

**Words (Manuscript): 3,700**

**Words (Abstract): 236**

**Tables: 3**

**Figures: 2**

**References: 57**

## **Advancing Paternal Age and Risk of Autism**

### **New Evidence from a Population-Based Study and a Meta-Analysis of Epidemiological Studies**

Christina M Hultman<sup>1</sup>, Sven Sandin<sup>1</sup>, Stephen Z. Levine<sup>2</sup>, Paul Lichtenstein<sup>1</sup>, Abraham Reichenberg<sup>3,4</sup>

1 - Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden

2 - Department of Criminology, Bar Ilan University, Israel

3 - Department of Psychosis Studies, Institute of Psychiatry, King's Health Partners, King's College London, UK

4 - Department of Psychiatry, Mount Sinai School of Medicine, New York ,NY, USA

#### **Corresponding author:**

Abraham (Avi) Reichenberg, Ph.D.

Department of Psychosis Studies

*Institute of Psychiatry, King's Health Partners, King's College London*

London, SE5 8Af, UK

Department of Psychiatry

*Mount Sinai School of Medicine*

New York, NY, 10029, USA

**E-mail:** [avi.reichenberg@iop.kcl.ac.uk](mailto:avi.reichenberg@iop.kcl.ac.uk) and [avi.reichenberg@mssm.edu](mailto:avi.reichenberg@mssm.edu)

This work was supported by a grant from the Swedish Council for Working Life and Social Research (guest research fellowship to A Reichenberg). Approval to access the registries was given by the Swedish National Board of Health and Welfare, and the study was approved by a local human subjects committee at the Karolinska Institutet (Stockholm, Sweden).

## ABSTRACT

Advanced paternal age has been suggested as risk factors for autism, but empirical evidence is mixed. This study examines whether the association between paternal age and autism in the offspring (I) persists controlling for documented autism risk factors, including family psychiatric history, perinatal conditions, infant characteristics and demographic variables; (II) may be explained by familial traits associated with the autism phenotype, or confounding by parity; and (III) is consistent across epidemiological studies. Multiple study methods were adopted. First, a Swedish 10 birth-years cohort (N=1,075,588) was established. Linkage to the National Patient Register ascertained all autism cases (N=883). Second, 660 families identified within the birth-cohort had siblings discordant for autism. Finally, meta-analysis included population-based epidemiological studies. In the birth-cohort autism risk increased monotonically with increasing paternal age. Offspring of men 50 years or older were 2.2 times (95% confidence interval: 1.26-3.88:  $p=0.006$ ) more likely to have autism than offspring of men 29 years or younger, after controlling for maternal age and documented risk factors for autism. Within-family analysis of discordant siblings showed that affected siblings had older paternal age, adjusting for maternal age and parity ( $p<0.0001$ ). Meta-analysis demonstrated advancing paternal age association with increased risk of autism across studies. These findings provide the strongest evidence to date that advanced paternal age is a risk factor for autism in the offspring. Possible biological mechanisms include de-novo aberration and mutations or epigenetic alterations associated with ageing.

**Keywords:** Autism, Epidemiology, Paternal age, Perinatal

## INTRODUCTION

Autism is a chronic disorder with onset by age 3, characterized by three main behavioral disturbances: social abnormalities, language impairments, and stereotyped, repetitive patterns of behavior (1). The cause of autism is unknown, however, twin and family studies provide compelling evidence for a strong genetic contribution (2), yet environmental influences may also be etiologically important (3).

This study investigates whether advancing paternal age at birth of offspring is associated with an increased risk of autism in offspring. Older paternal age at childbirth has been associated with several congenital disorders (4). In some cases a direct causal link has been argued (5). Therefore the main reason to examine the relationship between paternal age and autism is that it may provide clues to the biological pathways leading to autism.

Epidemiological findings on the association between advancing paternal age and risk of autism are mixed. Several studies report a strong association (6-10), yet others do not (11-13). The association may be unclear due to methodological limitations. These include high levels of missing-data, selection bias, and lack of statistical control for other risk factors (e.g., age of the other parent, birth order, perinatal complications, or presence of psychiatric disorders in parents). It has also been suggested that the association is due to fathers of autistic individuals delaying paternity because they carry traits associated with the autism phenotype (14). For example, traits such as shyness and aloofness which may limit interactions with women have been described in fathers of autistic children (15, 16).

## Running title: Advancing Paternal Age and Risk of Autism

Here we report data on the association between advancing paternal age and autism using multiple study methods. First, using a birth-cohort we examined whether the association is explained by any of previously identified parental or perinatal autism risk factors. Second, within the birth-cohort a family-based study examined the hypothesis that the association is due to delayed paternity resulting from traits associated with the autism phenotype, or due to confounding by parity. Finally, a meta-analysis examined the extant data on paternal age and autism.

## METHODS

### Birth Cohort Study

#### *Design*

A birth-cohort of all children born in Sweden from January 1, 1983 to December 31, 1992 and followed up until December 31, 2002 was established using data obtained from four Swedish national registries: **Medical Birth Register**, **Multi-generation Register**, **National Patient Register** and **Statistics Sweden**. The registries are detailed in **Table 1**. All Swedish live-born children and new residents are assigned a unique personal identification number. The number is used in all contacts with authorities and national registers rely on this number, ensuring accurate and complete linkage between registries. Some individuals in the analysis were studied regarding perinatal complications and parental psychiatric disorders and autism in case-control studies (10, 17). Unlike past research, the current study addresses different research questions, and uses cohort and family-based designs.

*Autism diagnosis*

All infants and preschool children are regularly seen at well-child care clinics and undergo routine medical and developmental screening. All children aged 4 undergo routine general health screening, that includes mandatory developmental assessment (motor, language, cognitive and social development) conducted by a nurse and pediatrician. Children with any suspected psychiatric disorder (including autism) are referred for further assessment by a specialized team in a child psychiatry unit. During the study period diagnoses were made by hospital-based diagnostic teams with a psychiatrist, clinical psychologist, and speech pathologist or occupational therapist, depending on clinical manifestations. The instruments include parental interviews, cognitive testing of the child, and observations in naturalistic settings, including the home or the unit. Diagnostic information is entered to the Hospital-Discharge-Register.

For the study years International Classification of Diseases, 9<sup>th</sup> (18) and 10<sup>th</sup> revisions (19) (ICD-9 and ICD-10) diagnostic codes were used in national registers. Specific criteria for autism slightly vary between ICD-9 and ICD-10. Both ICD versions, however, explicitly require the presence of three symptom areas of impairment in social interaction, communication and restricted range of interests and behaviors in childhood for the diagnosis of autism. To maximize diagnostic consistency across classification schemes and increase accuracy, the present investigation focuses on the narrow diagnosis of infantile/childhood autism (ICD-9 diagnostic codes 299A or ICD-10 diagnostic codes F84.0–F84.1), and does not include other forms of autism-spectrum disorders. As no specific code of autism was available before ICD-9 was introduced in 1987, the study was restricted to include subjects diagnosed from 1987.

## Running title: Advancing Paternal Age and Risk of Autism

The Hospital-Discharge-Register has shown high reliability for the diagnosis of adult-onset psychiatric disorders (e.g., schizophrenia) (20). Although we were unable to reassess subjects in our cohort because data used for analysis were anonymized, as part of another study we have been assessing the validity of autism diagnosis in the registry. We randomly ascertain cases with autism that appear in the Hospital-Discharge-Register, and implement a validation method developed by the Centers for Disease Control and Prevention (CDC) and utilized in a recent validation study of autism diagnoses in a similar register in Denmark (21-23). Medical records and any other available clinical information for each case are collected according to a systematic coding scheme which is based on the Diagnostic and Statistical Manual 4th Edition (DSM-IV) (1). Information is collected about symptoms regarding social interaction, communication, and stereotyped, restricted, repetitive behaviour. The collected information is reviewed and coded by two experts in autism diagnosis, and a consensus diagnosis is determined. For subjects who were born during the years of the present study cohort, diagnostic criteria for autism according to the (DSM)-IV criteria were upheld (methods and results are available from the authors on request).

### *Selected covariates*

Potential covariates were identified from a systematic review of epidemiological evidence for demographic, parental, perinatal and infant characteristics and autism risk (24), and selected for the analysis if they enhanced comparability with previous studies, or were recognized as potential confounders. Covariates included paternal education level, parental country of birth, parental psychiatric hospitalization history, maternal age, birth weight, birthweight for gestational age, fetal distress, and parity (see also **Table 2**). Year of birth was also included as a

## Running title: Advancing Paternal Age and Risk of Autism

covariate following recent debate about the potential confounding of paternal age and autism associations due to cohort effects (25-27).

### *The analytic cohort*

Individuals born in Sweden (n=1,075,588) over the 10-year period were identified through the Medical Birth Register. The Multigeneration-Register permitted the identification of parents, and to obtain information on the parents' birth-date, socioeconomic status, and national origin from Statistics Sweden. Through linkage with the Hospital-Discharge-Register information was obtained on psychiatric history in both children and their parents. 14,025 cohort members had a psychiatric diagnosis other than autism in the Hospital-Discharge-Register, and were excluded from the analysis to avoid possible bias (28). A further 2.5% of the entire cohort had missing data on one or more covariates, leaving 1,035,487 individuals in the analytic cohort. Patterns of missing data did not differ between non-psychiatrically-hospitalized controls and autism cases. Percent missing data on a single covariate (1.3% vs 1.7%), and two or more covariates (1.2% vs 0.9%), respectively, were similar.

### *Data analysis*

The association between paternal age and offspring autism was first examined using splines (29). A spline model estimates the response relation without assuming that the data follow a particular form, such as linear or cubic. This allows for an evaluation of the functional form of associations, and characterization of risk pattern including points of non-linearity (threshold effects).

## Running title: Advancing Paternal Age and Risk of Autism

We then examined paternal age using a categorical measure to allow for a nonlinear effect of paternal age on the risk of autism. Based on the spline model, paternal age was categorized into the following age groups: 15 to 29 (referent category), 30 to 39, 40 to 49, and 50 years or older. In a secondary analysis we further extended the paternal age categories to 50 through 54 and 55 or older. We fitted GEE (Generalized Estimating Equations) regression models (30, 31). Odds ratio (OR) and associated two-sided 95% Wald type confidence intervals (CIs) were computed, and significance level was set at  $p < 0.05$  (2-sided). GEE modeling is robust since it requires no assumptions about data distribution, and adjusts for correlations among siblings (55% of the cohort). For the unadjusted results, we fitted paternal age as the only predictor of autism. For the adjusted results, maternal age was first included in the model. A subsequent model included maternal age, and the additional a-priori selected covariates. Analyses were conducted using the SAS software version 9.2 on an AIX mainframe.

### Family Based Study

We modeled the parental age association with autism in a subset of families from the birth cohort with an autistic child and at least one non-autistic sibling. This study was carried for three reasons. First, to robustly test the paternal age effect: Family conditions are more similar for siblings than for unrelated children, which provide important control of confounding. Second, to carefully test the possibility that the paternal age effect pertains only to first born children, and consideration of whether ‘stoppage’ (i.e., after recognizing that a child has a neurodevelopmental problem stopping having subsequent children) might create an artefact of increased likelihood that a child with autism from an older father will also have a low birth order (6). Third, to address the hypothesis that fathers of autistic individuals delay paternity because of traits associated with the autism phenotype. An association between advancing

## Running title: Advancing Paternal Age and Risk of Autism

paternal age and autism within affected families would suggest that deferred paternity due to genetic autism-related traits is unlikely to explain advancing paternal age effect in autism.

### ***Data analysis***

To compare paternal age between autistic and non-autistic siblings linear mixed effect models were fitted allowing for correlated response between successive births within each family.

Means and standard deviations were computed. In subsequent models maternal age and parity were added as covariates.

To further explore the paternal age and parity effects a Generalized Linear Mixed-effect Model (GLMM) was fitted (32). This analysis modeled the conditional probability of an autistic child in families with an autistic child and at least one more child as a function of two parameters: (1) paternal age at the time of birth of the father's 1<sup>st</sup> child (whether this child was autistic or not) and (2) number of years since the birth of the 1<sup>st</sup> child (*For model specification see supplemental material*). If a statistically-significant effect is observed for the second parameter in the model this will provide evidence for a paternal age effect beyond the first child.

### **Meta-Analysis**

Meta-analysis, based on recommended guidelines (33), was used to examine pooled present-study and all previous peer-reviewed epidemiological results on the association between paternal age and autism.

Data sources: Published peer-reviewed studies were identified through a variety of methods that included: (1) computerized MEDLINE and PsycINFO searches for English-language

## Running title: Advancing Paternal Age and Risk of Autism

biomedical articles that examined prenatal and perinatal conditions in autism up to August 2010. This follows the recommendation to search multiple databases to maximize the number of relevant citations (34) (for search terms see *Supplemental Material*); (2) screening reference lists of original articles; and (3) manual search of relevant journals likely to publish such epidemiological studies. When necessary, authors were contacted with requests for additional study information.

Study selection: Studies were included in the meta analysis if they fulfilled the following inclusion criteria: (1) a well-defined sample of cases drawn from population-based registry or cohort; (2) comparison subjects drawn from the general population with information on parental age obtained from the same source; (3) use of a standardized format for presentation of data, allowing for comparisons between studies and calculation of crude odds ratios (ORs); and (4) presentation of results for paternal age.

### ***Data analysis***

Data was analyzed using standard meta-analytic modeling in R statistical software and the R-Meta package (35). Weighted odds ratios (ORs) and 95% CIs were calculated. Three sensitivity analyses were conducted including the removal of studies with (a) the largest magnitude of association; (b) that included autism and high rates of autism spectrum disorders (6, 7, 36); and (c), high rates of missing data on paternal age (6, 8, 13).

## **RESULTS**

### **Birth-Cohort Study**

Among all individuals in the birth-cohort the rate of autism was 8.3 cases per 10,000 persons (860 cases), increasing monotonically from 6.5 cases per 10,000 persons to 9.2 cases per 10,000

## Running title: Advancing Paternal Age and Risk of Autism

persons between 1983 and 1992. Two epidemiological surveys of autism prevalence that applied robust case-ascertainment methods were conducted in western Sweden during the early and late years of the birth-cohort (37, 38). The prevalence rates reported in those surveys (5.6 and 9.1 per 10,000, respectively) are similar to those in the national register, supporting the registry's representativeness. Also, the total rate is consistent with a review of epidemiological studies of autism reporting a median rate of 9.5 per 10,000 in those decades (39).

Characteristics of offspring with or without autism in relation to demographic, parental, perinatal and infant characteristics (covariates) are presented in **Table 2**. After controlling for the effects of all other characteristics and paternal age, risk of autism was independently associated with paternal birth in non-Western and non-Scandinavian countries; paternal and maternal psychiatric hospitalization; the offspring being born small-for-gestational age and intrauterine hypoxia or birth asphyxia (**Table 2**). Risk of autism was not associated with paternal education level, maternal age, maternal birth in non-Western and non-Scandinavian countries, low birth weight or parity.

### Paternal age

Spline regression was implemented to obtain a detailed risk assessment pattern (**Figure 1A**). This indicated that risk started to increase at the paternal of age 30, showed a plateau after age 40 and further increased from the age of 50.

Based on the spline regression, the effect of paternal age on autism risk was examined using 10-year age categories. Unadjusted and adjusted ORs for each category, relative to the group aged 15 to 29 years were computed (**see Table 3**). There was a statistically significant increase in the

## Running title: Advancing Paternal Age and Risk of Autism

risk of autism with advancing categories of paternal age. For example, fathers older than 50 years had 2.7 times increased risk of having an offspring with autism. The association between paternal age and risk of autism persisted after controlling for maternal age (**Table 3**). The association persisted while also controlling for the effects of parental psychiatric history, perinatal conditions and infant characteristics, year of birth and socioeconomic status (**Table 3**). Paternal - compared to maternal - wider reproductive age did not explain the observed association (see *Supplemental Material Tables S1-S3*).

When the effect of paternal age on autism risk was estimated in the extended paternal age groups (paternal age, 50 to 54 years and  $\geq 55$  years), increased risk was most marked in the oldest paternal age group. Fathers older than 55 years had 4.4 times increased risk of having an offspring with autism, after controlling for the effects of maternal age, parental psychiatric history, perinatal conditions and infant characteristics, year of birth and socioeconomic status (**Table 3**). Caution is warranted regarding the latter risk estimate due to low precision indicated by the wide 95% confidence intervals. Nevertheless, the results suggest that the relationship between paternal age and risk of autism is monotonic but possibly nonlinear. A formal analysis of a nonlinear monotonic association was not conducted because the sample was too small to permit a reliable statistical test. Inclusion of the cohort members who had a psychiatric diagnosis other than autism in the analytic cohort did not change the reported risk estimates related to paternal age.

### Family-based Study

The paternal age effect was examined within a subset of families of individuals with autism, who had at least one more non-autistic sibling in the birth cohort (N=660 families). Within

## Running title: Advancing Paternal Age and Risk of Autism

these families, paternal age when the offspring with autism was born was higher than the paternal age at the time the unaffected sibling/s were born [Mean age:  $32.7 \pm 6.3$  vs.  $30.8 \pm 6.4$  in affected and unaffected siblings, respectively,  $t(653)=10.96$ ,  $p<0.0001$ ]. The difference between affected and unaffected siblings in paternal age remained statistically significant after adjustment for maternal age [ $t(652)=5.52$ ,  $p<0.0001$ ]. The difference between affected and unaffected siblings in paternal age remained statistically significant also after adjustment for parity [ $t(629)=6.84$ ,  $p<0.0001$ ]. For convergence this analysis required restriction of birth order to be equal or lower than 4 thus slightly reducing the number of degrees of freedom.

The GLMM model showed statistically significant effects of father's age at the time the 1<sup>st</sup> child was born [ $F(3,631)=2.40$ ,  $p=0.049$ ], and of time since the birth of the 1<sup>st</sup> child [ $F(5,929)=12.01$ ,  $p<0.001$ ] demonstrating that paternal age does not pertain only to first born children. For descriptive purposes the modeled conditional probability of autism in the offspring was plotted as a function of father's age at the time of birth of the 1<sup>st</sup> child, and years since birth of 1<sup>st</sup> child (i.e., paternal age at subsequent births) in five paternal age categories (**Figure 1B**). Risk of autism at the time of birth of the 1<sup>st</sup> child increased monotonically with increasing categories of paternal age. Risk of autism continued to increase with each additional year of paternal age within all but the last age category. The latter observation suggests that stoppage may occur, but only in the oldest fathers, and is therefore unlikely to cause substantial artefactual effects.

### Meta-analytic Study

Twelve articles from 7 different countries (6-8, 10-13, 26, 36, 40-42) fulfilled all 4 inclusion criteria (for studies characteristics see *Supplemental Material Table S4*). Two studies were excluded from the meta-analysis although both demonstrated a statistically significant association

## Running title: Advancing Paternal Age and Risk of Autism

between advancing paternal age and autism. The study from Sweden (10) was excluded because of concerns for under-ascertainment of autism cases from the study's source population due to changes in autism services in Sweden during the time-period of the study (see reference 10, pg. 1360). The study from the US (41) was excluded because it substantially overlapped with another study (26) and the latter examined a considerably larger cohort.

**Figure 2 (A-C)** depicts results of the meta-analysis conducted on the association between paternal age and autism. Between-study variance was low (less than 0.1), but two of the tests for heterogeneity across studies were statistically significant ( $p < 0.05$ ), and therefore results of the random effects models are reported. Compared to fathers aged 29 years or younger, the random effects pooled estimates of risk of autism were as follows: 1.22 for offspring of fathers 30 to 39 years old (95%CI: 1.05-1.42); 1.78 for offspring of fathers 40 to 49 years old (95%CI: 1.52-2.07), and 2.46 for offspring of fathers 50 years or older (95%CI: 2.20-2.76). Sensitivity analyses did not attenuate the original results of the meta-analysis.

## DISCUSSION

### Paternal age as a risk factor for autism

The present investigation evaluated the hypothesis that risk of autism in the offspring increases with advancing paternal age. First, using data from a birth cohort, controlling for a range of documented risk factors, including parental, perinatal and socioeconomic variables and year of birth, the results demonstrated a strong monotonic relationship between increasing paternal age at birth of offspring and risk of autism in offspring. Second, pooled results of meta-analysis were consistent with this effect. These findings from multiple large data sources provide

## Running title: Advancing Paternal Age and Risk of Autism

substantial evidence toward confirming that paternal age is involved in the development of autism.

It has been suggested that parental traits related to the autism phenotype may explain the association between paternal age and autism. Such traits (e.g., shyness) could manifest as reduced ability for social interaction and may result in an older paternal age (14). Our family-based analysis examined this hypothesis by comparing paternal age in siblings with and without autism. The observed association between older paternal age and autism within families appears to be generally consistent with the paternal age effect in autism not resulting from deferred paternity due to genetic autism-related traits, but is not definitive.

### **Potential etiological mechanisms**

One possible explanation for the paternal age effect is an increased occurrence of spontaneous genomic alterations. It is thought that spermatogonial stem cell divisions occurring over the life-course result in higher mutational rates and cytogenetic abnormalities in the sperm of older men (43, 44). Numerous neurological and psychiatric disorders have been related to genomic alterations (45). Several studies have uncovered an increased prevalence of de-novo copy-number variants (CNVs), and other forms of genomic alterations in autistic children (46, 47). Interestingly, the genomic alterations mechanism predicts that the association between paternal age and autism should not pertain only to first born children. Carefully examined in the family-based study, advancing paternal age was associated with autism risk even when adjusting for birth order, and was evident in first as well as later born children. A descriptive analysis, however, indicated that this effect was not present in the group of fathers 40 years or older.

## Running title: Advancing Paternal Age and Risk of Autism

Thus the within family analysis appears to be generally consistent with the genomic alterations hypothesis, but this is not conclusive.

An alternative explanation is that epigenetic dysfunction underlies some paternal age effects. Epigenetic dysfunction has been associated with several neuropsychiatric disorders (48), and is also implicated in single-gene disorders, including Rett and Fragile X syndromes, characterized by autistic-like features in some patients (45). A study by Flanagan and colleagues (49) reported intra- and inter-individual epigenetic variability in the male germline, and found a number of genes that demonstrated age-related DNA-methylation changes. It is also possible that the accumulated exposure to various environmental toxins over the life-course could result in genomic and/or epigenetic alterations in the germ cells of older parents. Toxins have been shown to induce DNA damage, germline mutations and global hypermethylation in germ cells (50).

The main strength of this study is in combining a large birth-cohort, a family study and a meta-analysis allowing for greater confidence in observed relationships between paternal age and autism outcome. Additionally the youngest member of the cohort was followed up to the age of 10, ensuring coverage of the age range during which autism is likely to be diagnosed. Finally, the statistical models adjusted for correlations among siblings. The findings should, however, be interpreted in light of some limitations. First, we did not examine whether parental age was related to diagnoses within autism spectrum disorders (other than autism). Results may therefore not be generalized to autism spectrum disorders. Second, information was not available about clinical features such as severity of mental retardation and language level. Research suggests that most people with autism also exhibit varying degrees of mental

## Running title: Advancing Paternal Age and Risk of Autism

retardation (51). Whether severity of mental retardation modifies the association between paternal age and autism remains to be examined. Nevertheless, research has reported (52) an association between advancing paternal age and autism in a large sample of autism cases with IQ within-normal-range ( $>70$ ). Third, autism prevalence rates have increased dramatically since the time of the birth-cohort (39). Nevertheless, the association between advancing paternal age and autism has been reported in contemporary cohorts (6, 7). Fourth, information about possible associated medical condition (e.g., Fragile-X, Tuberous Sclerosis) was not available. It is noted, however, that epidemiological studies and meta-analysis have concluded that the overall proportion of autism cases that may be attributed to known medical disorders is low (39, 51). Finally, this study found no evidence for increased autism risk in the offspring of old mothers. Epidemiological studies on the association between advancing maternal age and risk of autism have reported mixed results (6-13, 26, 40, 41, 53, 54). The lack of consistency across studies could be due to limitations of sample size as well as other methodological differences, including autism case definitions and inclusion criteria and the ability to control for important confounders. This heterogeneity in findings requires further examination in a separate series of studies and/or meta-analyses.

## CONCLUSIONS

Based on data from a birth-cohort, a family-based study and a meta-analysis we provide the strongest and most consistent evidence available that advancing paternal age at the time of birth of offspring increases the risk of autism. De novo germline mutations, epigenetic alterations and life course toxic exposure may partly explain the observed association. The evidence is substantial enough to justify a search for the underlying mechanisms in both human and animal models (55, 56).

## Acknowledgments

**Funding/Support:** This work was supported by a grant from the Swedish Council for Working Life and Social Research (guest research fellowship to A Reichenberg).

**Role of the Sponsor:** The Swedish Council for Working Life and Social Research was not involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

**Author Contributions:** Drs Hultman, Reichenberg and Sandin had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Reichenberg, Sandin, Hultman, Lichtenstein.

**Acquisition of data:** Hultman, Sandin.

**Analysis and interpretation of data:** Hultman, Reichenberg, Sandin, Lichtenstein, Levine.

**Drafting of the manuscript:** Hultman and Reichenberg.

**Critical revision of the manuscript for important intellectual content:** Hultman, Reichenberg, Sandin, Lichtenstein, Levine.

**Statistical analysis:** Sandin, Levine, Reichenberg.

**Obtained funding:** Hultman.

**Administrative, technical, or material support:** Hultman, Lichtenstein.

**Study supervision:** Hultman, Reichenberg.

**Table 1:** National registries used for the study

<b>Registry</b>	<b>Description</b>
<b><i>Medical Birth Register</i></b>	The Medical Birth Register, established in 1973, contains data on pregnancy and birth for all births in Sweden, and has been described in detail elsewhere ( <a href="http://www.socialstyrelsen.se/en/">www.socialstyrelsen.se/en/</a> ). More than 95% of the Swedish pregnant population attend antenatal care before the 15th gestational week, and the register covers over 99% of all births. This register includes information collected prospectively, starting with the first antenatal visit through the time when mother and child are discharged from the hospital after delivery. Antenatal care routines are standardized and the information is provided through antenatal, obstetrical, and neonatal records, and classified according to the International Classification of Diseases (ICD) version 8 (57) until 1986, version 9 (ICD-9) (18) from 1987 to 1996, and ICD-10 (19) subsequently.
<b><i>Multi-Generation Register</i></b>	The Multigeneration-Register contains information about the entire Swedish population. Children born after January 1st 1932 are linked to their biological parents. The register comprises 9 million children (index persons), and 11 million unique individuals. Importantly the register includes family information (e.g., identification of parents, siblings and offspring) allowing linkage to other population based registers, which include information on health (e.g. psychiatric hospitalizations), demographic variables (e.g., date of birth, country of origin), and socioeconomic data (e.g., employment, education).
<b><i>National Patient Register</i></b>	Sweden has universal health insurance coverage that guarantees equal access to health services, regardless of employment status or socioeconomic status. The register has a nationwide coverage of inpatient treatment facilities and includes care in psychiatric as well as somatic hospitals. There are no private psychiatric hospitals in Sweden. The Swedish National Patient Register contains details on virtually all psychiatric hospitalizations since 1973, including admission and discharge dates and the discharge diagnosis made by the treating physician. Diagnostic information is coded using the ICD codes. The standard procedure dictates that diagnosis will be given by a consultant (equivalent of an attending) psychiatrist at the time of discharge from hospital. The diagnostic assessment is then forwarded on a computer medium to the National Patient Register. These routines are standardized across Sweden.
<b><i>Statistics Sweden</i></b>	Individual demographic and socio-economic data is maintained by Statistics Sweden (Total Population Register, Education Register and Census) allowing linkage of information on parental socio-economic status, education, country of origin and citizenship.

**Table 2:** Demographic, parental, perinatal and infant characteristics and their association with autism (Data are presented as number (percentage) of children).

Characteristics	Non-Psychiatrically Hospitalized Cohort (N=1,034,627)	Autism Cases (N=860)	Unadjusted OR <sup>4</sup> (95% CI)	Adjusted OR <sup>4</sup> (95% CI)
<b>Demographic and Parental Characteristics</b>				
<i>Paternal Education Level (Indicator of SES)</i>				
Less than primary school (less than 9 years)	55,193 (5.33%)	58 (6.74%)	1.34 (1.02-1.75)	0.99 (0.74-1.32)
Primary school (9 years)	164,271 (15.88%)	126 (14.65%)	0.97 (0.79-1.18)	0.95 (0.77-1.16)
Upper secondary school (10-12 years)	523,860 (50.63%)	417 (48.49%)	1.0	1.0
Higher education (more than 12 years)	291,303 (28.16%)	259 (30.12%)	1.14 (0.98-1.32)	1.13 (0.96-1.33)
<i>Maternal Country of Birth</i>				
Sweden	909,900 (87.94%)	711 (82.67%)	1.0	1.0
Scandinavia or Western	57,845 (5.59%)	54 (6.28%)	1.13 (0.86-1.48)	1.04 (0.77-1.40)
Other	66,882 (6.46%)	95 (11.04%)	1.86 (1.51-2.29)	1.25 (0.91-1.73)
<i>Paternal Country of Birth</i>				
Sweden	904,303 (87.40%)	703 (81.74%)	1.0	1.0
Scandinavia or Western	56,915 (5.50%)	50 (5.81%)	1.09 (0.82-1.44)	1.07 (0.79-1.44)
Other	73,409 (7.10%)	107 (12.45%)	1.89 (1.54-2.31)	1.54 (1.14-2.09)
<i>Maternal Psychiatric Hospitalization<sup>1</sup></i>				
No	984,268 (95.13%)	775 (90.11%)	1.0	1.0
Yes	50,359 (4.87%)	85 (9.89%)	2.11 (1.70-2.63)	1.91 (1.51-2.40)
<i>Paternal Psychiatric Hospitalization<sup>1</sup></i>				
No	978,402 (94.56%)	783 (91.04%)	1.0	1.0
Yes	56,225 (5.44%)	77 (8.96%)	1.58 (1.27-1.98)	1.51 (1.18-1.92)
<i>Maternal age</i>				
15-29	544,062 (52.59%)	422 (49.07%)	1.00	1.0
30-39	467,554 (45.19%)	413 (48.02%)	1.14 (0.99-1.31)	0.98 (0.93-1.16)
≥40	23,011 (2.22%)	25 (2.91%)	1.40 (0.93-2.09)	0.93 (0.59-1.44)
<b>Perinatal and Infant Characteristics</b>				
<i>Birth Weight</i>				
≤1,500gr	6,434 (0.62%)	14 (1.63%)	2.52 (1.51-4.23)	1.15 (0.58-2.28)
1,501-2,500gr	37,432 (3.62%)	56 (6.51%)	1.78 (1.36-2.35)	1.04 (0.71-1.52)
2,501-3,500gr	452,181 (43.70%)	383 (44.53%)	1.0	1.0
3,501-4,500gr	505,804 (48.89%)	374 (43.49%)	0.90 (0.78-1.04)	0.95 (0.82-1.11)
≥4,501gr	32,776 (3.17%)	33 (3.88%)	1.18 (0.82-1.71)	1.04 (0.66-1.64)
<i>Birth Weight for Gestational Age<sup>2</sup></i>				
Small	28,292 (2.73%)	57 (6.63%)	2.52 (1.93-3.29)	2.12 (1.52-2.98)
Normal	973,847 (94.13%)	766 (89.07%)	1.0	1.0
Large	32,488 (3.14%)	37 (4.30%)	1.42 (1.02-1.97)	1.42 (0.95-2.13)
<i>Fetal distress<sup>3</sup></i>				
Yes	23,994 (2.32%)	34 (3.95%)	1.76 (1.26-2.45)	1.44 (1.02-2.05)
No	1,010,633 (97.68%)	826 (96.05%)	1.0	1.0
<i>Parity</i>				
1	425,775 (41.15%)	329 (38.26%)	1.0	1.0
2	365,981 (35.37%)	320 (37.21%)	1.13 (0.97-1.32)	1.14 (0.97-1.34)
3	171,387 (16.57%)	125 (14.53%)	0.94 (0.77-1.16)	0.89 (0.71-1.11)
4+	71,484 (6.91%)	86 (10.00%)	1.51 (1.19-1.92)	1.29 (0.99-1.68)

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

1- Information on psychiatric admission of parents was obtained from the Hospital Discharge Register. A parent was defined as having a psychiatric history if a psychiatric diagnosis had been recorded either before or after the date that autism was diagnosed in the child.

2- Small or large size for gestational age: 2 standard deviations below or above the mean birth weight for the gestational age according to Swedish birth weight standards.

3- Intrauterine hypoxia or birth asphyxia.

4 – Each measure is adjusted for paternal and maternal age and all other variables in the table.

**Table 3:** Association Between Paternal Age and Risk of Autism

Paternal Age Group (years)	Non-Psychiatrically Hospitalized Cohort (N=1,034,627)	Autism Cases (N=860)	Rate	Unadjusted Model (OR and 95%CI)	p-value	Model Adjusted for Maternal Age (OR and 95% CI)	p-value	Model Adjusted for All Potential Confounders <sup>1</sup> (OR and 95% CI)	p-value
15-29	349,122	245	7.0:10,000	1.00	--	1.00	--	1.00	--
30-39	588,075	501	8.5:10,000	1.22 (1.05-1.42)	0.01	1.22 (1.03-1.46)	0.023	1.19 (1.00-1.42)	0.05
40-49	90,009	100	11.1:10,000	1.58 (1.24-2.00)	0.0002	1.58 (1.21-2.09)	0.001	1.42 (1.07-1.87)	0.014
≥50 <sup>#</sup>	7,421	14	18.8:10,000	2.66 (1.55-4.58)	0.0004	2.68 (1.53-4.68)	0.0005	2.21 (1.26-3.88)	0.006
50-54	5,270	6	11.4:10,000	1.60 (0.71-3.63)	0.25	1.61 (0.71-3.76)	0.25	1.34 (0.59-3.05)	0.49
≥55	2,151	8	37.1:10,000	5.27 (2.60-10.67)	<0.0001	5.30 (2.56-10.97)	<0.0001	4.36 (2.09-9.09)	<0.0001

Abbreviations: CI: Confidence Interval; OR: Odds Ratio

1- Adjusted for: Maternal age, maternal country of birth, maternal history of psychiatric illness, paternal country of birth, paternal history of psychiatric illness, birth weight, being small/large for gestational age, fetal distress, SES, birth order, and year of birth of the offspring.

<sup>#</sup> - Includes older age groups

## REFERENCES

1. American-Psychiatric-Association. *Diagnostic and Statistical Manual of Mental Disorders*. Fourth edn. APA: Washington DC, 1994.
2. Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E *et al*. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995 Jan; **25**(1): 63-77.
3. Newschaffer CJ, Fallin D, Lee NL. Heritable and nonheritable risk factors for autism spectrum disorders. *Epidemiol Rev* 2002; **24**(2): 137-153.
4. Lian ZH, Zack MM, Erickson JD. Paternal age and the occurrence of birth defects. *Am J Hum Genet* 1986 Nov; **39**(5): 648-660.
5. Tolarova MM, Harris JA, Ordway DE, Vargervik K. Birth prevalence, mutation rate, sex ratio, parents' age, and ethnicity in Apert syndrome. *Am J Med Genet* 1997 Nov 12; **72**(4): 394-398.
6. Durkin MS, Maenner MJ, Newschaffer CJ, Lee LC, Cunniff CM, Daniels JL *et al*. Advanced parental age and the risk of autism spectrum disorder. *Am J Epidemiol* 2008 Dec 1; **168**(11): 1268-1276.
7. Croen LA, Najjar DV, Fireman B, Grether JK. Maternal and paternal age and risk of autism spectrum disorders. *Arch Pediatr Adolesc Med* 2007 Apr; **161**(4): 334-340.
8. Reichenberg A, Gross R, Weiser M, Bresnahan M, Silverman J, Harlap S *et al*. Advancing paternal age and autism. *Arch Gen Psychiatry* 2006 Sep; **63**(9): 1026-1032.
9. Lauritsen MB, Pedersen CB, Mortensen PB. Effects of familial risk factors and place of birth on the risk of autism: a nationwide register-based study. *J Child Psychol Psychiatry* 2005 Sep; **46**(9): 963-971.
10. Daniels JL, Forssen U, Hultman CM, Cnattingius S, Savitz DA, Feychting M *et al*. Parental psychiatric disorders associated with autism spectrum disorders in the offspring. *Pediatrics* 2008 May; **121**(5): e1357-1362.
11. Larsson HJ, Eaton WW, Madsen KM, Vestergaard M, Olesen AV, Agerbo E *et al*. Risk factors for autism: perinatal factors, parental psychiatric history, and socioeconomic status. *Am J Epidemiol* 2005 May 15; **161**(10): 916-925; discussion 926-918.

12. Glasson EJ, Bower C, Petterson B, de Klerk N, Chaney G, Hallmayer JF. Perinatal factors and the development of autism: a population study. *Arch Gen Psychiatry* 2004 Jun; **61**(6): 618-627.
13. Maimburg RD, Vaeth M. Perinatal risk factors and infantile autism. *Acta Psychiatr Scand* 2006 Oct; **114**(4): 257-264.
14. Puleo CM, Reichenberg A, Smith CJ, Kryzak LA, Silverman JM. Do autism-related personality traits explain higher paternal age in autism? *Mol Psychiatry* 2008 Mar; **13**(3): 243-244.
15. Piven J. The broad autism phenotype: a complementary strategy for molecular genetic studies of autism. *Am J Med Genet* 2001 Jan 8; **105**(1): 34-35.
16. Bailey A, Palferman S, Heavey L, Le Couteur A. Autism: the phenotype in relatives. *J Autism Dev Disord* 1998 Oct; **28**(5): 369-392.
17. Hultman CM, Sparen P, Cnattingius S. Perinatal risk factors for infantile autism. *Epidemiology* 2002 Jul; **13**(4): 417-423.
18. World-Health-Organization. *International Classification of Diseases, Ninth Revision (ICD-9)*. World Health Organization: Geneva, Switzerland, 1977.
19. World-Health-Organization. *International Statistical Classification of Diseases, Tenth Revision (ICD-10)*. World Health Organization: Geneva, Switzerland, 1992.
20. Ekholm B, Ekholm A, Adolfsson R, Vares M, Osby U, Sedvall GC *et al*. Evaluation of diagnostic procedures in Swedish patients with schizophrenia and related psychoses. *Nord J Psychiatry* 2005; **59**(6): 457-464.
21. Lauritsen MB, Jorgensen M, Madsen KM, Lemcke S, Toft S, Grove J *et al*. Validity of childhood autism in the Danish Psychiatric Central Register: findings from a cohort sample born 1990-1999. *J Autism Dev Disord* 2010 Feb; **40**(2): 139-148.
22. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. *Pediatrics* 2001 Nov; **108**(5): 1155-1161.
23. Madsen KM, Hviid A, Vestergaard M, Schendel D, Wohlfahrt J, Thorsen P *et al*. A population-based study of measles, mumps, and rubella vaccination and autism. *N Engl J Med* 2002 Nov 7; **347**(19): 1477-1482.
24. Kolevzon A, Gross R, Reichenberg A. Prenatal and perinatal risk factors for autism: a review and integration of findings. *Arch Pediatr Adolesc Med* 2007 Apr; **161**(4): 326-333.

25. King MD, Fountain C, Dakhllallah D, Bearman PS. Estimated autism risk and older reproductive age. *Am J Public Health* 2009 Sep; **99**(9): 1673-1679.
26. Grether JK, Anderson MC, Croen LA, Smith D, Windham GC. Risk of autism and increasing maternal and paternal age in a large north American population. *Am J Epidemiol* 2009 Nov 1; **170**(9): 1118-1126.
27. Reichenberg A, Gross R, Sandin S, Susser ES. Advancing paternal and maternal age are both important for autism risk. *Am J Public Health* 2010 May; **100**(5): 772-773; author reply 773.
28. Rothman KJ, Greenland S. *Modern Epidemiology*. 2 edn. Lippincott Williams & Wilkins, 1998, 737pp.
29. Hastie TJ, Tibshirani RJ. *Generalized Additive Models*. Chapman & Hall: New-York, NY, USA, 1990.
30. Liang KY, Zeger SL. Longitudinal Data Analysis Using Generalized Linear Models. *Biometrika* 1986; **73**: 13-22.
31. Diggle PJ, Liang KY, Zeger SL. *Analysis of longitudinal data*. Oxford Science Publications: Oxford, UK, 1994.
32. Pawitan Y. *In All Likelihood: Statistical Modelling and Inference Using Likelihood*. Oxford University Press: Oxford, UK, 2001.
33. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D *et al*. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000 Apr 19; **283**(15): 2008-2012.
34. McDonald S, Taylor L, Adams C. Searching the right database: a comparison of four databases for psychiatry journals. *Health Libr Rev* 1999; **16**: 151-156.
35. Lumley T. R-Meta Package. 2.14 ed, 2008.
36. Sasanfar R, Haddad SA, Tolouei A, Ghadami M, Yu D, Santangelo SL. Paternal age increases the risk for autism in an Iranian population sample. *Mol Autism*; **1**(1): 2.
37. Bohman M, Bohman IL, Bjorck PO, Sjöholm E. Childhood psychosis in a northern Swedish county: Some preliminary findings from an epidemiological survey. In: Schmidt MH, Remschmidt H (eds). *Epidemiological Approaches in Child Psychiatry*. Georg Thieme Verlag: Stuttgart, 1983, pp 164-173.

38. Gillberg C, Steffenburg S, Schaumann H. Is autism more common now than ten years ago? *Br J Psychiatry* 1991 Mar; **158**: 403-409.
39. Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord* 2003 Aug; **33**(4): 365-382.
40. Lundstrom S, Haworth CM, Carlstrom E, Gillberg C, Mill J, Rastam M *et al.* Trajectories leading to autism spectrum disorders are affected by paternal age: findings from two nationally representative twin studies. *J Child Psychol Psychiatry* 2010 Feb 26.
41. Shelton JF, Tancredi DJ, Hertz-Picciotto I. Independent and dependent contributions of advanced maternal and paternal ages to autism risk. *Autism Res* 2010 Feb; **3**(1): 30-39.
42. Bilder D, Pinborough-Zimmerman J, Miller J, McMahon W. Prenatal, perinatal, and neonatal factors associated with autism spectrum disorders. *Pediatrics* 2009 May; **123**(5): 1293-1300.
43. Crow JF. The origins, patterns and implications of human spontaneous mutation. *Nat Rev Genet* 2000 Oct; **1**(1): 40-47.
44. Buwe A, Guttenbach M, Schmid M. Effect of paternal age on the frequency of cytogenetic abnormalities in human spermatozoa. *Cytogenet Genome Res* 2005; **111**(3-4): 213-228.
45. Reichenberg A, Mill J, MacCabe J. Epigenetics, Genomic Mutations and Cognitive Function. *Cognitive Neuropsychiatry* In Press.
46. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J *et al.* Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008 Feb; **82**(2): 477-488.
47. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T *et al.* Strong association of de novo copy number mutations with autism. *Science* 2007 Apr 20; **316**(5823): 445-449.
48. Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L *et al.* Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am J Hum Genet* 2008 Mar; **82**(3): 696-711.
49. Flanagan JM, Pependikyte V, Pozdniakovaite N, Sobolev M, Assadzadeh A, Schumacher A *et al.* Intra- and interindividual epigenetic variation in human germ cells. *Am J Hum Genet* 2006 Jul; **79**(1): 67-84.

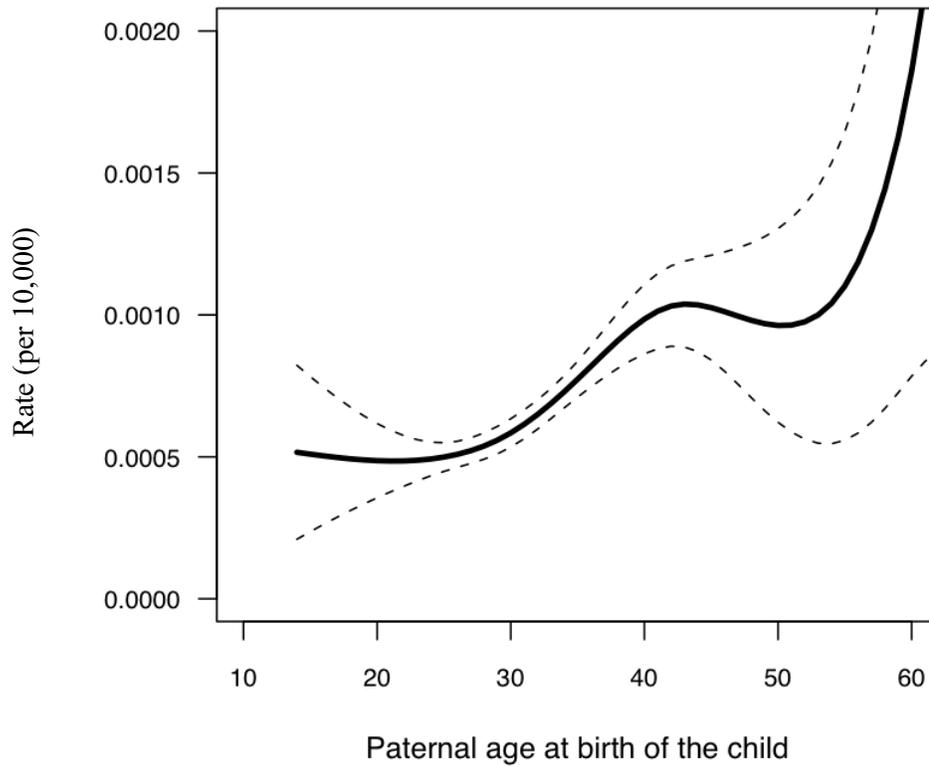
50. Yauk C, Polyzos A, Rowan-Carroll A, Somers CM, Godschalk RW, Van Schooten FJ *et al.* Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *Proc Natl Acad Sci U S A* 2008 Jan 15; **105**(2): 605-610.
51. Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children. *JAMA* 2001 Jun 27; **285**(24): 3093-3099.
52. Tsuchiya KJ, Matsumoto K, Miyachi T, Tsujii M, Nakamura K, Takagai S *et al.* Paternal age at birth and high-functioning autistic-spectrum disorder in offspring. *Br J Psychiatry* 2008 Oct; **193**(4): 316-321.
53. Croen LA, Grether JK, Selvin S. Descriptive epidemiology of autism in a California population: who is at risk? *J Autism Dev Disord* 2002 Jun; **32**(3): 217-224.
54. Eaton WW, Mortensen PB, Thomsen PH, Frydenberg M. Obstetric Complications and Risk for Severe Psychopathology in Childhood. *Journal of Autism and Developmental Disorders* 2001; **31**(3): 279-285.
55. Smith RG, Kember RL, Mill J, Fernandes C, Schalkwyk LC, Buxbaum JD *et al.* Advancing paternal age is associated with deficits in social and exploratory behaviors in the offspring: a mouse model. *PLoS One* 2009; **4**(12): e8456.
56. Foldi CJ, Eyles DW, McGrath JJ, Burne TH. Advanced paternal age is associated with alterations in discrete behavioural domains and cortical neuroanatomy of C57BL/6J mice. *Eur J Neurosci* Feb; **31**(3): 556-564.
57. World-Health-Organization. *Manual of the International Statistical Classification of Diseases, Eight Revision (ICD-8)*. World Health Organization: Geneva, Switzerland, 1967.

## Figures Legends

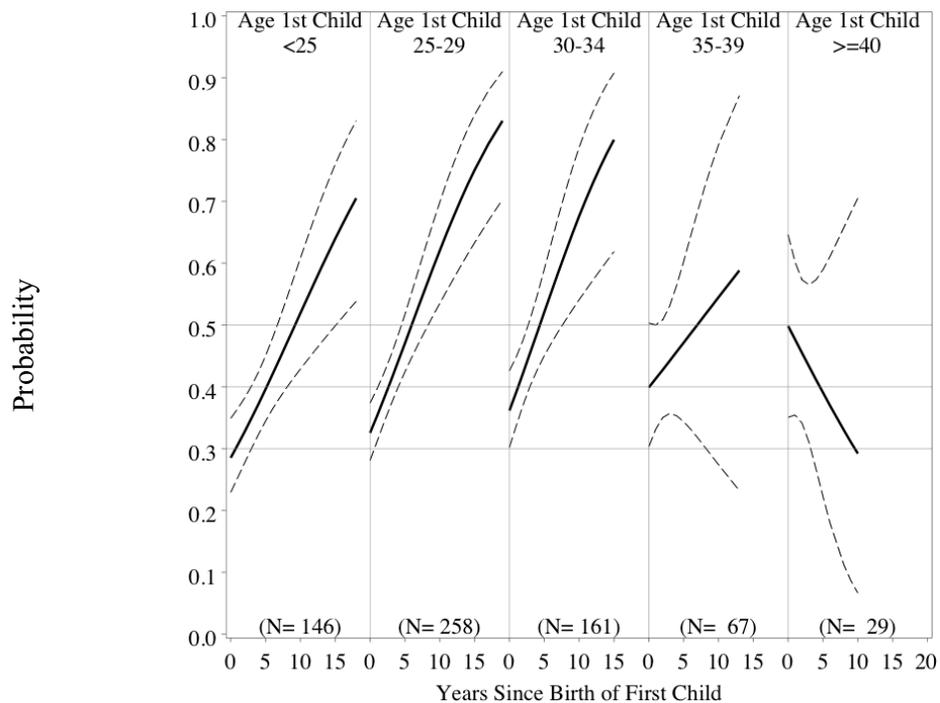
**Figure 1:** Autism According to Paternal Age at Birth of Offspring. Presented are: (A) Univariate rates in the birth-cohort calculated by spline model. (B) Conditioned probability for having an autistic child as a function of father's age at the time of birth of the first child and number of years since the birth of the first child in the family study (families with an autism child and at least one more non-autistic sibling). Solid line represents point estimates and dashed lines represent 95% confidence intervals.

**Figure 2:** Meta-analysis pooling results across 11 independent studies (providing data from 12 cohorts) summarizing the association between advancing parental age and risk of autism. Presented are forest plots of odds ratios and 95% confidence intervals comparing: **A.** Paternal age 30-39 to 29 and below. **B.** Paternal age 40-49 to 29 and below. **C.** Paternal age 50 and above to 29 and below (only 6 studies (providing data from 7 cohorts) provided information for this analysis). (Symbols are proportional to sample size)

**A.** Spline model: Univariate autism rates



**B.** Conditioned probability for having an autistic child as a function of father's age at the time of birth of the first child and number of years since the birth of the first child.



**A. Paternal Age: 30-39 vs. 29 or younger**

UK (94-96)  
Sweden (92-98)  
USA-Utah (94)  
Denmark (90-99)  
USA (94)  
Denmark (73-94)  
Australia (80-95)  
Sweden (83-92)  
Israel (80-85)  
Iran (98-00)  
California (89-02)  
California (95-99)  
**Average**

**B. Paternal Age: 40-49 vs. 29 or younger**

UK (94-96)  
Sweden (92-98)  
USA-Utah (94)  
Denmark (90-99)  
Sweden (83-92)  
Iran (98-00)  
USA (94)  
Denmark (73-94)  
Australia (80-95)  
California (89-02)  
California (95-99)  
Israel (80-85)  
**Average**

**C. Paternal Age:  $\geq 50$  vs. 29 or younger**

Australia (80-95)  
Denmark (90-99)  
Sweden (92-98)  
California (89-02)  
Sweden (83-92)  
UK (94-96)  
Israel (80-85)  
**Average**

