Social demographic change and autism*

Kayuet Liu[1], Noam Zerubavel[l], Peter Bearman[1]

[1]Paul F. Lazarsfeld Center for the Social Sciences,
Columbia University

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ABSTRACT

Parental age at child's birth – which has increased for children born in the 1992-2000 birth cohorts is strongly associated with an increased risk of autism. By turning a social demographic lens on the historical patterning of concordance amongst twin pairs, we identify a central mechanism for this association -- *de novo* mutations -- deletions, insertions and duplications of DNA in the germ cells that are not present in the parents' DNA. Along the way we show that a demographic eye on the rising prevalence of autism gives rise to three major discoveries. The first is that social demographic change can yield genetic changes that at the population level combine to contribute to the increased prevalence of autism. The second is that the estimated heritability of autism has been dramatically overstated. Third, we show that heritability estimates can change over remarkably short periods of time due to increases in germ-cell mutations.

INTRODUCTION

This article considers and provides a solution to an intriguing puzzle that involves squaring the following four contradictory observations. The first observation is that in the scientific community, autism is widely regarded to be the most genetic of all neuropsychiatric disorders (Lamb et al. 2000). The second observation is that autism prevalence has increased rapidly (roughly a ten-fold increase) over the past four decades (Cohen et al. 2005). The third observation is that fundamental changes in the human gene pool are highly unlikely in one or two generations and have never been observed previously. Thus, if autism is related to genes, it seems axiomatic that the genetic foundation for the increased prevalence of autism rests on a gene-environment interaction involving a change in the environment. The fourth observation is that molecular genetic research has identified the genetic basis for less than 15% of autism cases, and no single known genetic cause explains more than 1-2% (Abrahams and Geschwind 2008; Wang et al. 2009). A typical response to such a riddle might be to wait for the molecular genetic research to catch up and identify a genetic cause of autism. The evidence presented in this article suggests instead we can start to square this circle of conundrums by focusing our attention towards a new, and profoundly different, observation – specifically the observation of genetic influence on autism through *de novo* mutations arising from social demographic change. Here the change of interest is increased parental age.

There is a strong relationship between parental age and autism. The one study (King et al. 2009) that decomposes maternal and paternal age -- and confounding cohort effects -- identifies maternal age as riskier than paternal age (utilizing the California data deployed in this analysis). Specifically, the categorical risks associated with maternal age over 40 years ranged from 1.27

(95% CI=0.95, 1.69) to 1.84 (95% CI=1.37, 2.47), and the risk associated with advanced paternal age ranged 1.29 (95% CI=1.03, 1.6) to 1.71 (95% CI=1.41, 2.08) over the study period reported here. Over the same time, the proportion of children born whose parents were age 35 or over at birth increased rapidly: from 24.3% in 1992 to 36.2% in 2000. This article proposes that the relationship between advanced parental age and increased autism risk arises at least in part from de novo mutations, and uses a twin design to assess that possibility.[1]

To anticipate the main results of this article, we first demonstrate that autism heritability – defined in the narrow sense as the difference in concordance for autism between monozygotic (MZ) and dizygotic (DZ) twins -- is not as significant as typically believed. This finding points to the need to consider prenatal, social and environmental influences on autism risk. One social factor – parental age at child's birth – which has increased substantially for children born over the 1992-2000 birth cohorts is strongly associated with an increased risk of autism. We identify a central mechanism for this association -- *de novo* mutations -- deletions, insertions and duplications of DNA in the germ-cells (sperm or egg) that are not present in the parents' DNA. Affecting the offspring's DNA sequence, *de novo* mutations may lead to genetic predisposition

[1]Note that our twin design limits our inferences to twin births. While it is likely that the increased risk for autism is associated with de novo mutations, we cannot rule out an unobserved factor, for example fertility treatments.

to autism, yet do not require fundamental changes in the human gene pool.[2] We analyze the temporal pattern of concordance for autism in twin pairs over time and show that this pattern reveals increasing genetic effects on autism. Finally, we design and report findings of a critical test that provides exceptionally detailed support for the idea that *de novo* mutations are associated with autism. Along the way we show that a sociological eye on the rising prevalence of autism gives rise to three major discoveries. The first is that the estimated heritability of autism – from family studies, where heritability refers to MZ-DZ difference in concordance -- has been dramatically overstated. Second, we show that heritability estimates can change over short periods of time due to the rising frequency of germ-cells mutations and there is evidence that the heritability of autism is increasing. Third we show that social demographic change in parents can yield genetic changes that at the population level combine to contribute to the increased prevalence of autism. In the discussion we consider how a demographic lens, sensitive to temporality, can shed new light on autism and perhaps therefore influence the direction of subsequent research.

BACKGROUND

Autism is a developmental disorder that profoundly limits the ability of those with the disease to communicate, to form and maintain social relations, and to respond to environmental

[2] As one of our anonymous reviewers helpfully points out, *de novo* mutations are genetic and they may be heritable (in the sense that they may be passed on to children of the next generation) but they are unlikely to be identified with genomic association studies in so far as they are independently arising mutations likely to involve a wide variety of loci on the genome.

stimuli. The incidence of autism in the United States (and elsewhere) has increased rapidly over the past two to three decades. In California, where our data arise and where the most systematic records have been kept, the number of autism cases increased 634% between 1987 and 2002 (California Department of Developmental Services 2003). This is a striking increase. Equally striking is the absence of consensus with respect to causes for increased prevalence. In fact, with the exception of being male and parental age – factors long recognized to be associated with increased risk – newer studies routinely report findings in tension with previous research.

Hundreds of studies have investigated hundreds of factors believed to be associated with both the incidence and increased prevalence of autism. In addition to genetic predisposition, scholars have argued that prenatal conditions, obstetric complications, parental characteristics, environmental toxins, and the availability of school and community resources could all potentially be associated with the number of autism cases (Grandjean and Landrigan 2006; Kolevzon, Gross and Reichenberg 2007; Palmer et al. 2005; Reichenberg et al. 2006). Covariates identified in previous work are extensive, ranging from premature birth, breech birth, and low Apgar score at five minutes to parental characteristics, such as socioeconomic status, education, age, race, occupation, and history of schizophrenia (Croen, Grether and Selvin 2002; Larsson et al. 2005). At the community level, resources available for screening, increased service availability, the density of pediatricians in a community, environmental toxicity, the number of students per school, and percentage of students receiving a free lunch have been tied to rates of autism (Lathe 2006; Palmer et al. 2005). Most of these associations are not causally related to increased prevalence. Instead, a disproportionate body of research seemingly confuses correlated time series with cause producing an efflorescence of factors associated with autism; a veritable

laundry list of variables to consider. One reason for this state of epidemiological confusion is that autism, by virtue of increased prevalence, is a moving target; the composition of those with autism changes over time, and hence our understanding of risk factors is also temporally sensitive, dependent on the observation window and spatial context in which the study is based (King et al. 2009). In this article we show how a more nuanced understanding of temporal dynamics leads to new insights.

In broad sweep, three ideas motivate most of the current accounts of the increased prevalence of autism. The first account is that increased prevalence arises from diagnostic dynamics, driven by process of diagnostic change, substitution and drift (Shattuck 2006). Support for this hypothesis arises from the fact that early on in the epidemic one could not observe the classic SES-health gradient and that autism spectrum disorders appeared to be a diagnosis of choice for middle and upper class parents whose children would be otherwise diagnosed as mildly or severely retarded, a disorder now associated with increased stigma. King and Bearman (2009) estimate that roughly 25% of the increased prevalence of autism arises from diagnostic change on the mental retardation (MR) pathway. Using a different estimation strategy (Bishop et al. 2008) suggest that one-third of the caseload arises from diagnostic dynamics. Consistent with this idea is the corresponding claim that the real incidence of autism in the population was previously under-reported to avoid the stigma associated with autism when it was perceived to be a psychogenic rather than developmental disorder.

The second account centers around environmental toxins, in interaction with genetic factors, none of which are well understood. Most of the evidence for such a link is indirect. Base metals that are clearly implicated in developmental disorders are most commonly suspected, and

at this writing, 272 candidate toxins have been identified as potentially linked to autism (Grandjean and Landrigan 2006). The environmental impact hypothesis remains robust principally because the increase in autism caseloads is consistent with observed increase in environmental degradation. Finally, there is clear evidence that autism has a significant family and genetic component net of gene-environment interactions, though molecular genetic research has identified a genetic basis for less than 15% of all autism cases, and no single known genetic cause accounts for more than 1-2% of all cases (Abrahams and Geschwind 2008; Wang et al. 2009). The key support that autism is a genetic disorder arises from the reported difference in concordance rates for autism in identical and fraternal twin pairs, and a high recurrence risk in siblings.

There is no reason to believe that any one of these frameworks is wrong and many reasons to believe that the increased prevalence of autism is the outcome of multiple self-reinforcing processes that invoke all three of these core explanations. We can, however, make progress by recognizing that the increase in reported autism prevalence – at least that component not arising from increased surveillance and ascertainment – must be tied to a social change process that invokes a biological mechanism associated with neuro-developmental processes. This is the strategy undertaken in this article, where we consider twin, full-sibling, and half-sibling concordance for autism over time in order to understand how social and genetic factors intersect to induce the rising prevalence of autism.

The Evidence for High Heritability of Autism

To date, the strongest evidence supporting the idea that autism is a genetic disorder arises from twin and family studies. Previous twin studies on full syndrome autism have reported high

pairwise concordance rates in identical (MZ) twins (36-96%) and low concordance rates in fraternal (DZ) twin pairs (0-31%) (Bailey et al. 1995; Folstein and Rutter 1977; Ritvo et al. 1985; Steffenburg et al. 1989). Because MZ twins share 100% of their genes while DZ twins share only around 50%, a large difference between MZ and DZ concordance rates is regarded as strong evidence for genetic influences. The recurrence risk of autism in siblings is reported to range from 3-9%, much higher than the population rate of 10 in 10,000 children (Baird and August 1985; Bolton et al. 1994; Piven et al. 1990; Ritvo et al. 1989).[3] Relatives of a child with autism are also more likely to have broadly defined autism spectrum traits than controls (Szatmari et al. 2000).

One significant problem with these reports is that they arise from small convenience samples and/or referrals. Where recruitment of pairs into studies is based on such samples, heritability estimates are biased upwards, since pairs more similar on unobserved characteristics are more likely to be enrolled. In this case, the conclusion that the concordance of full-spectrum autism is much higher in MZ than DZ twins have arisen from studies with a total combined population of 110 pairs. This is not a robust platform from which to make inference. Moreover, the epidemiological patterns of autism do not conform to simple Mendelian expectations: most autism cases are sporadic (i.e., with no history in the family) and the pattern of observed

[3] Other studies have used a population based sample to look at the concordance for more broadly defined autistic spectrum disorders or autistic traits (e.g., Hoekstra et al. 2007; Taniai et al. 2008). To our knowledge, no population-based study has been conducted on full syndrome autism.

concordance rates in identical twins, fraternal twins, full-siblings and relatives are inconsistent with dominant, recessive, or X-linked transmission models (Skuse 2000). This suggests that the genetic influence on autism is likely to be complex.

One important idea that we explore in detail is that Mendelian inheritance is not likely to be the core mechanisms for genetic influence. Interactions between multiple loci are much more likely to be the underlying mechanisms (Abrahams and Geschwind 2008), and recent studies suggest that the process is likely to be driven by genetic changes arising in part from *de novo* mutations of germcells. Recent studies conducted by Sebat et al.(2007), Jacquemont et al. (2006), and others (deChristian et al. 2008; Kumar et al. 2008; Marshall et al. 2008; Morrow et al. 2008; Szatmari et al. 2007; Weiss et al. 2008) suggest *de novo* mutations occurring in a wide range of genomic locations contribute to autism, adding support to the evidence that autism has heterogeneous genetic causes. Consequently, simple (and static) models of genetic inheritance appear inadequate to account for autism.

From a social demographic perspective, this makes sense. The autism research community has long understood that parental age is a significant risk factor for autism. But the age of parents is not something that "gets under the skin" by itself; so net of the impact of parental age (and SES) on the probability of diagnosis and thus ascertainment – it follows that if broad social changes associated with increased age of parents is playing a role in the autism epidemic that a dynamic multiple-locus model is more likely to identify the operative mechanism than the traditional Mendelian framework. This is the possibility explored in this article where we consider concordance of twin pairs over time, and reveal how rising parental age, which leads to greater probability of *de novo* mutations, accounts for the changes in concordance we observe

over time. As discussed subsequently, we exploit the fact that *de novo* mutations are rare events to design a critical test of their role in the etiology of autism.

Concordance in Twins and Recurrence Risk in Siblings

Measures of genetic influence based on fragile empirical foundations are suspect. In order to generate robust estimates of genetic influences, we need to measure concordance rates (casewise and pairwise) for autism in twins and recurrence risk in siblings in a representative sample. These are our central outcome measures. Casewise concordance (Pcw) measures the probability that a co-twin will be affected (with a given disorder) given the other twin is affected. Pairwise concordance (Ppw) measures the proportion of concordant (both twins are affected) pairs in all pairs with at least one twin who is affected.

Twins may be concordant or discordant on some characteristic of interest, in this case, autism. Let n_c be the number of concordant pairs and n_d be the number of discordant pairs. It follows that in same sex (SS) twins, the casewise concordance rate is equal to $[2n_c/(2n_c+n_d)]$ since we can assume interchangeability of twins. This means we can assume that the risk of twin A to be affected given that twin B is affected is the same as the risk of twin B to be affected given that twin A is affected. For many disorders, interchangeability of twins can be assumed for opposite sex (OS) twins as well, but for autism, interchangeability cannot be assumed because boys are four times more likely than girls to be affected. Thus separate casewise concordance rates need to be estimated to measure (i) the likelihood that a male co-twin would also have autism given that his female twin sibling was affected $[2n_c/(2n_c+$ all pairs with an affected female$)]$; and (ii) the likelihood that a female co-twin would also have autism given that her male twin sibling was affected $[2n_c/(2n_c+$all pairs with an affected male$)]$. In (i) the female twin with

autism is referred to as the proband and in (ii) the male twin with autism is referred as the proband, by convention.

An advantage of casewise concordance rates in twins is that they are directly comparable to recurrence risk estimated in other relatives. Recurrence risk is a measure of the likelihood that a condition recurs in the family by affecting another sibling. We determine recurrence risk amongst sibling pairs by the later sibling method (Ritvo et al. 1989), which is the proportion of younger siblings born after an older child with autism (again referred to as the proband) who also have autism. Stoppage – parents stopping to have more children after having an affected child – is less of an issue with this method as the recurrence risk is only evaluated among families with children born after an child with autism. We further differentiated recurrence risk in full- and half-siblings. To examine the effect of gender and potential differential genetic liability, we calculated casewise concordance rates and recurrence risk by the sex pairing of the proband and the co-twin/sibling. Casewise concordance rates are reported with 95% confidence intervals estimated based on asymptotic (MLE) variance. Recurrence risk is reported with Wilson 95% confidence intervals (Agresti and Coull 1998).

In short, we are interested in the extent to which autism runs in families. As with all other things that may run in families, one efficient way to consider if they do is to calculate twin concordance and sibling recurrence risk. For this, it is critical to work with large representative samples that do not arise from self-selection, self-nominated membership in twin registries, or convenience samples of twins, all of which care biased towards selection of similar pairs, and hence amplify the extent to which pairs are concordant on some trait.

DATA AND METHODS

Our data arise from California. In California, the 21 regional centers of the statewide Department of Developmental Services (DDS) provide services to the vast majority (estimated to be >85%) of people with autism.[4] This study was based on the DDS client data for the roughly 20,000 persons born between 1992 and 2000 who were diagnosed with autism. The DDS provides service to patients with Autistic Disorder. Individuals diagnosed with other developmental disorders, including Asperger's Disorder, Childhood Disintegrative Disorder, Rett's Disorder and Pervasive Developmental Disorder - Not Otherwise Specified (PDD-NOS) are ineligible for services provided through the DDS. Thus our study focuses on "full-syndrome" autism – where diagnostic dynamics associated with increased prevalence are less intense -- not spectrum disorders, likely subject to more diagnostic movement.

Identifying Twins and Siblings

To identify the co-twins and siblings of all individuals with autism, we first linked the DDS data of all individuals with autism to the birth master files on the 4,906,926 children born between 1992 and 2000 in California. Over time, the mean age of diagnosis has fallen precipitously, from roughly six to three. Even so, we chose 2000 as the latest cohort to minimize ascertainment biases arising from missing older children: we estimated at least 95% of the 2000 birth cohort with autism would have been diagnosed by June 2006. 16,352 out of the 19,436

[4] See Croen, Grether and Selvin (2002). The estimation was based on a comparison between the DDS data and California's special education database. The case definition of autism in the special education is unclear and is likely to include many children that do not meet the full syndrome criteria. Thus 85% is a conservative estimate.

(84%) DDS patients born 1992 and 2000 who ever had an autism diagnosis were successfully linked to the birth records data. Record linkage was performed by matching probabilistically using information on patient's names, gender, date of birth and race. Potential mismatches where manually verified. The vast majority of individuals not matched were born outside of California.

Exact matching of twin pairs on date of birth, hospital code, mother's names, mother's date of birth, mother's country of birth yielded 39,035 SS and 17,596 OS twin pairs, of which 503 pairs had at least one child with full-syndrome autism. Exact matching on mother's names, mother's date of birth and mother's country of birth yielded 9,496 sibling groups with at least one child with autism. Sibling groups with older siblings born before 1992 (3,871) were excluded from the analysis (since we have no information on the diagnostic status of these older siblings), yielding 5,625 eligible siblings groups with at least one child with autism. The excluded group has a slightly lower prevalence of autism than our study population because 1st born children have a higher risk for autism, but with respect to all other characteristics are comparable to those included. Father's surname and date of birth were used to differentiate full siblings and maternal half-siblings. For our calculation of parental age, we averaged the ages of both parents where present. When fathers age was missing, we used mothers age. In short, the data on which this study is based is large and representative. Consequently, the inferences we are able to make are likely more robust than those arising from prior work.

RESULTS

Twin Concordance and Sibling Recurrence Risk Implicates Social/Environmental Factors

Table 1 reports twin concordance and sibling recurrence risk in autism. SS twins had the highest casewise concordance rates (48.8% for males and 42.4% for females). There was no significant difference between the casewise concordance in SS males and SS female twins (p=.398).

Table 1 about here

The concordance in OS twins is necessarily *lower* than in SS-DZ twins due to their gender composition: while half of the OS pairs twins must be girls (who have one-fourth of the risk of autism than do boys), SS-DZ twin samples with at least one twin affected consist mostly of males because of the higher prevalence of autism in boys. Just the effect of gender alone would lead to a higher concordance rate in SS-DZ than OS pairs. Against this background, it is striking that the pairwise concordance in OS twins (10.1%) observed in this study is higher than the often cited 0% concordance in the SS-DZ twin samples (Bailey et al. 1995).[5] The casewise concordance rate for OS twins is 18.4%, fully 2.6 times higher than the recurrence risk in OS full-siblings (7.2%). As OS twins and OS full-siblings have the same genetic relatedness, this indicates that in addition to genetic factors, perinatal, prenatal, social and other environmental

[5] Bailey et al.(1995) argued that the true concordance rate in DZ twins should be close to the recurrence risk of 2.9% in siblings, citing Bolton et al.'s (1994) finding. As mentioned before, the low concordance in DZ twins in these earlier studies may be explained by sampling bias.

factors must contribute to autism.

The administrative data we work with do not have a direct measure of zygosity so we do not know from these data whether twins are MZ or DZ, which is central to the estimation of genetic influence. Luckily, a simple and well-established rule that has been shown to give robust zygosity estimates has been developed for this purpose and has been widely used in research on twinning rates and a range of other research questions (Scarr-Salapatek 1971; Tong, Caddy and Short 1997; Westergaard et al. 1997). The rule exploits the known fact that all OS twin pairs are DZ twins. Since DZ twins have an equal probability of being male and female – just as all births have such an equal probability -- it follows that the number of DZ twins in SS pairs in the population equals the total number of OS twin pairs.[6] Minor deviations from the equal sex ratio have a negligible impact on the resulting estimates (Fellman and Eriksson 2006). As mentioned above, there were 39,035 SS twin pairs and 17,596 OS pairs born between 1992 and 2000 in California. Let r be the proportion of monozygotic twins in the SS group. Using this general rule

[6] The rule is the Weinberg rule. In societies where there is a markedly skewed sex ratio arising from strong preferences for males, for example, this rule does not apply.

it follows that r= [(39,035-17,596)/39,035]=0.55.[7] This gives a MZ twinning rate of 4.4 in 1000 pregnancies (21,469 out of a total of 4,847,467 pregnancies). Unlike DZ twinning rates, MZ twinning rates are stable across countries and over time (Bortolus et al. 1999). Our estimated MZ twinning rate corresponds closely to the figure based on a large scale study with direct zygosity measures--4.5 per 1000 maternities (Derom et al. 1987).

Having estimated the value of r and given the fact that the SS concordance rate is a

[7] We estimated r based on all twin pairs born in California during the study period, not our sample of twin pairs with at least one with autism. Applying the Weinberg method to this study's sample of twin pairs is erroneous because boys are 4-5 times more likely to have autism than girls. Hence, DZ-SS female pairs have a lower probability to be in our sample than OS pairs, merely due to the fact that both girls have a lower probability of autism than the male twin in an OS pair, who thus can qualify the OS pair's inclusion in the sample. It is then imperative that the number of DZ-SS pairs would *not* equal to the number of OS pairs in a sample of twin pairs with at least one twin having autism. Yet *within* the male-male (or female-female) pairs in our sample, the proportion of MZ pairs equals to the r estimated from the population data, as it should be the case in all random samples of SS pairs. Only when zygosity has a direct impact on the risk of autism (e.g., the biological process of zygotic splitting increases the risk of autism) will r estimated from the population data be a biased estimate. Note that r in our sample is independent of concordance rates. Even though the concordance rate of autism is higher in MZ twins than DZ twins, a DZ *pair* is as likely to be included as a MZ *pair*, as having *one* twin with autism is the sufficient condition for inclusion.

weighted average of the concordance rates in MZ twins and SS fraternal twins, we can estimate the MZ concordance rates by simple linear transformation. We know that the risk of autism depends on the child's sex, but not the sex of the proband.[8] Given this, the casewise concordance rate for MZ male pairs and MZ female pairs are estimated to be 57.0% and 67.2%, respectively. The corresponding pairwise concordance rates are 39.9% and 50.6% respectively. These concordance rates are substantially lower than the commonly cited range of 80-100%. On the other hand, our estimate of the casewise concordance in DZ twins is 32.9%, substantially higher than the previously reported figure of 0%.

Our estimations of MZ and DZ concordance rely on two assumptions. The first is that the sex ratio is equal at birth. The second is that the risk of autism depends on the sex of the child, not on the sex of his or her twin sibling. Minor to moderate deviations from these assumptions would not lead to different estimates. For example, if we over– or under–estimated the proportion of MZ twins in the SS twin group twins (r) by 5%, the resulting change in our estimate of casewise concordance would have been less than 1% for MZ male twin pairs — the gender in which most cases of autism occur — and less than 6% in MZ female twin pairs. We

[8] The recurrence risk for the male siblings of diagnosed female and male probands in our sample is 18.4% and 13.8%, respectively. Among female siblings, the recurrence risk is 3.5% when the proband is female and 5.1% when the proband is male. For both male and female siblings, there is no statistically significant difference by the sex of the proband ($p>0.05$). These results confirm previous reports that sibling recurrence risk does not differ by the sex of the proband (Goin-Kochel, Abbacchi and Constantino 2007; Pickles et al. 2000; Szatmari et al. 2000).

observe similar robustness in estimates when the assumption about proband's sex and genetic liability was violated. Even if sisters posed 30% higher risk to their co-twin than did brothers, the estimated pairwise concordance rates for MZ male pairs and MZ female pairs would be as low as 47.4% and 53.3%, respectively. Given our data, to yield a MZ pairwise concordance rate of 80% or above (reported in other, smaller studies), the male co-twin of a female proband would have to be exposed to at least *150 times* higher risk as compared to the male co-twin with a male proband. This is improbable; all of the biological mechanisms would point to enhanced risk -- if any -- flowing in the opposite direction.[9]

A heritability estimate is a population measure of the proportion of the overall phenotypic variance attributable to genotypic variance, and it is specific to the time and the population (although this is not widely recognized). Bailey et al. (1995) estimated that heritability of autism to be over 90% under the standard ACE model, which partitions the variance into additive (allelic) genetics, shared, and non-shared environment components. For comparison, we estimated heritability by applying the ACE model to our data, using the expected distributions of

[9] Hypothetical deviations from assumptions and their impact on estimated concordance:

		Monozygotic Male Twin Pairs		Monozygotic Female Twin Pairs		SS Dizygotic Twin Pairs	
		Casewise (%)	Pairwise (%)	Casewise (%)	Pairwise (%)	Casewise (%)	Pairwise (%)
Current estimates		57.0	39.9	67.2	50.7	32.9	19.7
Deviation from the estimated proportion of MZ pairs in SS pairs	+ 5%	56.1	39.0	61.4	44.3	-	-
	-5%	57.9	40.7	67.7	51.2	-	-
Additional risk posed by female probands vs. male probands	+10%	59.9	42.7	68.1	51.7	30.1	17.7
	+20%	62.3	45.2	68.9	52.5	27.8	16.2
	+30%	64.3	47.4	69.5	53.3	25.9	14.9

MZ and DZ twin pairs by their joint diagnostic status. In males, the heritability of autism is estimated to be 19%. Among females it is 63%. If the genetic liability posed by female probands were 30% higher than male probands, the heritability of autism in males would be under 35%; for females heritability would remain greater than 50%. Thus an outer-bound estimate of heritability is 19-35% for males and 50-63% for females.

Heritability estimates can provide useful information for social scientists. Our estimates are the proportions of variance attributable to genetics within each gender. Higher heritability in females does not necessarily mean affected females have a higher genetic liability than males; it can also arise if environmental factors are less important for girls than for boys. Differences in developmental and social environments can elicit distinct genetic expressions and therefore marked difference in heritability. It is also possible that different sets of genes (and their interactions) are needed to elicit autism in males and females.[10]

[10] As female MZ twins are not phenotypically identical due to X-inactivation (i.e., a process by which one of the two X chromosomes in the first 70-100 cells in female fetus development is randomly inactivated (Lyon 1961)), the similarity of concordance rates for MZ males and females could suggest that the key genes for autism are unlikely to be on the X chromosome. However, if the locus of interest is not subject to random X-inactivation (Puck and Willard 1998), the theory that an imprinted X-link locus of paternal origin (i.e., a gene is expressed only when inherited from the father) is protective against autism (Skuse 2000) is consistent with a high concordance in MZ female twins, a lower concordance in DZ female twins than DZ male twins, and a lack of significant difference to the risk in the siblings of female and male probands.

Independent of the differences between males and females – which are likely of sociological interest – it is clear that the heritability of autism has been wildly over-estimated in previous work. Autism is still very heritable, but not more than other neuro-developmental disorders. This is significant since the discovery that autism is not overwhelmingly heritable means that prenatal, social, and environmental factors likely play a substantial role in the expansion of the caseload. We now consider how one social demographic factor, a relatively subtle change in age of parents at birth is associated with autism through de novo mutations.

Age of Parents at Child's Birth and Relative Risk for Autism

De novo mutations are positively associated with parental age. So if age at child's birth changes over time, we have a reason to consider whether *de novo* mutations can account for some of the increased prevalence of autism we observe over time. As noted earlier, advanced maternal age and paternal age are significant risk factors for autism in almost all birth cohorts from 1992 to 2000 (King et al. 2009) and maternal and paternal age at child's birth increased over the period from 1992 to 2000. Mean maternal age was 26.9 (±6.03) in 1992 and increased to 27.7 (± 6.33) by 2000. Similarly, paternal age increased from 29.6 (±6.84) to 30.6 (± 7.11) over the same period. The increasing standard deviation, particularly of paternal age, is noteworthy since parents' age at birth is strongly bounded on the left tail of the distribution. Thus there has been a considerable increase in advanced paternal age, extending the length of the right tail of the distribution.

The Logic of De Novo Mutations in the Context of Concordance

Putting three facts together -- *de novo* mutations are associated with neuro-developmental disorders; parental age is associated with *de novo* mutations; and autism is associated with increasing parental age -- leads one to wonder whether or not parental age is associated with autism via *de novo* mutations.[11] This is the hypothesis we directly test by focusing on the pattern of concordance across twin pairs. Other strategies for observing (from population data) evidence for a relationship between *de novo* mutations and autism focus on the sex ratio of children with autism. Following Anello, et al (2009), who show that the sex ratio for a sample of 393 children with autism spectrum disorder (ASD) is increasingly balanced with paternal age – a sign of a potentially increased role played by *de novo* mutations, we first consider whether we observe a

[11] Other factors could cause the association between parental age and autism. Older parents could choose neighborhoods to live in that are selective for autism, from environmental degradation or enhanced ascertainment; older parents could be more worried than younger parents about their children and expose them to differential testing. Subsequently we consider whether these competing explanations fit with the detailed evidence described in this article.

similar pattern in our data.[12] Not surprisingly, we observe the same pattern. Specifically, for fathers younger than 35 the M:F ratio is 4.92 (2,841/13,930); whereas for fathers older than 35 the M:F ratio is 4.38 (3,941/16,719). For mother's age we observe a similar pattern 4.90 vs. 4.24). Both differences are statistically significant.

De novo mutations are extremely rare events. This fact allows us to design a critical test of the role they may play in increasing autism prevalence. Since mutations are rare events, the same *de novo* mutations should always be present in both MZ co-twins and lead to high concordance for the expression of autism. Since increasing parental age should be associated with a higher rate of *de novo* mutations, we expect rising concordance in MZ twins across time. Although counter-intuitive, the rising rate of *de novo* mutations should lead to lower concordance in DZ twins over time. This is because such rare mutations should almost never independently co-occur in DZ co-twins even at high parental ages.

[12] Interpreting the relationship between parental age and the sex ratio of children with autism as a sign of de novo mutations requires the assumption that de novo mutations affect males and females more equally than the inheritance of liability genes(Anello et al. 2009). For instance, if instead the *de novo* mutation of concern affects the inactivation of genes on the extra X chromosome – a process only necessary in girls – and if such problems with inactivation are associated with autism, then the mutation will increase the chance of autism in girls more than boys (Brooks 2005).

In addition, the *de novo* mutation hypothesis has clear predictions for the changing patterns in the numbers of concordant and discordant pairs among MZ and DZ twins over time. Because *de novo* mutations are very unlikely to be shared by DZ twins, such sporadic mutations should generate discordant DZ twin pairs: concordance should decrease as *de novo* mutations increase. Thus we expect the OS concordance rate to decline due to an increase in the number of discordant twin pairs, while the number of concordant pairs should not be affected. It follows that examining twin concordance rates in a time of rising parental age can test the contribution of *de novo* mutations to the etiology of autism and as an explanatory factor involved in increased autism prevalence.

Increasing Heritability over Time

Figure 1 reports SS and OS concordance rates in 1992-94, 1995-1997 and 1998-2000. Recall that all OS twins are DZ twins while around 55% of the SS twins are monozygotic. Therefore any observed trend for concordance of SS twins will be muted by the combined trends in the concordance rates in MZ and DZ twins.

Figure 1, Panels A, B, and C about here

As shown in Figure 1, panel A, the casewise concordance in SS twins has increased over time, while decreasing in OS twins, suggesting autism is becoming more genetically determined due to

the *de novo* mutation mechanism.[13] A z-test of proportions was used to test whether there are significant differences in SS and OS concordance across time. There is no significant difference in the concordance rate between -SS pairs and OS pairs in 1992-1994 (z=1.155, p=0.248), but there were significant differences in 1995-1997 (z=2.963; p=0.003) and 1998-2000 (z=4.393; p=0.000). This is precisely what we expect to observe if such mutations are shaping the pattern of concordance.

In panel B, we report change in mean parental age at twin births, which increases steadily during the same period. Recall that because MZ twins are developed from a single pair of matched egg and sperm cells, any *de novo* mutations will be found in both twins. In contrast, DZ twins develop from two distinct pairs of egg and sperm cells. As *de novo* mutations are rare events, the chance that both DZ twins will share the same *de novo* mutation is extremely low. If *de novo* mutations have an increasing causal share in the etiology of autism over time, we should expect an increase in the difference between MZ and DZ concordance rates. One mechanism that

[13] We can estimate changing heritability by zygosity, of course. First we need to take into account the general rise in DZ twinning rates in developed countries that have been attributed to increased use of artificial fertility treatments (Tong and Short 1998). Adjusting for the increasing proportion of DZ twins in SS pairs over time (from 42% to 47%) and assuming equal genetic liability for male and female probands, the estimated MZ casewise concordance rates increased from 37% in 1992-1994 to 75% in 1998-2000. If the genetic liability posed by female probands was 30% higher than male probands, the MZ casewise concordance rates are 41%, 59% and 79% in 1992-94, 1995-97 and 1998-2000.

accounts for *de novo* mutations' increasing share of autism etiology is the rise in parental age over our study period which is likely to lead to increased mutation rates.[14]

To test this, we fit trend lines to the 5-year moving averages of the casewise concordance rates and mean parental age as shown in Figure 1, panel C. Concordance in SS twins begins to increase with parental age after 35.[15] Over the same period, OS concordance begins to decrease after age 35. The R^2 for the fitted SS line is 0.23, for the fitted OS line 0.33. We note here that a better fit could be obtained *ex post* by fitting higher polynomials or splines. This might look better, but would involve *ad hoc* theorizing. Recall that the prediction tested here is limited to older parents and so a square term is sufficient to capture change at the tails of the distribution. The pattern of decreasing concordance in OS twin pairs observed in Figure 1, panel C is

[14] One may argue that the temporal trends in the concordance rates can be the results of other demographic trends such as a growing population of Hispanics in California and/or rising education level. Yet the diverging pattern of the concordance rates persists after excluding the Hispanics (SS casewise: 31%, 50% and 50%; OS casewise: 28%, 22% and 19% in 1992-1994, 1995-1997; and 1998-2000, respectively). It is also the case when we only look at the population with above high school education (SS casewise: 41, 49% and 48%; OS casewise: 28%, 24% and 13 in 1992-1994, 1995-1997 and 1997-2000, respectively).

[15] The women who have children before 35 and after may be different, given the common perception about "appropriate" maternal age in this period. But if there is this selection – which seems reasonable – we do not have a way to capture it with our data.

precisely what we would expect if *de novo* mutations are associated with the increased risk of autism. It is less clear why the SS concordance should increase rapidly with parental age, as the increase in SS concordance due to the increase in identical twins should be muted by the decrease in fraternal twins. It may be due to a higher penetration rate among the identical twins who inherited the susceptible genotype, or other risk factors that are specific to the offspring of advanced age parents.

Finally, recall that our hypothesis also has clear predictions for the changing patterns in the numbers of concordant and discordant pairs among SS and OS twins. First, as *de novo* mutations will not be shared by OS twins, a single mutation should generate discordant twin pairs. Therefore, the OS concordance rate should decline due to an increase in the number of discordant twin pairs, while the number of concordant pairs should not be affected. This is the case. While the number of OS concordant pairs remained stable over time, the number of discordant pairs consistently increased: there were 7, 6, and 6 concordant OS pairs and 46, 50 and 73 discordant OS pairs born in 1992-94, 1995-97 and 1998-2000, respectively. Turning to SS twins, we know that because a de novo mutation necessarily affects both twins in MZ pairs, it will follow that if the proportion of autism cases caused by such mutations increased over our study period, we should observe a surge in the number of concordant MZ pairs. However, SS twin pairs are comprised of DZ and MZ twins. Consequently, if *de novo* mutations are driving increased prevalence, we would expect both rising numbers of same concordant pairs (MZ effect) and discordant pairs (DZ effect). As predicted, we observe that both concordant and discordant pairs increased during the study period: there were 15, 36 and 47 SS concordant pairs and 55, 76 and 86 discordant pairs in 1992-94, 1995-97 and 1998-2000, respectively.

DISCUSSION

This article provides substantial population level support for the link between increasing probability of *de novo* mutations and autism. To our knowledge, no previous studies have examined changing concordance for autism across time.[16] Modeling changing concordance over time allows us to construct a test of an interaction between genes and the social demographic environment that predicts increasing genetic influence. This is precisely what we observe.

Prior twin studies have suggested a high heritability of autism based on reports that the concordance for MZ twins is many times higher than that for DZ twins. The empirical foundations for these claims arise from absurdly small samples. Adding together all twin pairs from the four previous studies yields a total of 110 pairs. All four studies have relied on referrals or convenience samples,[17] which tend to recruit similar pairs and are thus biased towards concordant twins, leading to inflated estimates of true concordance. In contrast, the results reported here arise from a large population-based sample. Our results show substantial MZ concordance, providing evidence that there is some genetic etiology for autism. Nevertheless, the results show that the reported high heritability for autism (>0.9) is seriously overestimated.

[16] Actually, no studies to our knowledge have ever considered changing concordance of anything over time. This is one reason why a sociological lens on genetics is useful.

[17] Ritvo et al's (1985) subjects were recruited from advertisements. The other three studies' subject were primarily through referrals from medical doctors, psychiatrists and advocacy organizations, including Steffenburg et al's (1989) study, which is often mistaken to be as a population-based study.

A reduced heritability estimate matters as it implicates social and/or environmental drivers as playing a substantial role in the increased prevalence of autism. Prenatal factors are likely to be important environmental drivers. Concordance for OS twins and full-siblings ought to be equivalent from a genetic perspective as DZ twins are full-siblings who happen to be born at the same time. This is not the case. The observation that the OS concordance rate in twins is 2-3 times higher than the recurrence risk in full-siblings suggests the importance of risk factors related to the prenatal environment or the larger social environment.

Although the genetic influence on autism has been overestimated, it has increased over time due to non-allelic mechanisms. While the human gene pool does not change substantially over one or two generations, *de novo* germ-line mutation rates are much more susceptible to rapid social and/or environmental changes such as rising parental age, and thus can explain the increase in the heritability of autism. Of importance is the fact that while age of parents at birth of twins was significantly higher in 2000 than in 1992, age of parents at the birth of their second-born did not increase over the same period. Thus, the difference between the trends of OS twin concordance and full-sibling recurrence risk may be associated with age of parents. Since the utilization of assisted reproductive technologies (ART) is associated with the age of parents and has increased radically over the same time period, ART may be implicated in the increased prevalence of autism. Our data show that the increase in the percentage of children with autism born in multiple births (from 3.6% in 1992 to 5.7% in 2000) exceeded that of the percentage of multiple births in all births in California (from 2.1% in 1992 to 2.9% in 2000). This implication requires future investigation.

It remains possible that other factors have contributed to the diverging trends in the SS and OS

concordance. A virus or a toxin experienced *in utero* could yield the results we observe. Specifically, an increasingly prevalent virus (or toxin) associated with a small risk of autism would lead to increasing concordance of SS twins (who often share the same placenta) and decreasing concordance of OS twins. Similarly, interactions between genes and an increasingly common environmental trigger could also generate the same pattern. While these accounts are possible, we believe an increase of *de novo* mutations due to rising parental age is more efficient given the documented rise in parental age, recent findings that link *de novo* mutations and autism, and the observed associations between concordance rates and parental age reported in this article.

The temporal concordance trend reported in this article is not predicted by a diagnostic expansion theory. If ascertainment and surveillance dynamics rest behind the increase in SS concordance, we would expect to observe increasing rather than decreasing concordance for OS twin pairs over time. The observation of decreasing concordance over time in opposite sex twins challenges the idea that the results we observe are an artifact of reduction of error in diagnosis as a consequence of enhanced surveillance or clearer understanding of diagnostic markers.[18] First,

[18] The calculation of recurrence risk was restricted to younger siblings born within 36 months of the proband's birth month to standardize the window of time in which the younger sibling(s) were born. Families with the proband born in 1998 were excluded as the observation window was less than 36 months. No statistically significant time trend was detected in recurrence risk ($p=0.204$).

there is no evidence that diagnostic errors have been reduced; second, if this were the case, we should observe the same effect across all pair types. Finally, increasing ascertainment and surveillance would predict heightened recurrence risk for siblings over time. We do not observe any increase in such risk.

Instead, we observe how a relatively subtle social change, the population level shift in mean age of parents at birth of their twins, is associated with enhanced risk of autism. This suggests that our image of gene-environment interactions needs to be substantially broadened to include in the relevant environment a broad array of fundamental social processes that taken together make up the social structures in which we live, and shape the health outcomes that we, and our children experience.

For social scientists there are three important discoveries. First we show that a sociological eye on the role of genetics yields the insight that de novo mutations may play a significant role in autism etiology. Only by observing changing patterns of concordance over time – that is historicizing genetic influences rather than essentializing them -- could we find

		Recurrence risk (%)	***n* with autism/*n* younger siblings**
Proband's year of birth	1992	12.4	12/97
	1993	13.0	14/108
	1994	13.2	19/144
	1995	10.9	14/128
	1996	15.1	23/152
	1997	12.1	21/174

Chi-sq test of linear trends in proportion =1.613; p=0.204

evidence of a new causal mechanism underlying autism. Second, by working with a large population based data set, as versus small clinical samples, we have been able to properly estimate the true heritability of autism. These estimates show that autism is far less heritable than previously thought, and that as a consequence explanations for the precipitous increase in prevalence must turn towards environmental and social dynamics often ignored by the scientific research community. And third, we show that the identification of the mechanisms by which social processes operating at the macro-level – in this case, increases in parental age – "get under the skin" and shape health outcomes is a proper social science activity.

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Table 1. Twin concordance and sibling recurrence rates

A. Twin concordance

	Casewise concordance (%, 95% CI)	Pairwise concordance (%)	*n* concordant pairs/*n* all pairs
Same sex (SS)	47.5 (41.6-53.4)	31.1	98/315
Two boys (SSM)	48.8 (42.1-55.5)	32.3	80/248
Two girls (SSF)	42.4 (29.2-55.6)	26.9	18/67
Opposite sex (OS)	18.4 (11.3-25.5)	10.1	19/188
Male cotwins of a female proband	38.8 (25.2-52.4)	-	19/49
Female cotwins of a male proband	12.1 (7.0-17.2)	-	19/158

B. Estimates of monozygotic (MZ) and dizygotic (DZ) concordance rates

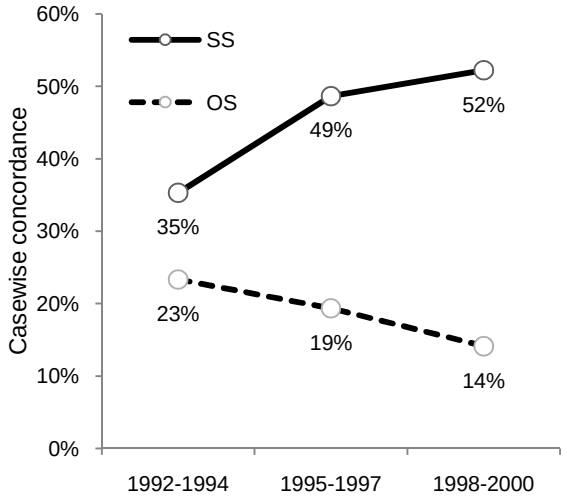
	Casewise concordance (%, 95% CI)	Pairwise concordance (%)	
Monozygotic males (MZM)	57.0 (40.5-73.5)	39.9	-
Monozygotic females (MZF)	67.2 (42.8-91.6)	50.6	-
Same sex dizygotic (SSDZ)	32.9 (10.5-48.7)	19.7	

C. Recurrence risk in full-and half-maternal siblings

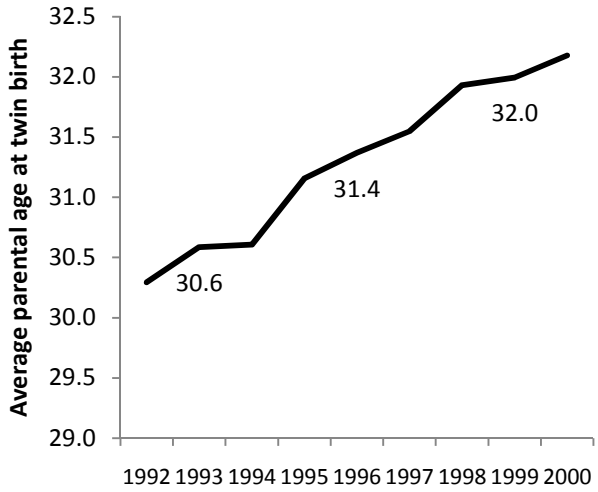
	Recurrence risk (%, 95% CI)	*n* with autism/total n of siblings
All Full-siblings	9.7 (8.7-10.9)	272/2796
SS full-siblings	12.2 (10.5-13.9)	173/1427
SS male full-siblings	13.8 (12.0-15.9)	165/1196
SS female full-siblings	3.5 (1.8-6.7)	8/231
OS siblings	7.2 (6.0-8.7)	99/1369
Male full-siblings of a female proband	18.4 (13.8-24.0)	40/218
Female full-siblings of a male proband	5.1 (4.0-6.6)	59/1151
Half-maternal siblings	3.4(1.6-6.9)	7/208

Figure 1 (Panels A-C): Temporal trends of autism concordance and parental age

Panel A



Panel B



Panel C