Review

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Advancing parental age and autism: multifactorial pathways

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Converging evidence from epidemiological, genetic, and animal studies supports the hypothesis that advancing parental age, both of the father and mother, increases the risk of autism spectrum disorders (ASD) in offspring. Paternal age has received considerable attention, with whole-genome sequencing studies linking older fathers to higher rates of *de novo* mutations and increased risk of ASD. The current evidence suggests that the increased risk of ASD in the offspring of older mothers may be related to mechanisms different from those operating in older fathers. Causal pathways probably involve the interaction of multiple risk factors. Although the etiology of ASD is still poorly understood, studies of parental age provide clues into the genetic and environmental mechanisms that mediate the risk of ASD.

The epidemiological evidence

Prevalence estimates of autism spectrum disorders (ASD) have consistently increased across the decades, from five cases per 10 000 persons in the 1980s [1] to the latest CDC estimates of one in 68 [2]. While the prevalence estimates are vulnerable to methodological issues [3], it is undeniable that more cases of ASD are observed now than before. This increase in prevalence has been attributed to a non-mutually exclusive combination of secular trends in factors such as changes in diagnostic practice [4,5] and heightened awareness [6], and/or a true increase in the disorder. Given the apparent increase in ASD prevalence that has occurred in recent decades, risk factors that have experienced parallel increases over the same time-period are of great interest as potential causal factors of the rise in ASD. Advancing parental age has been the subject of considerable attention given the noticeable trend toward delayed reproduction in many countries worldwide. To illustrate, in 1980 the proportion of births from mothers above the age of 35 years in Spain was approximately 14%, but increased to nearly 25% by 2007 [7]. Similarly, in England in 1993, the proportion of births from fathers aged 35–54 was 25%, increasing to 40% by 2003 [8].

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To date, nearly 40 epidemiologic studies across North America, Europe, Asia, and Australia have found evidence linking advancing paternal age (APA) and/or advancing maternal age (AMA) with an increased risk of ASD in the offspring. Individual studies are not wholly consistent in direction or magnitude of findings: various studies have reported increased risk of ASD with both older fathers and older mothers [9]; older fathers but not older mothers [10]; older mothers but not older fathers [11]; or, neither older fathers nor older mothers [12]. The discrepancies in findings are likely influenced by issues such as sample size and characteristics, missing data, case ascertainment, and covariate adjustment. For example, not all studies adjusted for confounders such as socioeconomic status, a key predictor of receipt of ASD diagnosis, or co-parental age, an important covariate necessary to disentangle independent contributions of AMA versus APA (because maternal and paternal ages are highly correlated).

Regardless, the general consensus supported by meta-analysis is that APA and AMA are independently associated with increased risk of ASD, and that a dose–response effect exists such that the risk monotonically increases with parental age. In a 2012 meta-analysis focused on AMA using data from over 25 000 ASD cases and eight million controls aggregated from ten studies, investigators found that mothers $\geq$35 years of age had 1.5-fold increased odds (95% CI: 1.1–1.9) of having a child with ASD compared to mothers 25–29 years old [13]. A similarly large 2010 APA meta-analysis of data from 11 studies found that fathers aged 40–49 had a 1.8-fold (95% CI: 1.5–2.1) increased risk of a child with ASD compared to fathers $\leq$29 years [14]. The results of these two meta-analyses confirm the findings of an earlier, independent meta-analysis [15].

Thus, the question is not *if* advancing parental age is associated with an increased risk of ASD, but rather *how* advancing parental age might increase the risk of ASD. Innovative animal models and advances in sequencing technologies have provided converging lines of evidence, and there are striking parallels in that advancing parental age is associated with increased risk of multiple neuropsychological disorders in offspring, including bipolar disorder, schizophrenia, and hyperkinetic disorders [16]. Nevertheless, several questions remain unanswered. In this review we examine the current evidence concerning mechanisms

linking advancing parental age with ASD and discuss areas for further research. Different mechanisms likely underlie maternal and paternal age-effects, and uncovering such mechanisms will shed light into the genetic and environmental bases of ASD.

Animal models related to APA

Animal studies provide an opportunity to explore clues from epidemiology under controlled, experimental conditions. For example, if APA does cause altered brain development, studying brain-related outcomes in animal models where paternal age is delayed could provide clues to mechanisms of action [17]. We have argued elsewhere that schizophrenia and autism epidemiology needs developmental neurobiology [18].

One early study [19] based on the Wistar rat found that APA (sire age 23 months) was associated with decreased learning capacity in the offspring. Since then, most animal model work related to APA has been based on the mouse. A decrease in learning in the offspring of older sires aged 25–30 months was confirmed, as well as a decrease in motor activity in the offspring of 30 month old sires [20]. Behavioral and brain structural effects have been observed in the offspring of 'younger old' sires as well. Two groups have examined the offspring of sires aged 10–18 months versus control sires aged 2–4 months. In the first study, the offspring of older sires (sire age 10 months) had reduced social activity and reduced exploratory behavior, but no change in locomotion [21]. In another study of offspring of older sires (12–18 months), increased anxiety-related behavior and exploration, and an altered coping strategy to an aversive environment (the Forced Swim test) were observed [22]. This study also examined brain structure with magnetic resonance imaging (*ex vivo* imaging, 16.4 Tesla) and found that the trajectory of cortical development was altered in male offspring of older sires. As neonates, the offspring of older sires had significantly thinner cortices than control males. This appeared to reverse by adulthood. The altered pattern of cortical growth in the offspring of older sires is of interest with respect to the links between APA and ASD because altered cortical growth has been linked to ASD [23,24].

Apart from exploring behavioral and brain structural changes, animal models have provided clues related to the genetic and epigenetic correlates of APA. A mouse model recently identified an increased risk of *de novo* copy-number variants (CNV) in the offspring of older sires [25]. Based on genome-wide microarray screening technology, seven distinct CNVs were identified in a set of 12 offspring and their parents. Competitive quantitative PCR confirmed these CNVs in the original set and also established their frequency in an independent set of 77 offspring and their parents. On the basis of the combined samples, six *de novo* CNVs were detected in the offspring of older sires (12–16 months), whereas none were detected in the control group (sire age 3 months). Two of the CNVs were associated with behavioral and/or neuroanatomical phenotypic features. Curiously, one of the *de novo* mutations was a deletion within *AUTS2*, a gene linked to risk of autism [26]. In recent studies based on brain tissue, investigators found altered gene transcription and DNA methylation in the offspring of older fathers [27,28], supporting that epigenetic changes to brain-expressed loci may potentially underlie the APA effect in neuropsychiatric disorders. These studies, while based on small samples, provide a template for future studies.

However useful animal models are, there may be an upper limit in how much knowledge can be extracted regarding whether advancing parental age influences the risk of ASD. In general, it can be difficult to extrapolate from animal models to humans owing to differences in physiology, anatomy, and neurodevelopmental timing. Considering that a complex human neurobehavioral disorder such as ASD is diagnosed only through careful observation by trained experts, it does not lend itself readily to animal modeling. Finally, animal models of parental age-effects can study only biological mechanisms, whereas parental age in humans is both social and biological in nature, and as such will involve multifactorial pathways that may differ from animals.

Insight into APA effects from genetic and reproductive studies

One of the leading theories regarding the mechanism of action between APA and risk of neurodevelopmental disorders relates to age-related mutagenesis in the male germ cell. While the germ cells of females undergo 22 mitotic cell divisions *in utero*, male germ cells undergo 30 during embryogenesis, then divide every 16 days from puberty onwards [29]. Because the male germ cell undergoes many more cell divisions across the reproductive age range, it has been proposed that copy-errors in the germ cell result in *de novo* mutations in the offspring of older males, with implications for ASD risk (Box 1). Multiple studies utilizing whole-genome or -exome sequencing have found that higher numbers of *de novo* loss-of-function single-nucleotide variants are seen with increasing paternal age [30–34]. While the null expectation is 30–100 *de novo* point mutations for a newborn, about 1–2 additional mutations are seen for each additional year of paternal age [31,35]. Because paternal age and maternal age are highly correlated, more *de novo* mutations naturally would be expected with older maternal age, even if no independent AMA effect existed. To disentangle APA from AMA effects on number of *de novo* mutations, two family-based studies used multiple regression adjusted for co-parental age (i.e., both maternal and paternal ages entered as predictors) and found that APA remained a meaningful predictor of the number of *de novo* mutations, but not AMA [31,35]. These findings are in concordance with a recent analysis of over 2500 simplex families (only one individual affected in the family) that estimated that the large majority (approximately 3:1) of *de novo* nonsense, frameshift, and splice-site mutations are of paternal origin [36], further supporting that *de novo* mutations are primarily a paternal contribution and therefore one potential means by which APA could influence ASD risk.

The evidence for APA effects on *de novo* mutations appears to be more consistent for base-substitution mutations and less so for larger structural variants. Although an earlier mentioned mouse study found evidence that *de novo* CNVs occurred at a greater frequency in the offspring of older fathers [25], APA does not appear to uniformly affect