significantly associated with use of assisted reproduction in the CNV group. In the subsample of individuals with gene mutation status, maternal age trended toward a significant association with use of assisted reproduction and was found to be a potential contributor. This variable reached statistical significance when comparing maternal age and presence of autism-associated gene-disrupting events in the ASD group exposed to assisted reproduction. As alluded to previously, it is possible that the association between assisted reproduction and ASD found in other reports is related to characteristics of the parents using assisted reproduction that increases ASD risk and not assisted reproduction itself. Specific to this report, it is possible that parental age—not assisted reproduction—is associated with an increased risk of autism-associated gene disruptions.

It was somewhat unexpected to see maternal age associated with gene mutations, given the already mentioned studies linking paternal age to autism-associated de novo CNV. However, the population studied in this report is more likely to have maternal age as a primary driver of the use of assisted reproduction. Indeed, the results above showed that in the group with gene mutation status no relation between assisted reproduction usage and paternal age was seen but a trend was observed suggesting that mothers using assisted

reproduction were older than mothers not using assisted reproduction.

There are limitations to the conclusions that can be reached from these data. It is possible that some patients with ASD and assisted reproduction may not have developed ASD or any type of autism-associated genetic event had they not been exposed to assisted reproduction. Phrased differently, it is possible that ASD is an oligogenic disorder and that the group exposed to assisted reproduction will not have a different genotype than the group not exposed to assisted reproduction, but that in the group exposed to assisted reproduction it was assisted reproduction that caused the last mutation necessary to reach a pathological threshold. With that in mind, it would have been helpful to have a typically developing control group in this study. Additionally, although all currently known autism-associated genetic events were considered, there are likely to be more of these events discovered. Furthermore, although the study was sufficiently powered to look at assisted reproduction as a broad category of risk, it was not sufficiently powered to look at any subtype of assisted reproduction. With that acknowledged, as presented in Table 1, no type of assisted reproduction appeared grossly different from another in terms of CNV or mutation rates. Finally, this retrospective study did not incorporate medical records to verify parent report, which would have been ideal.

This study adds valuable information because it is the first to compare the genotype of patients with ASD who have also been exposed to assisted reproduction with patients with ASD who have not been exposed to assisted reproduction. In doing so, no association was found between rates of autism-associated genetic events and assisted reproduction, and no a major genotypic difference were found between patients with ASD exposed to assisted reproduction as compared to patients with ASD not exposed to assisted reproduction.

CONCLUSION

In this large sample, there was no increased rate of ASD-associated genetic events in ASD patients conceived by assisted reproduction compared with ASD patients conceived without assisted reproduction. Additionally, maternal age was identified as a potential contributor to ASD associated genetic events in the context of assisted reproduction. This study is the first on the subject to concurrently examine the genotype of individuals with ASD in relation to their exposure to assisted reproduction versus spontaneous conception and therefore adds valuable, reassuring evidence to the argument that assisted reproduction does not increase the risk of ASD.

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