The Age of the Father

In 1912, Wilhelm Weinberg observed a higher incidence of disease among sporadic cases of achondroplasia in the later-born children of a family [Weinberg, 1912]. The cause of this parental age effect remained a mystery until a correlation was found between maternal age and the risk for children with Down, Patau, or Edwards syndrome due to chromosomal nondisjunctions, leading to trisomy 21, 13, and 18, respectively. Epidemiological studies, on the other hand, indicated both maternal and paternal age effects for autism spectrum disorder (ASD) and other neuropsychiatric disorders [Grether et al., 2009; Buizer-Voskamp et al., 2011]. In a large North American study, a 10-year increase in maternal age was associated with a 38% increase in risk for ASD, and a 10-year increase in paternal age with a 22% increase in ASD risk [Grether et al., 2009]. A population registry-based study in the Netherlands found a significant association with increased paternal age for ASD, schizophrenia, and major depressive disorder, but not for bipolar disorder [Buizer-Voskamp et al., 2011]. Several hypotheses, including de novo mutations, epigenetic alterations, and selection into late fatherhood have been put forward to explain this paternal age effect [de Kluiver et al., 2017].

Children with ASD and probable de novo mutations had a significantly higher mean parental age and paternal age at birth, and the likelihood of a de novo mutation increased with 7–8% per year of parental or paternal age [Geier et al., 2016]. Autistic children with de novo mutations showed significant improvement in sociability with increasing age, whereas patients without de novo mutations showed significant worsening in sociability with increasing age [Geier et al., 2016]. This finding points towards a genetic component in the parental age effect for autism and other neuropsychiatric disorders. In children

with neurodevelopmental disorders, such as ASD, 76% of CNVs originated on the paternal allele [Hehir-Kwa et al., 2011]. Interestingly, this paternal bias was less pronounced and not statistically significant for recurrent CNVs flanked by segmental duplications (64%). This suggests that the molecular mechanisms provoking rare de novo CNVs may be dependent on the parent of origin. Thus, the increase in rate of rare de novo CNVs with paternal age may involve replication-based mechanisms during spermatogenesis [Hehir-Kwa et al., 2011]. This is in agreement with the different biology of the female and the male germline [Goriely and Wilkie, 2012]. Since the germ cells in the developing ovary have essentially completed their proliferative history, maternal mutagenic events consist mostly of chromosome nondisjunction and defects in recombination. In contrast, with the onset of puberty, spermatogenesis requires 23 divisions per year of spermatogonial stem cells during the entire male reproductive life [Goriely and Wilkie, 2012]. Round spermatids of the rat showed a strongly diminished antioxidant defense [Den Boer et al., 1990]. Higher levels of abortive apoptosis, telomere erosion, micronucleus formation, and p53 inactivation have been proposed for the male versus female germline [Pellestor et al., 2011, 2014; Poot and Haaf, 2015]. Sequencing entire genomes of parent-offspring trios from the general population and from families with a single ASD patient corroborated the paternal bias for de novo mutations and their increase with advancing paternal age at conception [Kong et al., 2012; O’Roak et al., 2012]. Whole genome sequencing of 200 ASD parent-child trios confirmed that the majority of germline de novo mutations (75.6%) originated from the father and increased significantly with paternal age only [Yuen et al., 2016]. In ASD patients versus healthy con-

trols, predicted damaging de novo mutations were found significantly more often in intergenic noncoding (15.6%) and genic noncoding (22.5%) regions. Untranslated regions of genes, boundaries involved in exon-skipping, and DNase I hypersensitive regions were the most enriched for these de novo mutations. Interestingly, 2 out of 185 (1.1%) of the ASD cases showed aberrant DNA methylation profiles. These 2 individuals also carried de novo mutations in the ASD-risk and ASD-epigenetic genes *DNMT3A* and *ADNP* [Yuen et al., 2016]. The latter finding points towards a possible involvement of hitherto under-recognized epigenetic inheritance in ASD.

To study epigenetic inheritance as a possible mechanism underlying the paternal age effect, Atsem et al. [2016] selected *DRD4*, *NCOR2*, *NKX2-5*, *KCNA7*, *GET4*, *DMPK*, *PDE4C*, *TNXB*, and *FOKK1* from a set of genes with age-related changes in DNA methylation because of their association with neuropsychiatric and other disorders [Jenkins et al., 2014]. To calibrate their data, repetitive LINE-1 elements, comprising roughly 17% of the human genome, were used as a surrogate marker for global DNA methylation. Average methylation levels of these 10 loci in 162 IVF/ICSI sperm samples were quantified by bisulfite pyrosequencing. The *DMPK*, *FOKK1*, *KCNA7*, and *NCOR2* genes showed significant decreases in DNA methylation with advancing paternal age. *FOKK1* and *KCNA7* revealed the strongest decrease in DNA methylation, with correlation coefficients of -0.35 and -0.34 , respectively. The other 5 genes and the LINE-1 elements did not show a significant correlation between average DNA methylation and paternal age. In a second cohort of 188 independent sperm samples, both *FOKK1* ($r^2 = -0.25$; $p < 0.001$) and *KCNA7* ($r^2 = -0.37$; $p < 0.001$) DNA methylation correlated negatively with paternal age. By allele-specific DNA methylation analyses of fetal cord blood samples, the authors found that methylation of the paternal *FOKK1* allele correlated negatively with paternal age, whereas maternal allele methylation was insensitive to age. Since *FOKK1* duplication is associated with autism, fetal cord blood *FOKK1* methylation was analyzed in 74 children with autism and 41 age-matched controls. In the autism group, the *FOKK1* promoter showed that a trend ($p = 0.07$) towards *FOKK1* demethylation was accelerated during the first years of life (2–5 years). Dual luciferase reporter assay showed that *FOKK1* methylation suppresses gene expression. Since nonimprinted genes are also involved in transgenerational epigenetic inheritance, these results suggest that postzygotic reprogramming is incomplete and that sperm epigenetic alterations can persist in the differentiated somatic cells of the offspring. These

findings are corroborated by genome-wide DNA methylation screens comparing sperm from young and old mice [Smith et al., 2013; Milekic et al., 2015]. A significant loss of DNA methylation in older mice in regions associated with transcriptional regulation correlated with reduced exploratory and startle behaviors as well as similar brain DNA methylation abnormalities as observed in the paternal sperm [Smith et al., 2009, 2013; Milekic et al., 2015]. Offspring from older fathers also showed transcriptional dysregulation of developmental genes implicated in autism and schizophrenia. Thus, this study demonstrated age-related changes in DNA methylation in sperm, which can be transmitted to the next generation and may contribute to the increased risk for neuropsychiatric and other complex diseases in offspring of older fathers.

This study clearly needs recapitulation in independent cohorts with more and preferably genome-wide-distributed loci. Still, it provides evidence for epigenetic inheritance complementing de novo mutations as possible molecular mechanisms underlying the paternal age effect for disease risk. In addition, this study points towards a source of the “missing heritability” of complex diseases such as intellectual delay, ASD, and schizophrenia [Manolio et al., 2009]. Notwithstanding incremental improvements in CNV and single nucleotide variant detection, a considerable fraction of patients, e.g., with ASD, does not receive a satisfactory genetic diagnosis [Pinto et al., 2010; Hochstenbach et al., 2011; O’Roak et al., 2011; Iossifov et al., 2014; Krumm et al., 2014]. This may be due to “transmitted factors” in families with ASD and comorbidities as demonstrated by investigation of parents and siblings with the Social Responsiveness Scale [van Daalen et al., 2011]. In hindsight, such transmitted factors may very well relate to aberrant methylation at specific genomic loci in the brain [Schneider et al., 2012, 2014, 2016; Berko et al., 2014]. Another ramification of the study by Atsem et al. [2016] may be possible phenotypic differences in patients with genetic and epigenetic mutations. In ASD patients with or without comorbid conditions, diverse subphenotypic manifestations of CNVs have been inferred and detected [Poot, 2013; Merikangas et al., 2015]. In contrast to patients with de novo mutations, those without showed significant worsening in sociability with increasing age [Geier et al., 2016]. Thus, complementing current genome-wide mutation analyses with searches for aberrant DNA methylation may provide clues towards individually tailored treatments for ASD and other complex disorders.

Martin Poot

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