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Trajectories leading to autism spectrum disorders are affected by paternal age: findings from two nationally representative twin studies

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Background: Despite extensive efforts, the causes of autism remain unknown. Advancing paternal age has been associated with various neurodevelopmental disorders. We aim to investigate three unresolved questions: (a) What is the association between paternal age and autism spectrum disorders (ASD)?; (b) Does paternal age moderate the genetic and environmental etiological factors for ASD? (c) Does paternal age affect normal variation in autistic-like traits? **Methods:** Two nationally representative twin studies from Sweden (n = 11, 122, assessed at age 9 or 12) and the UK (n = 13, 524, assessed at age 9) were used. Categorical and continuous measures of ASD, autistic-like traits and autistic similarity were calculated and compared over paternal age categories. **Results:** Both cohorts showed a strong association between paternal age and the risk for ASD. A U-shaped risk association could be discerned since the offspring of both the youngest and oldest fathers showed an elevation in the risk for ASD. Autistic similarity increased with advancing paternal age in both monozygotic and dizygotic twins. Both cohorts showed significantly higher autistic-like traits in the offspring of the youngest and oldest fathers. **Conclusions:** Phenomena associated with paternal age are clearly involved in the trajectories leading to autistic-like traits and ASD. Mechanisms influencing the trajectories might differ between older and younger fathers. Molecular genetic studies are now needed in order to further understand the association between paternal age and ASD, as well as normal variation in social, language, and repetitive behaviors in the general population. **Keywords:** Autism spectrum disorders, paternal age, autistic traits, behavioral genetics.

Autism spectrum disorders (ASD) are chronic syndromes characterized by social abnormalities, communication impairments, and stereotyped and repetitive patterns of behavior (Wing, 1981). To qualify for a diagnosis of autistic disorder all three of these must be present to a considerable degree (APA, 1994). ASD also include Asperger's disorder, pervasive development disorder not otherwise specified (PDD-NOS) and disintegrative disorder, which represent variations in manifestation of the triad of impairments.

The causes of ASD are unknown; however, results from twin and family studies provide compelling evidence for a strong genetic contribution (Folstein & Rutter, 1978; Constantino & Todd, 2003; Ronald, Happe, Price, Baron-Cohen, & Plomin, 2006). Investigations of non-heritable factors suggest that environmental influences may also be pathogenetically important (Ronald, Happé, Bolton, et al., 2006). Advancing paternal age may provide clues to the biological pathways leading to ASD. For example, some etiological factors in ASD are related to de novo mutations and chromosomal abnormalities (Eichler & Zimmerman, 2008; Jamain et al., 2003; Sebat et al., 2007). Advancing paternal age has been associated with increased rates of de novo genetic mutations in the germlines of older fathers (Walter, Intano, McCarrey, McMahan, & Walter, 1998), and several studies have reported an association between advancing paternal age and narrowly defined autism (Durkin et al., 2008; Reichenberg et al., 2006). Other studies, however, did not find such an association (Larsson et al., 2005). A definitive conclusion about the hypothesized association between paternal age and ASD is thus precluded.

If advancing paternal age is indeed related to pathogenetic processes in ASD, variations in paternal age may influence the degree to which genetic and environmental influences play a role in ASD. This can be examined using the twin methodology comparing the within-pair similarity in ASD severity.

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If the similarity between monozygotic (MZ) and/or dizygotic (DZ) twins varies with advancing paternal age, paternal age possibly moderates genetic and environmental effects.

Conceptualizing impairments characteristic of ASD as dimensions rather than categories has been proposed by several research groups (Anckarsäter et al., 2008; Constantino & Todd, 2003; Gillberg, 1991; Posserud, Lundervold, & Gillberg, 2006). Thus, a diagnosis of ASD is assigned when a certain number of autistic-like traits surpasses a distinct threshold. An unresolved question involves the association between paternal age and autistic-like traits. The dimensional approach to ASD would predict that the effect of paternal age would manifest not only with the categorical disorder, but also more broadly, through an association with autistic-like traits in the general population.

In this study, two large-scale population-based twin cohorts were used for the following aims: (1) examine paternal age as a risk factor for ASD using contemporary cohorts and diagnostic instruments, (2) investigate whether paternal age influences twin similarity for ASD, and (3) assess whether paternal age is associated with variations in autistic-like traits in the general population.

Methods

Cohorts

This study builds on two nationally representative cohorts of twins from Sweden (Child and Adolescent Twin Study in Sweden – CATSS (Lichtenstein et al., 2006)), and the United Kingdom (Twins Early Development Study – TEDS (Oliver & Plomin, 2007)). The characteristics of each cohort are described in detail in Table 1.

Definition of autism spectrum outcome

Autism spectrum outcome in CATSS. Parents were interviewed with the Autism – Tics, AD/HD, and other Co-morbidities inventory (A-TAC) (Hansson, Svanstrom Rojvall, Rastam, Gillberg, & Anckarsäter, 2005), a psychiatric telephone interview. The A-TAC has been clinically evaluated and has good test–retest and excellent inter-rater reliability. The A-TAC has excellent validity for ASD (area under the curve .95 for ASD, Larson et al., 2010) when administered by laymen over the phone. For the outcome of ASD, 12 items were used. Items are based on the DSM-IV criteria for autistic disorder (299.00), modified for interviewing purposes. Items are scored '1' for 'yes', '.5' for 'yes, to some extent' and '0' for 'no'. The cut-off score for possible ASD is 4.5 or higher. Cohort members receiving a score lower than 4.5 were counted as non ASD.

Autism spectrum outcome in TEDS. Parents were given the Childhood Autism Spectrum Test (CAST; Williams et al., 2006). The CAST is a 37-item parental self-completion screening questionnaire. Thirty-one items are scored '1' for ASD positive responses, and '0' for a negative response. The remaining 6 control items are questions on general development and are not part of the autism screening. The cut-off score for possible ASD is 15 or higher. At that cut-off score the CAST has 100% sensitivity and 97% specificity, with a 50% positive predictive value against research diagnosis.

A further follow-up was carried out in families with twins with possible ASD. This allowed for better diagnostic specificity for the outcome of autism. Briefly, the Development and Well-Being Assessment (DAWBA; Goodman, Ford, Richards, Gatward, & Meltzer, 2000), which is a package of interviews, questionnaires and rating techniques designed to generate ICD-10 and DSM-IV psychiatric diagnoses on 5–17-year-olds, was used. Trained lay interviewers conducted a phone assessment with parents. Two experienced clinicians then reviewed the cases and decided independently on diagnostic status of each twin. They then compared their diagnoses and resolved differences whenever possible. For the present study, ASD was defined as receiving a diagnosis of ASD by clinicians following the DAWBA interview. Cohort members receiving a score lower than 15 in the CAST-screening were counted as non ASD.

Informed consent has been appropriately obtained in both studies.

Statistical analysis

Association between paternal age and ASD. The association between paternal age and ASD was examined using generalized estimating equations (GEE) regression models. Odds ratio (OR) and associated two-sided 95% Wald type confidence intervals (CIs) were computed. The GEE model fitting technique is robust since the data need not follow a particular parametric distribution such as Poisson or binomial. The GEE also adjust for correlations among twins. Since previous work has suggested that the paternal age effect might be particularly robust when fathers are older than 50 (Reichenberg et al., 2006), paternal age was categorized

Table 1 Cohort description

Cohort	Country	ASD measure	Participants	*N*	Zygosity
Child and Adolescent Twin Study in Sweden (CATSS; Lichtenstein et al., 2006)	Sweden	Autism – Tics, AD/HD, and other Co-morbidities inventory (A-TAC) (Hansson et al., 2005)	Nation-wide cohort of all 9- and 12-year-old twins born from July 1992 to June 1998, assessed at ages 9 or 12	11,122	30% Monozygotic
Twins Early Development Study (TEDS; Oliver & Plomin, 2007)	UK	The Childhood Autism Spectrum Test (CAST; Williams et al., 2005)	Nationally representative cohort of families with twins born 1994–1996, assessed at ages 8–9	13,524	56% Monozygotic

into the following age groups: younger than 25, 25–34, 35–44, 45–50 and 51 or older. GEE models included maternal age, zygosity, sex and socio-economic status (SES) as covariates.

Moderating role for paternal age in genetic and environmental pathways to ASD. Twin data provided an opportunity to assess whether paternal age moderates the relative contribution of genetic and environmental influences on ASD. In twin studies, genetic influences are implied if the correlation between pairs of MZ twins is greater than the correlation between DZ twins. Tetrachoric correlations were computed, via SAS PROC CORR, for ASD outcome in MZ and DZ twins stratified by paternal age categories. Tetrachoric correlations are the categorical (binary) equivalent for ordinary Pearson correlations between continuous measures. Since there were no twins concordant for ASD that had fathers who were 45 or older at the time of birth, we had to modify our grouping. The following categories of paternal age were used: younger than 25, 25–34, 35–39 and 40 and older.

Paternal age and autistic-like traits. The association between paternal age and autistic-like traits in the general population was examined using linear mixed effects models. Means and standard deviations were computed. Mixed effects models are commonly used for the analysis of continuous outcome measures when adjusting for within-family correlations is required. Paternal age was categorized into the following age groups: younger than 25, 25–34, 35–44, 45–50 and 51 or older. Paternal age categories were the fixed effect, and family was the random effect. Maternal age, zygosity, sex and SES were included as covariates in the models. In both cohorts, ASD cases as defined above were excluded from the analysis.

Results

Association between paternal age and ASD

We first examined the effect of paternal age on the risk of being screen-positive for an ASD. The unadjusted and adjusted ORs for each of the categories, relative to the group aged 25 to 34 years in the two twin studies, are presented in Table 2. There was an increase in the risk of ASD with advancing categories of paternal age in both twin cohorts. For example, fathers older than 50 years had 3 times increased risk of having an offspring with ASD. This association persisted after adjustment for maternal age, zygosity, gender and SES, and was statistically significant in the CATSS cohort; and although not significant ($p = .105$), the UK sample was at a comparable level. In both twin studies there was also evidence for an increase in risk of ASD in young fathers (<25). Therefore, for descriptive purposes we also fitted regression models which examined linear and non-linear relationship between paternal age and ASD. There was evidence for a non-linear, U-shaped association between paternal age and ASD in both cohorts (Sweden $p = .035$, UK $p = .055$, one-tailed), thus, the full sample test of the U-shaped relationship was statistically significant in the Swedish series and a near significant trend in the UK series. There was no association between maternal age and risk of ASD after adjustment for potential confounders (data not shown).

Moderating role for paternal age in genetic and environmental etiologies of ASD

The tetrachoric correlations were higher for MZ than DZ twins in all paternal age groups in both cohorts (Table 3). In addition, there was increased MZ and DZ twin similarity for ASD in groups with higher paternal age. This was reflected by a positive Pearson correlation between paternal age categories and MZ correlation coefficients (Sweden $r = .72$, $p = .14$; UK $r = .94$, $p = .029$), and paternal age categories and DZ correlations coefficients (Sweden $r = .92$, $p = .13$; UK DZ sample was too small to allow formal statistical testing).

Association between paternal age and autistic-like traits

A U-shaped association was observed between paternal age categories and autistic-like traits score in both the Swedish and UK cohorts (Figure 1a). This

Table 2 Associations between paternal age and risk of ASD in two cohorts of twin: A) CATSS (Sweden), and B) TEDS (UK)

Paternal age group, years	Non-ASD cohort	ASD cases	Risk	Unadjusted OR (95% CI)	*p*-value	Adjusted* OR (95% CI)	*p*-value
Sweden							
<25	467	15	32:1000	2.49 (1.34–4.64)	.0038	1.93(.87–4.30)	.105
25–34	6235	80	12:1000	1.00	–	1.00	–
35–44	3690	56	15:1000	1.17 (.81–1.71)	.385	1.21 (.79–1.85)	.380
45–50	358	9	25:1000	1.88 (.78–4.56)	.159	1.90 (.73–4.92)	.185
≥51	108	4	37:1000	3.24 (0.–93–11.19)	.062	3.37 (1.02–11.14)	.046
UK							
<25	467	6	13 :1000	2.16 (.82–5.70)	.118	1.91 (.88–4.17)	.101
25–34	6577	38	6 :1000	1.00	–	1.00	–
35–44	3968	17	4 :1000	.74 (.39–1.37)	.338	.81 (.41–1.58)	.547
45–50	376	3	8 :1000	1.43 (.43–4.73)	.549	1.66 (.47–5.82)	.425
≥51	104	2	19 :1000	2.81 (.37–20.95)	.312	3.59 (.37–34.46)	.26

Note: CI = confidence interval; OR = odds ratio. *OR adjusted for maternal age, zygosity and SES 1–4 (four as highest, based on education).

Table 3 Tetrachoric correlations (95% CI)[1] for autism outcome stratified by paternal age categories in A) CATSS (Sweden), and B) TEDS (UK) twin cohorts. *N* = affected cases

Paternal age group, years	MZ	*N*	DZ	*N*
Sweden				
<25	.71 (.35–.1.00)	6	*	3
25–34	.81 (.65–.96)	16	.35 (.03–.66)	24
35–39	.71 (.40–.1.00)	7	.65 (.40–.89)	15
≥40	.99 (.97–1.00)	9	.70 (.45–.95)	12
UK				
<25	.83 (.35–.98)	8	*	9
25–34	.86 (.71–.94)	41	.32 (–.04–.61)	44
35–39	.88 (.63–.98)	15	.52 (–.03–.86)	13
≥40	.97 (.74–1.00)	8	*	14

[1]Rarity of concordant ASD pairs lead to overlapping confidence intervals between MZ and DZ estimates for almost all age groups.
* No twin pairs concordant for ASD. Tetrachoric correlation could not be calculated.

was supported by statistically significant quadratic associations (p values < .003). Specifically, offspring of old (≥50), and young (<25) fathers had significantly (p < .05) higher autistic-like trait scores compared with offspring of fathers aged 25–34. Similar U-shaped associations were observed for the social, communication and non-social stereotyped and repetitive behavioral domains in the Swedish cohort (Figure 1b-d), and for the communication and non-social stereotypes and repetitive behavioral domains in the UK cohort (Figure1c-d).

Discussion

The findings of this study add to what is known about the association between paternal age and ASD in four ways: First, the study findings replicate previous reports of an association between advancing paternal age and ASD. Second, younger paternal age also appears to be associated with ASD. Third, advancing paternal age appears to influence twin similarity for ASD. Fourth, paternal age is associated with social functioning, language functioning and stereotyped and repetitive behaviors in the general population.

Our study has several strengths. The strength of an epidemiological study lies within its population

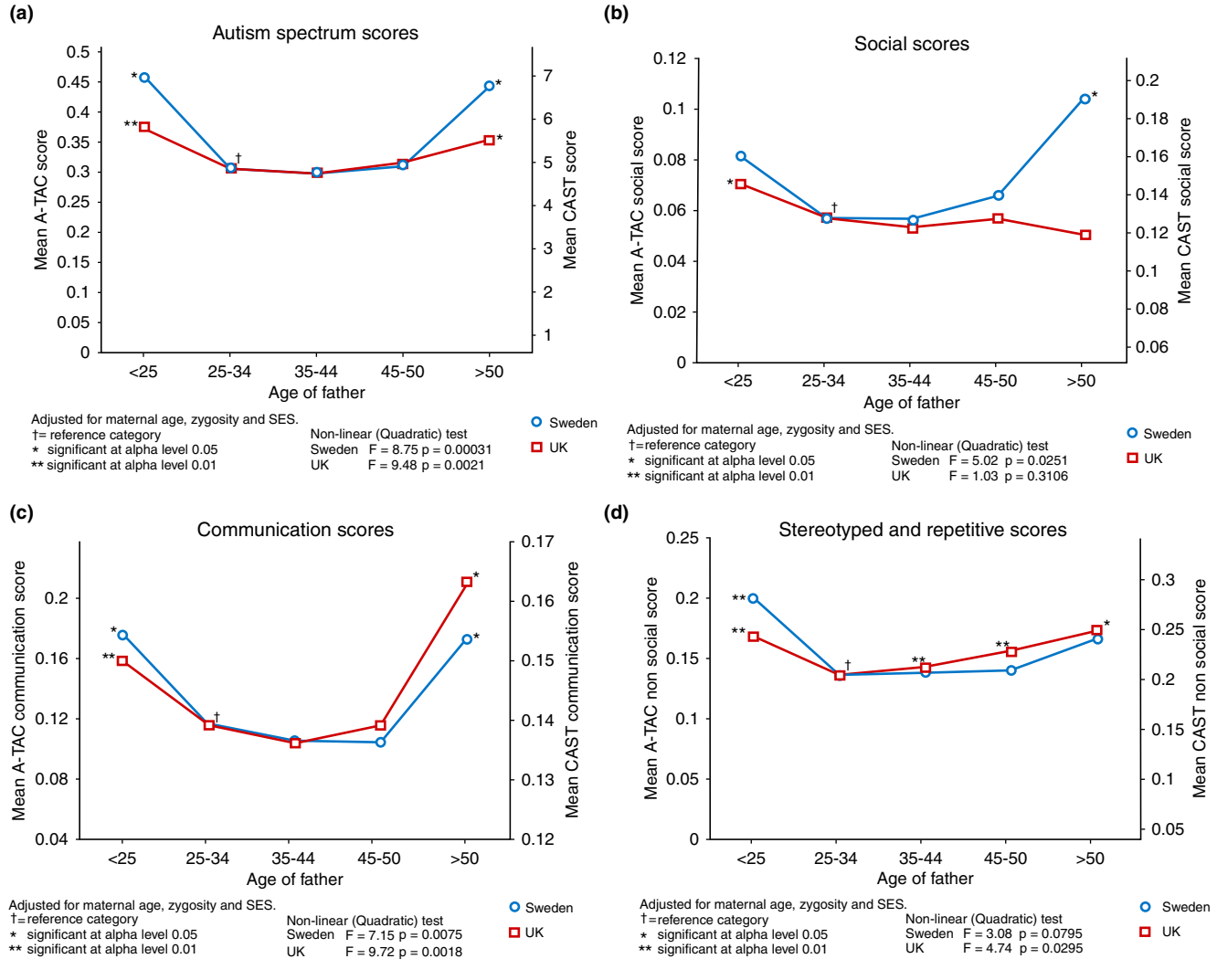

Figure 1 Association between paternal age and autistic like traits in two cohorts of twins: CATSS (Sweden. In blue), and TEDS (UK, in red). Presented are adjusted means for (a) Autistic-like traits total score, and (b) Social, (c) Communication and (d) Non-social stereotyped and repetitive behavioral domains scores

representation. Our two twin cohorts are representative for Sweden and the UK. The study capitalizes on large birth cohorts. This design uses prospective data collection, which contributes to the reliability and validity of information. Furthermore, our design includes different instruments for ASD/autistic-like traits assessment in the different cohorts, but concludes similar results. Taken together, these factors allow for greater confidence in observed relationships between exposure and outcome.

Nevertheless, the results need to be interpreted in the light of several limitations. First, we cannot rule out diagnostic misclassification since the instruments used do not have perfect sensitivity or specificity. However, it is unlikely that paternal age would be associated with differential assessment of ASD. Second, our assessment relies on parental reports as the only data source; complementary teacher and/or child reports would be optimal. Third, we do not differentiate between autistic disorder and other spectrum disorders, nor did we account for clinical features such as intellectual level or language severity. Fourth, our case sample is relatively small. This limits statistical power and increases the risk for type II errors. In addition, due to statistical power limitations we could not perform gender-specific analyses. It would be interesting to explore whether there are sex-specific effects of paternal age (Croen, Najjar, Fireman, & Grether, 2007; Reichenberg et al., 2006). Finally, autistic-like traits in the parents might delay reproduction, and our findings might reflect a genetic familiarity rather than a phenomenon directly associated with increased paternal age. This was, however, investigated in a recent study (Puleo, Reichenberg, Smith, Kryzak, & Silverman, 2008), which indicated that autistic-like traits in the parents did not account for the high paternal age effect on ASD.

With these cautionary notes in mind, we will go on to consider the implications of our findings to molecular genetic studies and to etiological models in ASD.

Advancing paternal age and ASD

One most commonly proposed explanations for the replicated paternal age effect in ASD and other disorders is an increasing rate of *de novo* germline mutations across the reproductive life-course in men. The hypothesis is that the ongoing spermatogonial stem cell divisions in males result in higher mutation rates (Crow, 2000) and cytogenetic abnormalities in the sperm of older men (Buwe, Guttenbach, & Schmid, 2005), although to date no systematic genome-wide survey of such changes has been performed. Studies in mice have shown a significant increase in the overall male germ cell mutation frequency in older fathers (Walter et al., 1998). Interestingly, a number of studies have uncovered an increased prevalence of *de novo* copy number variants (CNVs) in children with autism (Sebat et al., 2007), supporting the notion that novel mutational events occurring in the paternal germline may be important in pathogenesis.

Studies in a number of other disorders that show a clear paternal age effect, however, suggest that mutations in sperm cannot fully explain the association (Tiemann-Boege et al., 2002). An alternative explanation is that epigenetic dysfunction underlies the paternal age effect. 'Epigenetics' refers to the reversible regulation of gene expression mediated principally through changes in DNA methylation and chromatin structure. Interestingly, a study by Flanagan et al. (2006) uncovered significant intra- and inter-individual epigenetic variability in the male germline, and found a number of genes that demonstrated age-related DNA methylation changes. While traditionally thought to be erased and reset during gametogenesis, recent evidence demonstrates that epimutations can be meiotically inherited and thus influence phenotype in an individual's offspring (Hitchins et al., 2007).

In addition to *de novo* mutations/epimutations, it is also possible that the accumulated exposure to various environmental toxins over the life-course could result in germline alterations in older men. Given that several environmental toxins have been shown in mice to induce germline mutations, DNA damage, and global hypermethylation (Yauk et al., 2008), it is highly plausible that such changes could increase with age via cumulative exposure and thus be more prevalent in older fathers.

A pattern of increased MZ *and* DZ twin similarity for ASD in groups with higher paternal age may potentially support the notion that environmentally induced genomic and epigenetic changes are likely to underlie the paternal age effect in ASD. However, due to a small number of cases and overlapping CIs no definite conclusion can be drawn.

Young fathers and ASD

While most focus has been on the effect of advancing paternal age and risk to the offspring, our observation that young paternal age also increases risk for ASD, and is associated with higher autistic-like traits scores, accords with epidemiological observations in several other disorders/traits. Abel, Kruger, and Burd (2002), for example, report a U-shaped risk profile for preterm birth and birth-weight in relation to paternal age. Chen et al. (2008) examined a large retrospective cohort study from the US and found that infants fathered by men younger than 20 years of age had an increased risk of preterm birth, low birth-weight, and various neonatal complications. These observations are interesting given the suggested links between pre- and peri-natal complications and the risk of developing ASD (Kolevzon, Gross, & Reichenberg, 2007).

It is plausible that the mechanisms underlying the effects of younger paternal age are different to those occurring in older fathers. Interestingly, it has been demonstrated that semen quality is lower in younger

men, particularly with regard to morphology (Chen et al., 2008). In addition, young men are more likely to be exposed to certain types of lifestyle risk factors (e.g., drug abuse) which have been linked to *de novo* mutations in the germline and overall sperm quality (Robbins et al., 2005). Alternatively, the offspring of younger parents might harbor a greater susceptibility for neurodevelopmental disorders. That is, younger parents might not be a random sample; younger parents may have elevated rates of psychiatric problems, thus giving rise to the possibility of passive gene–environment correlations where the parental personality influences the early reproduction but also passes on the genetic components for neurodevelopmental disorders.

Pathogenetic models

The diagnosis of autism is made if a child shows a triad of impairments: social difficulties, communication problems, and non-social restricted, repetitive behaviors. Twin studies using TEDS data have reported modest genetic overlap between social, communication and stereotyped and repetitive autistic-like traits when assessed in the general population and at the impaired extreme (Ronald, Happé, Bolton et al., 2006; Ronald, Happé, Price et al., 2006). These previous studies suggest that some degree of pathogenetic influences are common across the triad, but also that there are substantial genetic influences that are specific to each type of autistic-like traits. A family study of multiplex ASD families also reported some evidence for genetic heterogeneity between the autism triad, but with high familial overlap between social motivation and range of interest/flexibility subscales (Sung et al., 2005). Our findings, that paternal age was significantly related to all three autistic-like traits in the Swedish cohort, and two of the autistic-like traits in the UK cohort, suggest that the association with paternal age might involve a pathogenetic mechanism that is shared across the ASD triad.

Conclusions

These findings provide further evidence for the role of paternal age in the pathogenesis of ASD in the offspring. Paternal age was associated with categorical ASD, as well as with autistic-like traits as measured on a continuum, suggesting that molecular genetic studies of paternal age and ASD could be informative for both the clinical disorder and the normal variations in social, language, and repetitive behaviors in the general population.

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Key points

- Advancing paternal age is associated with an increasing risk for autism spectrum disorders in the offspring.
- Autistic-like traits in the normal population are affected by both young and advancing paternal age.
- Autistic similarity within twin pairs seems to increases with advancing paternal age.

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