Running title: Paternal Age Effect and *de novo* mutations

Review Title: The impact of paternal age on new mutations and disease in the next generation

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Capsule

We review recent data linking advanced paternal age, prevalence of spontaneous genetic disorders and the origins and frequency of new mutations and discuss the biological basis underlying the associations.

Key words

Paternal age effect; Selfish selection; Spermatogonial stem cells; Rare disorders; Complex disorders

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Abstract

Advanced paternal age is associated with an increased risk of fathering children with genetic disorders and other adverse reproductive consequences. However, the mechanisms underlying this phenomenon remain largely unexplored. In this review, we focus on the impact of paternal age on *de novo* mutations (DNMs). DNMs are an important contributor to genetic disease and can be studied both indirectly through large-scale sequencing studies and directly in the tissue in which they predominantly arise – the ageing testis. We discuss recent data that have helped establish the origins and frequency of DNMs, and highlight experimental evidence about the close link between new mutations, parental age and genetic disease. We then focus on a small group of rare genetic conditions, the so-called ‘paternal age effect’ disorders, which show a strong association between paternal age and disease prevalence, and discuss the underlying mechanism (“selfish selection”) and implications of this process in more detail. More broadly, understanding the causes and consequences of paternal age on genetic risk has important implications both for individual couples and for public health advice given that the average age of fatherhood is steadily increasing in many developed nations.

Introduction

It has long been known that older parents have a higher risk of having children with genetic disorders. The link between advanced maternal age and congenital abnormalities, particularly those associated with chromosomal aneuploidies, in offspring has received considerable attention, for example the strong association between maternal age effect and Down's syndrome (trisomy 21) prevalence (1). However, there is a growing body of evidence that, independent of maternal age, elevated paternal age is associated with difficulties conceiving, complications in pregnancy, an increased susceptibility of offspring to a wide range of conditions including spontaneous dominant disorders, congenital abnormalities, neurodevelopmental conditions, and various malignancies (2–12). The American Society of Reproductive Medicine, the British Andrology Society and the Canadian Fertility and Andrology Society have advised that the upper limit for sperm donors for assisted conception should be 40 years old as a precautionary measure "so that that potential hazards related to aging are diminished" on the basis of increased risk of genetic abnormalities in children (13–15).

In many developed countries, the average age of fatherhood has been steadily increasing, despite considerable demographic variation. In the United Kingdom, for example, the standardised mean age of fathers in 2020 was 33.7 years, the highest since data collection began and an increase from 29.7 years in 1970, while over the same period mean maternal age rose from 26.7 to 30.7 years (16). A similar picture is apparent in the United States, with one study indicating that mean paternal age has risen from 27.4 years in 1972 to 30.9 years in 2015 (with variation attributed to ethnicity/race, geographic location and education level), with 8.9% and 0.9% of fathers over the age of 40 and 50, respectively (17). Given this trend prevalent across the developed world, there is an ever-increasing need to evaluate the impact, and understand the causes and consequences, of advanced paternal age on genetic risk for both individual couples and public health advice. Moreover, this information is also crucial given the popularity of assisted reproductive technologies (ART) which offer couples the option to reproduce later in life, to provide accurate risks regarding delayed parenthood (18).

While epidemiological studies have shown a convincing correlation between paternal age and disease risk, in many cases, this association is ill-defined and (in some cases) not always reproducible (8,19–21). Often, the exact threshold of what consists of "advanced" paternal age is also poorly defined and varies from study to study (9). Additional factors can further cloud our interpretation of population-based studies, including the fact that maternal and paternal ages are often highly correlated with little variability between the age of the mother and the father, so unpicking the impact of one from the other in terms of disease association can be challenging. Importantly, correlations do not provide direct evidence for causality and the mechanisms underlying the effect of advanced paternal age on disease remain uncertain and are likely to be moderated by a complex interaction of factors (12). -

Over the last decade, thanks to advances and falling costs of next-generation sequencing (NGS) technology, it has become possible to interrogate and further dissect the components mediating the effect of paternal age on disease risk. Here, we focus largely on *de novo* point mutations (DNMs), DNA sequence variations that are "new" in offspring and are not apparently present in either parent. In this review, we examine how, why, where, and how often new mutations are introduced in our genomes, and the link between DNMs, disease and other negative reproductive outcomes. We then discuss the unusual properties of a small group of specific genetic disorders ('paternal age effect' (PAE) disorders) which have provided a paradigm to study DNMs directly in their tissue of origin and led to the discovery of the process of "selfish selection". DNMs are important contributors to human disease, and understanding their origins and the factors that influence their occurrence, such as advanced paternal age, have important implications for public health, assisted reproductive technology treatments, complex disease and, more generally, the evolution of our genome.

Origins and Frequency of New Mutations in Humans

The rate at which new mutations arise is crucial to our understanding of both genetic disease and genome biology. Much insight into the biology of DNMs has been gained from large-scale implementation of whole-genome sequencing (WGS) or whole-exome sequencing (WES) of mother-father-child family trios – sequencing of coding portions (WES) or the whole genomes (WGS) of a child and both biological parents (**Fig. 1a**). Such studies have convincingly shown that the number of new point mutations present in a newborn is on average 60 (ranging from ~30 to ~90, depending on parental age at conception), placing the average human germline mutation rate at ~1.2 x 10^{-8} per nucleotide per generation (22–27).

Overall, the number of DNMs increases steadily and relatively monotonically with parental age. It is also possible to determine the parental origin of DNMs using a haplotype phasing strategy. This can be performed directly using the WGS data from the family trio when an informative heterozygous single nucleotide polymorphism (SNP) is present in the vicinity of the DNM which allows the maternally- and paternally-derived alleles in the child to be distinguished (**Fig. 1b**) (28,29). Such phasing methods have shown that ~80% of all DNMs are present on the paternally-derived allele, and that the number of DNMs in a child is predominantly influenced by the age of the father at conception (**Fig. 1c**) (24,25,28).

On average, ~1-2 additional DNMs arise in the genome of a child per additional year in the age of the father (31). Juxtaposed to this, a smaller (but significant) maternal age effect has been reported (23,25,30) (**Table 1**). Aside from DNM datasets derived from family trios, WGS studies of individual multi-sibling families with large age differences between the first and last child have again shown that the predominant factor determining the number of DNMs in a child is the paternal age, and overall only a modest variability in familial mutation rates has been reported (24,26,33,34).

It has long been accepted that differences in the biology of the male and female germlines provide a compelling explanation as to why the vast majority (~80%) of DNMs are paternal in origin, in particular the number of germline cell divisions in the life history of a sperm compared to an egg (**Fig 1c, Table 1**) (35). All cell divisions required for the production of an oocyte take place during early embryogenesis. By contrast, spermatogonial stem cells (SSCs) within the seminiferous tubules of the testis divide continuously to sustain sperm production throughout a man's reproductive life, and so the number of genome replications increases with age. It can be estimated that the sperm produced by a 25-year old man would have undergone ~350 SSC replications, compared to ~750 in a 45-year old man (**Fig. 1c**) (35). In addition to the number of mitotic divisions in the male germline resulting in incidental copying errors, other factors have been proposed to contribute to the age effect, including damage-associated mutations (particularly oxidative stress) during environmental exposures, age-related reduction in DNA repair and epigenetic reprogramming of germ cells (27,36–38). However, molecular evidence derived from large WGS data is consistent with SSC replications being the predominant factor influencing the parental bias in DNM origin and the paternal age effect of DNMs. For example, large WGS mutation datasets have been used to derive “mutational signatures” (defined as specific DNA substitution patterns typically caused by distinct underlying mutational processes, such as DNA replication errors, DNA damage caused by UV-exposure or other exogenous/endogenous exposure, defective DNA repair pathways) (39). This approach shows that the most common signatures observed in DNMs are similar to those associated with spontaneous preneoplastic somatic mutations (so-called ‘mutation signatures 1 and 5’) (24–26,40). This supports the idea that stem cell cycling is the main mutational process operative in the germline and the principal contributor to DNMs (24,31,39). Mutational signatures associated with paternally- and maternally-derived DNMs are distinct from one another, pointing that they originate via different processes (**Table 1**) (24,25,27,31,41–43).

More recently, in a large WGS study comparing mutational load across distinct histological laser-microdissected tissues, Moore et al. showed that the overall mutation rate (estimated 1.35 mutations per year of paternal age) and mutational signatures derived directly from analysis of testicular stem cells was comparable to the mutation rate estimated from WGS trio studies (22–24,44). Hence, these data provide further evidence consistent with copying errors within SSCs being the main source of DNMs (44).

However, the mutational rate of the germline is 1-2 orders of magnitude lower than in any other somatic cell types implying that other mechanisms maintaining genomic integrity must be at play (44,45). Processes such as increased DNA repair capabilities or “transcriptional scanning” have been proposed to modulate the germline mutation signatures and mutation rate in human testes (27,46). Xi et al. proposed that widespread transcription in the testis (which express over 80% of protein-coding genes in humans) facilitates DNA repair and copy-error correction, thus reducing germline mutation rates (46–49).

DNMs and Genetic Disease

Although DNMs are rare events, they are important contributors to genetic disease. In a seminal publication from the UK Deciphering Developmental Disorders (DDD) study, a large-scale trio WES study of over 4000 families with severe, undiagnosed developmental disorders, the contribution of DNMs to spontaneous monogenic developmental disorders was estimated to be ~1 in 300 live births, greater than the combined impact of trisomies 13, 18 and 21 (50). Importantly, this study also showed a linear relationship between the prevalence of live births with dominant disorders caused by DNMs and parental age, doubling every 20 years (with prevalence estimated to range from ~1:448 (0.22% of all births) in young (20 yrs old) to ~1:213 (0.47%) in older (45 yrs old) couples), an almost identical slope to the relationship between paternal age and number of DNMs genome-wide (50).

Overall, data on DNMs obtained from WGS/WES studies of large family trios and/or testicular tissues show that a) we all acquire a small but consistent number (~30-90) of new mutations at each generation, b) there is a strong paternal bias in the origin of DNMs, c) most DNMs exhibit mutational signatures suggesting they occurred as copy-errors during stem cell cycling, d) paternal age is the main contributor to the number of DNMs in a child, in most cases showing a linear relationship, and e) the prevalence of spontaneous developmental disorders shows a trend with paternal age almost identical to that observed for the genome-wide number of DNMs. Taken together, these findings strongly support the proposal that for the majority of genetic disorders caused by DNMs, the effect of paternal age on disease prevalence is causally linked to the slow and steady accumulation of DNMs in SSCs over time.

There is robust epidemiological evidence that many other negative reproductive outcomes, such as preterm birth, low birth weight, poor Apgar scores, and increased morbidity, show a similar linear increase with paternal age; however, for these conditions, the link with DNM accumulation in SSCs is more tenuous (19,51–53). For example, a study of over 40 million live births in the US between 2007 and 2016 found a J-shaped association curve between paternal age and adverse perinatal outcomes after adjustment for maternal age, race, education, smoking status and number of prenatal visits, with the youngest fathers having poorer reproductive outcomes than men in their 20s, that was followed by a steady and linear increase in negative pregnancy outcomes with increased paternal age (51). Given the linear relationship between paternal age and increased number of DNMs, it is tempting to suggest that many of the aforementioned adverse reproductive consequences are also caused by the DNM accumulation as a man ages, but further evidence, including the role of genetic factors in these conditions, is required to support this hypothesis (see also below).

Germline Mosaicism, DNMs and Recurrence Risk

Germline mosaicism is now recognized as another important source of DNMs and therefore genetic disease (**Table 1**). Spontaneous mutations can occur during early embryonic mitotic divisions in either one of the two parents - either before specification of the primordial germ cells (PGCs) resulting in mixed somatic and germline mosaicism; or post-specification of PGCs, resulting in confined germline mosaicism; or may occasionally occur in the offspring post-fertilisation (**Fig. 1c**) (29,54). While mosaicism has a small contribution to the overall DNM load, it has important clinical implications for sibling recurrence risk (29,55). Because DNMs occurring spontaneously in the adult male or female germlines are rare events (mutation rate: ~1.2×10^{-8} per nucleotide/generation), the risk of the same DNM occurring as an independent mutational event is negligible. By contrast, in case of germline mosaicism, the DNM is present in multiple parental germ cells, leading to a substantial risk (up to 50%) of recurrence in future children (29). As a result, the average recurrence risk for conditions caused by DNMs is ~1-2% (24,26,29). A recent WGS study of paired blood-semen samples from 14 men showed that an average of ~30 mosaic variants were present (albeit most at very low levels) in their semen (56). DNMs with a mosaic origin exhibit no parental age effect and no parental bias (the ratio of paternal to maternal mutations is ~50:50, compared to the ~80:20 ratio for germline DNMs).

Selfish Selection and Paternal Age Effect (PAE) Disorders

Paternal age effect (PAE) and spontaneous Mendelian disorders

When we consider the paternal age effect in reference to genetic disease, there is a source of misunderstanding in the field which we seek to clarify. While so far we have described the linear and remarkably similar relationship between DNMs, paternal age and incidence of most developmental disorders, specific DNMs associated with a small subset of spontaneous Mendelian disorders show a non-linear relationship with paternal age (and therefore birth prevalence of the disorders rises sharply as paternal age at conception increases) (2,32). We previously define these disorders as "Paternal Age Effect" (PAE) disorders, and have proposed that additional mechanisms beyond a simple linear increase in DNMs across the genome arising from SSC replication underlies these conditions. The striking impact of advanced paternal age on these disorders has been recognised for over a century (2,35). As such, because PAE disorders are well-characterised recurrent conditions with elevated birth prevalence that have received considerable attention, they are often considered to be exemplars for other spontaneous disorders. However, we must emphasise that PAE disorders are exceptions that differ from the majority of spontaneous disorders already discussed in this review. Importantly the study of these rare disorders has provided novel insights into the intimate relationship that exists between the homeostatic regulation of SSCs and spermatogenesis, DNM prevalence and disease incidence.

The best known examples of PAE disorders are those caused by specific point mutations in *FGFR2* (causing Apert, Crouzon and Pfeiffer syndromes); *FGFR3* (achondroplasia, thanatophoric dysplasia, hypochondroplasia and Muenke syndrome); *RET* (multiple endocrine neoplasia types 2A and 2B); *PTPN11* (Noonan syndrome); and *HRAS* (Costello syndrome) (**Table 2**) (32). These disorders present with unusual features, including (32,57):

1. When the disorder is caused by a DNM, the causative mutations are (almost) exclusively inherited from unaffected fathers, pointing to the fact that the original mutational events occurred during spermatogenesis.
2. A strong epidemiological paternal age effect, whereby fathers of affected children are significantly older than the population average (approximately two to seven years older than the population mean).
3. A narrow mutational spectrum with the causative mutations encoding specific protein changes, typically associated with gain-of-function properties.
4. A high apparent germline mutation rate, with individual substitutions occurring up to 1000 times more frequently than the average germline mutation rate.

These features led to the hypothesis that the causative mutations should be present at elevated levels in testes and sperm and become more abundant in older men. Given the highly localized nature of the causative mutations (usually a specific point mutation), it was possible to test this hypothesis - although technically demanding, as the mutations are anticipated to be present at extremely low levels (ranging from ~10^{-4} to <10^{-6}, based on the birth prevalence of the associated disorders). Nonetheless, specific pathogenic mutations in 12 genes (**Table 2**) which fulfil the above criteria were detected at elevated levels in sperm and/or testes and showed the anticipated paternal age effect (58–72). Being able to quantify and visualise the mutations directly within the tissue in which they originate (sperm and testis) has allowed to define the mechanism by which these DNMs appear so frequently in the population, termed 'selfish selection'.

In selfish selection, rare, specific point mutations that confer functional properties to the encoded protein spontaneously occur in SSCs within a seminiferous tubule of the adult testis, providing a competitive advantage and leading to clonal expansion of mutant SSCs as the man ages (59,60,62,64,66–69,71,72). In turn, this leads to a sharp increase in the relative mutation abundance in sperm over time and a higher likelihood of fertilisation by a mutant sperm, resulting in the Mendelian disorder in the offspring (**Fig. 2a**). This process is equivalent to clonal growth observed in tumorigenesis but occurs in the germline rather than somatic tissues. Hence, selfish mutations have consequences not only for the individual in which they occur, where they occasionally cause a rare benign testicular tumor (spermatocytic tumor), but also for the next generation (32,64,73).

All selfishly selected mutations known to date cluster within the Receptor Tyrosine Kinase (RTK)-RAS-MAPK signalling pathway, the most frequently mutated pathway in cancer and a known regulator of testicular homeostasis, and the mutations all encode dominant gain-of-function, activating the pathway (57,72). Based on our current understanding of the mechanism, we predict that *any* gene/pathway expressed in SSCs which controls testicular homeostasis could be under selection in the testis, provided that the mutations are compatible with sperm viability and allow an embryo to develop. The technical demands in identifying mutations present at ultra-low levels in the male germline has precluded large-scale discovery screens, although rapid technical advances are opening the door to evaluating the process at scale (72). Over the last few years, single cell RNA-sequencing of human testes has allowed unbiased identification of the key molecular pathways controlling SSC homeostasis and provide important starting points for the discovery of additional selfishly selected genes (**Fig. 2b**) (74–78).

Advanced paternal age and complex disorders

Thus far, we have shown that for many monogenic disorders there is an intimate relationship between paternal age, DNMs, genetic disease, and homeostatic regulation of the male germline, which strongly suggests a biological link between these factors. However, the majority of genetic disease is not caused by DNMs in single genes. Rather, “complex” (or common) diseases such as diabetes, neurodevelopmental conditions including autism and schizophrenia, and multiple sclerosis, are caused by a combination of multiple genetic (inherited variations and possibly DNMs), epigenetic, environmental and lifestyle factors. Overall, these multifactorial disorders are poorly characterised, but for some of them, the association with advanced paternal age is robust and reproducible. For example, older fathers are at higher risk of having children with schizophrenia or autism (3,19,20,79–82). There is considerable debate as to whether the link between advanced paternal age and these disorders is due to: a) inherited genetic factors influencing the health of fathers and timing of reproduction (inherited model), b) testes-driven DNMs that arise randomly as a consequence of ageing (*de novo* model), c) environmental factors (such as oxidative stress), d) epigenetic factors that accumulate over time; or e) (more likely) a combination of the above (12,51,83–88). Unravelling the contribution of these factors to the paternal age effect in common disorders is challenging (and beyond the scope of this review), but before this can be attempted, a better understanding of the aetiology of these ‘complex’ disorders will be required (12,89). For example, Yoon et al. recently showed that stratification of autism into two subtypes (simplex/low-risk families with a single affected child *vs.* multiplex/high-risk with recurrent family history), provide an efficient way to identify families with likely causative *de novo* events, where they contribute to autism risk in up to 70% of the low-risk families (87).

For those complex disorders where the *de novo* contribution has been established or is likely to be high (12,85–87,90), it may be possible to ask whether selfish selection is a contributory factor to the paternal age effect. To assess this possibility, it is important to consider the functional consequence of a particular selfish mutation both for the testis (where it provides a competitive advantage to mutant SSCs) and on the fitness of the offspring who inherits the constitutive mutation. While ‘strong’ selfish mutations causing the PAE disorders discussed above provide a robust selective advantage to SSCs and become significantly enriched in the sperm of older men, they cause deleterious disease phenotypes and poor reproductive fitness in the offspring, such that the variants are rarely transmitted over multiple generations. However, ‘weaker’ selfish mutations, while enriched to a lesser extent in SSCs and sperm, are anticipated to cause more subtle and milder disease phenotypes. Importantly, these milder mutations can become a source of heritable material across several generations and contribute to the mutational burden characteristics of complex disorders (12,90,91). Further supporting this scenario are several studies which have shown that mutations in genes known to operate in selfish selection, such as the RTK-RAS pathway or more globally in SSCs regulation, have been implicated in the pathogenesis of neurodevelopmental disorders (92,93). Drawing from what we have learned from the study of PAE disorders, the general principles of age-related DNM accumulation and selfish selection predict that 'mild' functional/pathogenic DNMs in specific growth-controlling pathways will accumulate and/or synergise to eventually dysregulate specific pathways causing disease in future generations, via a mechanism we term "global anticipation" of mutation accumulation. The steady increase of age of paternity in most developed countries is anticipated to accelerate this process, with the possibility of disease phenotypes manifesting over fewer generations. Transmission of newly acquired, mildly deleterious variants across generations may provide a parsimonious explanation for the association between advanced grandparental age and neurodevelopmental disorders (80,94). Further evidence will be required to establish whether DNM accumulation and/or selfish selection provide plausible mechanistic links to the paternal age effect observed in some subclasses of neurodevelopmental (and more broadly, complex) disorders, but these hypotheses will become amenable to scrutiny as our understanding of these common human diseases broaden.

What are the wider implications of selfish selection?

There are several corollaries of selfish selection with potential medical implications that are worth considering. First, the recurrence risk for selfish DNMs caused by selection is lower than for other spontaneous disorders because the mutations arise and clonally expand during adulthood. Despite positive selection in the testis, the levels of selfish mutations in sperm will be lower than those observed for cases of parental mosaicism (**Table 1**). This has implications for genetic counselling and should be reassuring for affected families as the recurrence risk for PAE disorders is predicted to be <0.1% (29,95).

Second, the existence of selfish clones in the testes of all ageing men (71,72) may have implications for procedures involving biopsies for testicular sperm extraction combined with intracytoplasmic sperm injection (TESE-ICSI) as a treatment for male infertility. As an intriguing example, in a previous study we analysed a testis from a 90-year old man with severe atrophy caused by strangulation in an inguinal hernia, and identified very few seminiferous tubules containing germ cells, consistent with the clinical presentation. However, upon analysis, we showed that the remaining SSCs all carried the clonal FGFR3 p.K650E mutation, a known selfishly selected mutation that causes the lethal disorder thanatophoric dysplasia in offspring (and is associated with bladder carcinoma as a somatic event), implicating an apparent survival advantage of mutant compared to wildtype germ cells in this diseased testis (64,71). This anecdotal finding raises the possibility that localized testicular biopsies used for sperm retrieval in men with overall poor spermatogenesis may be more prone to carry selfishly selected DNM. Caution may need to be observed with biopsies in older men and depending on the likelihood of selecting such surviving clones, screening of these testicular biopsies for known selfish DNMs may be advisable prior to use in TESE-ICSI.

Conclusions and perspectives

Over recent years, concerns have been raised about the impact of advanced paternal age on reproductive outcomes, but remarkably little has been understood about the underlying biology driving the paternal age effect. Recent advances in NGS technologies, facilitating large-scale trio WES/WGS studies and characterization of DNMs at scale and in an unbiased manner. Such studies have shed light on some unique features of the germline, where the predominant contributor to the number of DNMs transmitted to offspring is the age of the father. While this effect is small (an additional ~1-2 DNMs per extra year of fatherhood), it has a significant impact on spontaneous disease. With DNMs contributing to ~1:300 live births for severe spontaneous developmental disorders, this amounts to ~500,000 births annually worldwide – a considerable disease burden that cannot be ignored. The mutational signature of these DNMs is consistent with copying errors arising during the turnover of SSCs. The strong correlation between paternal age, the observed number of DNMs and the prevalence of monogenic developmental disorders (and other negative reproductive outcomes) compellingly suggests that the steady accumulation of DNMs in the male germline contributes most significantly to the paternal age effect.

However, we have emphasized that the classic PAE disorders are outliers to the above model. The unusual features of these disorders have allowed us to investigate selfishly selected mutations directly within sperm and testes and propose a unified mechanism for clonal expansion of mutant SSCs. Selfish selection is a universal process occurring in the testes of all men as they age. To date, it has only been possible to demonstrate that a handful of mutations causing a few spontaneous dominant disorders within the RTK-RAS-MAPK pathway genes are selfishly selected, but the study of large cohorts and the rapid improvements in technology are paving the way to investigate this process on a much larger scale (96-97). Moreover, we speculate that increased age of fatherhood in the general population may lead to global anticipation of mutation accumulation that extends beyond the few rare PAE disorders characterized so far, to include a contribution to some complex/common disorders.

These findings have provided helpful insights to the biological mechanisms driving epidemiological observations but, as it is often the case in science, they have also raised new questions which should open novel research avenues. A key question in the field, which remains largely unanswered, is how the male germline maintains low mutation rate, despite SSCs dividing over decades, a process which is inherently mutagenic. While DNMs accumulate with increasing paternal age, it is important to note that this phenomenon occurs in the backdrop of extremely low mutation rates, several orders of magnitude lower than in any other somatic cell types analysed. Perhaps what is remarkable is not the fact that we mutate, but how few DNMs arise in our genome at each generation. Another notable feature of the germline that distinguishes it from somatic cells, is its resilience and robustness to mutagenic factors. Rather reassuringly, several WGS studies have shown that treatments with potentially mutagenic chemotherapeutic agents and/or exposure to ionizing radiation do not result in increased incidence of DNMs or congenital malformations in offspring (98–103). Confirming this expectation, a recent survey of DNM load from WGS data from ~22,000 family trios highlighted the remarkable consistency and monotonic linear increase with parental age of the numbers of DNMs. Indeed, only 12 individuals (0.05%) behaved as outliers, exhibiting 2-7 times more *de novo* SNVs than expected (27). Finally, there is no evidence of increased DNM load or altered mutation spectrums in mice or humans born as a result of ART compared to spontaneously conceived offspring (104,105).

Overall, the individual disease risk to offspring due to advanced paternal age remains small, but its impact on population health is non-negligible. Moreover, the consequences of raising the age of fatherhood may need to be considered over several generations. Because DNMs occur in every newborn, it will be difficult to circumvent the genetic risks associated with paternal age, but it is possible to anticipate the consequences of this phenomenon, for example, by offering prenatal screening for PAE conditions to older couples - similar to the testing provided routinely for conditions associated with advanced maternal age. Finally, it is important to acknowledge that a small amount of new functional variants in our genome may also be desirable as this ensures the introduction of beneficial alleles in our genomes (see for example (106)) and promotes genetic heterogeneity and diversity among individuals; these are essential attributes that have likely contributed to make us adaptable and successful as a species.

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References

1. Cuckle H, Morris J. Maternal age in the epidemiology of common autosomal trisomies. Prenat Diagn 2021; 41:573–83.

2. Risch N, Reich EW, Wishnick MM, McCarthy JG. Spontaneous mutation and parental age in humans. Am J Hum Genet 1987; 41:218–48.

3. Malaspina D, Harlap S, Fennig S, Heiman D, Nahon D, Feldman D, et al. Advancing Paternal Age and the Risk of Schizophrenia. Arch Gen Psychiatry 2001; 58:361–7.

4. Murray L, McCarron P, Bailie K, Middleton R, Davey Smith G, Dempsey S, et al. Association of early life factors and acute lymphoblastic leukaemia in childhood: historical cohort study. Br J Cancer 2002; 86:356–61.

5. Choi J-Y, Lee K-M, Park SK, Noh D-Y, Ahn S-H, Yoo K-Y, et al. Association of paternal age at birth and the risk of breast cancer in offspring: a case control study. BMC Cancer 2005; 5:143.

6. Yip BH, Pawitan Y, Czene K. Parental age and risk of childhood cancers: a population-based cohort study from Sweden. Int J Epidemiol 2006; 35:1495–503.

7. Green RF, Devine O, Crider KS, Olney RS, Archer N, Olshan AF, et al. Association of paternal age and risk for major congenital anomalies from the National Birth Defects Prevention Study, 1997 to 2004. Ann Epidemiol 2010; 20:241–9.

8. Lan K-C, Chiang H-J, Huang T-L, Chiou Y-J, Hsu T-Y, Ou Y-C, et al. Association between paternal age and risk of schizophrenia: a nationwide population-based study. J Assist Reprod Genet 2021; 38:85–93.

9. Bray I, Gunnell D. Advanced paternal age: How old is too old? J Epidemiol Community Health 2006; 60:851–3.

10. Toriello HV, Meck JM. Statement on guidance for genetic counseling in advanced paternal age. Genet Med 2008; 10:457–60.

11. Kovac JR, Addai J, Smith RP, Coward RM, Lamb DJ, Lipshultz LI. The effects of advanced paternal age on fertility. Asian J Androl 2013; 15:723–8.

12. Janecka M, Mill J, Basson MA, Goriely A, Spiers H, Reichenberg A, et al. Advanced paternal age effects in neurodevelopmental disorders—review of potential underlying mechanisms. Transl Psychiatry 2017; 7:e1019.

13. Guidelines for sperm donation. Fertil Steril 2004; 82:9–12.

14. British Andrology Society. British Andrology Society guidelines for the screening of semen donors for donor insemination (1999). Hum Reprod 1999; 14:1823–6.

15. Havelock J, Liu K, Levitan S, Petropanagos A, Khan L. Guidelines for the Third-Party Reproduction. In: Canadian Fertility & Andrology Society Clinical Practice Guidelines 2016, available at: https://cfas.ca/_Library/clinical_practice_guidelines/Third-Party-Procreation-AMENDED-.pdf.

16. Birth characteristics in England and Wales - Office for National Statistics [Internet]. [cited 2022 Apr 12]; Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthcharacteristicsinenglandandwales/2020

17. Khandwala YS, Zhang CA, Lu Y, Eisenberg ML. The age of fathers in the USA is rising: an analysis of 168 867 480 births from 1972 to 2015. Hum Reprod 2017; 32:2110–6.

18. Chan PTK, Robaire B. Advanced Paternal Age and Future Generations. Front Endocrinol 2022; 13:897101.

19. Chen X-K, Wen SW, Krewski D, Fleming N, Yang Q, Walker MC. Paternal age and adverse birth outcomes: teenager or 40+, who is at risk? Hum Reprod 2008; 23:1290–6.

20. Petersen L, Mortensen PB, Pedersen CB. Paternal age at birth of first child and risk of schizophrenia. Am J Psychiatry 2011; 168:82–8.

21. Thompson JA. The Effects of Parent Ages on Birth Defects. Acta Sci Paediatr 2020; 3:58–69.

22. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature 2012; 488:471–5.

23. Jónsson H, Sulem P, Kehr B, Kristmundsdottir S, Zink F, Hjartarson E, et al. Parental influence on human germline de novo mutations in 1,548 trios from Iceland. Nature 2017; 549:519–22.

24. Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, Al Turki S, et al. Timing, rates and spectra of human germline mutation. Nat Genet 2016; 48:126–33.

25. Goldmann JM, Wong WSW, Pinelli M, Farrah T, Bodian D, Stittrich AB, et al. Parent-of-origin-specific signatures of de novo mutations. Nat Genet 2016; 48:935–9.

26. Sasani TA, Pedersen BS, Gao Z, Baird L, Przeworski M, Jorde LB, et al. Large, three-generation human families reveal post-zygotic mosaicism and variability in germline mutation accumulation. eLife 2019; 8:e46922.

27. Kaplanis J, Ide B, Sanghvi R, Neville M, Danecek P, Coorens T, et al. Genetic and chemotherapeutic influences on germline hypermutation. Nature 2022; 605:503–8.

28. Goldmann JM, Veltman JA, Gilissen C. De Novo Mutations Reflect Development and Aging of the Human Germline. Trends Genet 2019; 35:828–39.

29. Bernkopf M, Abdullah UB, Bush SJ, Wood K, Ghaffari S, Giannoulatou E, et al. The PREGCARE study: Personalized recurrence risk assessment following the birth of a child with a pathogenic de novo mutation [pre-print] 2022 available from: https://www.biorxiv.org/content/10.1101/2022.07.26.501520v1

30. Wong WSW, Solomon BD, Bodian DL, Kothiyal P, Eley G, Huddleston KC, et al. New observations on maternal age effect on germline de novo mutations. Nat Commun 2016; 7(1):10486.

31. Acuna-Hidalgo R, Veltman JA, Hoischen A. New insights into the generation and role of de novo mutations in health and disease. Genome Biol 2016; 17:241.

32. Goriely A, Wilkie AOM. Paternal Age Effect Mutations and Selfish Spermatogonial Selection: Causes and Consequences for Human Disease. Am J Hum Genet 2012; 90:175–200.

33. Jónsson H, Sulem P, Arnadottir GA, Pálsson G, Eggertsson HP, Kristmundsdottir S, et al. Multiple transmissions of de novo mutations in families. Nat Genet 2018; 50:1674–80.

34. Goldmann JM, Hampstead JE, Wong WSW, Wilfert AB, Turner TN, Jonker MA, et al. Differences in the number of de novo mutations between individuals are due to small family-specific effects and stochasticity. Genome Res 2021; 31:1513–8.

35. Crow JF. The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet 2000; 1:40–7.

36. Crow JF. Age and sex effects on human mutation rates: an old problem with new complexities. J Radiat Res (Tokyo) 2006; 47:B75-82.

37. Ségurel L, Wyman MJ, Przeworski M. Determinants of mutation rate variation in the human germline. Annu Rev Genomics Hum Genet 2014; 15:47–70.

38. Aitken RJ, De Iuliis GN, Nixon B. The Sins of Our Forefathers: Paternal Impacts on De Novo Mutation Rate and Development. Annu Rev Genet 2020; 54:1–24.

39. Koh G, Degasperi A, Zou X, Momen S, Nik-Zainal S. Mutational signatures: emerging concepts, caveats and clinical applications. Nat Rev Cancer 2021; 21:619–37.

40. Francioli LC, Polak PP, Koren A, Menelaou A, Chun S, Renkens I, et al. Genome-wide patterns and properties of de novo mutations in humans. Nat Genet 2015; 47:822–6.

41. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013; 500:415–21.

42. Gao Z, Moorjani P, Sasani TA, Pedersen BS, Quinlan AR, Jorde LB, et al. Overlooked roles of DNA damage and maternal age in generating human germline mutations. Proc Natl Acad Sci U S A 2019; 116:9491–500.

43. Agarwal I, Przeworski M. Signatures of replication timing, recombination, and sex in the spectrum of rare variants on the human X chromosome and autosomes. Proc Natl Acad Sci U S A 2019; 116:17916–24.

44. Moore L, Cagan A, Coorens THH, Neville MDC, Sanghvi R, Sanders MA, et al. The mutational landscape of human somatic and germline cells. Nature 2021; 597:381–6.

45. Milholland B, Dong X, Zhang L, Hao X, Suh Y, Vijg J. Differences between germline and somatic mutation rates in humans and mice. Nat Commun 2017; 8:15183.

46. Xia B, Yan Y, Baron M, Wagner F, Barkley D, Chiodin M, et al. Widespread Transcriptional Scanning in the Testis Modulates Gene Evolution Rates. Cell 2020; 180:248-262.e21.

47. Melé M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, et al. The human transcriptome across tissues and individuals. Science 2015; 348:660–5.

48. Soumillon M, Necsulea A, Weier M, Brawand D, Zhang X, Gu H, et al. Cellular Source and Mechanisms of High Transcriptome Complexity in the Mammalian Testis. Cell Rep 2013; 3:2179–90.

49. Liu H, Zhang J. Higher Germline Mutagenesis of Genes with Stronger Testis Expressions Refutes the Transcriptional Scanning Hypothesis. Mol Biol Evol 2020; 37:3225–31.

50. McRae JF, Clayton S, Fitzgerald TW, Kaplanis J, Prigmore E, Rajan D, et al. Prevalence and architecture of de novo mutations in developmental disorders. Nature 2017; 542:433–8.

51. Khandwala YS, Baker VL, Shaw GM, Stevenson DK, Lu Y, Eisenberg ML. Association of paternal age with perinatal outcomes between 2007 and 2016 in the United States: population based cohort study. BMJ 2018; 363:k4372.

52. Astolfi P, De Pasquale A, Zonta LA. Paternal Age and Preterm Birth in Italy, 1990 to 1998. Epidemiology 2006; 17:218–21.

53. Urhoj SK, Andersen PK, Mortensen LH, Davey Smith G, Nybo Andersen A-M. Advanced paternal age and stillbirth rate: a nationwide register-based cohort study of 944,031 pregnancies in Denmark. Eur J Epidemiol 2017; 32:227–34.

54. Acuna-Hidalgo R, Bo T, Kwint MP, van de Vorst M, Pinelli M, Veltman JA, et al. Post-zygotic Point Mutations Are an Underrecognized Source of De Novo Genomic Variation. Am J Hum Genet 2015; 97:67–74.

55. Cao Y, Tokita MJ, Chen ES, Ghosh R, Chen T, Feng Y, et al. A clinical survey of mosaic single nucleotide variants in disease-causing genes detected by exome sequencing. Genome Med 2019; 11:48.

56. Yang X, Breuss MW, Xu X, Antaki D, James KN, Stanley V, et al. Developmental and temporal characteristics of clonal sperm mosaicism. Cell 2021; 184:4772-4783.e15.

57. Maher GJ, Goriely A, Wilkie AOM. Cellular evidence for selfish spermatogonial selection in aged human testes. Andrology 2014; 2:304–14.

58. Tiemann-Boege I, Navidi W, Grewal R, Cohn D, Eskenazi B, Wyrobek AJ, et al. The observed human sperm mutation frequency cannot explain the achondroplasia paternal age effect. Proc Natl Acad Sci U S A 2002; 99:14952–7.

59. Goriely A, McVean GAT, Röjmyr M, Ingemarsson B, Wilkie AOM. Evidence for Selective Advantage of Pathogenic FGFR2 Mutations in the Male Germ Line. Science 2003; 301:643–6.

60. Goriely A, McVean GAT, Pelt AMM van, O’Rourke AW, Wall SA, Rooij DG de, et al. Gain-of-function amino acid substitutions drive positive selection of *FGFR2* mutations in human spermatogonia. Proc Natl Acad Sci U S A 2005; 102:6051–6.

61. Qin J, Calabrese P, Tiemann-Boege I, Shinde DN, Yoon S-R, Gelfand D, et al. The molecular anatomy of spontaneous germline mutations in human testes. PLoS Biol 2007; 5:e224.

62. Choi S-K, Yoon S-R, Calabrese P, Arnheim N. A germ-line-selective advantage rather than an increased mutation rate can explain some unexpectedly common human disease mutations. Proc Natl Acad Sci U S A 2008; 105:10143–8.

63. Dakouane Giudicelli M, Serazin V, Le Sciellour CR, Albert M, Selva J, Giudicelli Y. Increased achondroplasia mutation frequency with advanced age and evidence for G1138A mosaicism in human testis biopsies. Fertil Steril 2008; 89:1651–6.

64. Goriely A, Hansen RMS, Taylor IB, Olesen IA, Jacobsen GK, McGowan SJ, et al. Activating mutations in FGFR3 and HRAS reveal a shared genetic origin for congenital disorders and testicular tumors. Nat Genet 2009; 41:1247–52.

65. Yoon S-R, Qin J, Glaser RL, Jabs EW, Wexler NS, Sokol R, et al. The Ups and Downs of Mutation Frequencies during Aging Can Account for the Apert Syndrome Paternal Age Effect. PLOS Genet 2009; 5:e1000558.

66. Choi S-K, Yoon S-R, Calabrese P, Arnheim N. Positive selection for new disease mutations in the human germline: evidence from the heritable cancer syndrome multiple endocrine neoplasia type 2B. PLoS Genet 2012; 8:e1002420.

67. Giannoulatou E, McVean G, Taylor IB, McGowan SJ, Maher GJ, Iqbal Z, et al. Contributions of intrinsic mutation rate and selfish selection to levels of de novo HRAS mutations in the paternal germline. Proc Natl Acad Sci U S A 2013; 110:20152–7.

68. Shinde DN, Elmer DP, Calabrese P, Boulanger J, Arnheim N, Tiemann-Boege I. New evidence for positive selection helps explain the paternal age effect observed in achondroplasia. Hum Mol Genet 2013; 22:4117–26.

69. Yoon S-R, Choi S-K, Eboreime J, Gelb BD, Calabrese P, Arnheim N. Age-dependent germline mosaicism of the most common noonan syndrome mutation shows the signature of germline selection. Am J Hum Genet 2013; 92:917–26.

70. Eboreime J, Choi S-K, Yoon S-R, Arnheim N, Calabrese P. Estimating Exceptionally Rare Germline and Somatic Mutation Frequencies via Next Generation Sequencing. PloS One 2016; 11:e0158340.

71. Maher GJ, McGowan SJ, Giannoulatou E, Verrill C, Goriely A, Wilkie AOM. Visualizing the origins of selfish de novo mutations in individual seminiferous tubules of human testes. Proc Natl Acad Sci USA 2016; 113:2454–9.

72. Maher GJ, Ralph HK, Ding Z, Koelling N, Mlcochova H, Giannoulatou E, et al. Selfish mutations dysregulating RAS-MAPK signaling are pervasive in aged human testes. Genome Res 2018; 28:1779–90.

73. Giannoulatou E, Maher GJ, Ding Z, Gillis AJM, Dorssers LCJ, Hoischen A, et al. Whole-genome sequencing of spermatocytic tumors provides insights into the mutational processes operating in the male germline. PloS One 2017; 12:e0178169.

74. Guo J, Grow EJ, Yi C, Mlcochova H, Maher GJ, Lindskog C, et al. Chromatin and Single-Cell RNA-Seq Profiling Reveal Dynamic Signaling and Metabolic Transitions during Human Spermatogonial Stem Cell Development. Cell Stem Cell 2017; 21:533-546.e6.

75. Guo J, Grow EJ, Mlcochova H, Maher GJ, Lindskog C, Nie X, et al. The adult human testis transcriptional cell atlas. Cell Res 2018; 28:1141–57.

76. Sohni A, Tan K, Song H-W, Burow D, de Rooij DG, Laurent L, et al. The Neonatal and Adult Human Testis Defined at the Single-Cell Level. Cell Rep 2019; 26:1501-1517.e4.

77. Shami AN, Zheng X, Munyoki SK, Ma Q, Manske GL, Green CD, et al. Single-Cell RNA Sequencing of Human, Macaque, and Mouse Testes Uncovers Conserved and Divergent Features of Mammalian Spermatogenesis. Dev Cell 2020; 54:529-547.e12.

78. Di Persio S, Tekath T, Siebert-Kuss LM, Cremers J-F, Wistuba J, Li X, et al. Single-cell RNA-seq unravels alterations of the human spermatogonial stem cell compartment in patients with impaired spermatogenesis. Cell Rep Med 2021; 2:100395.

79. McGrath JJ, Petersen L, Agerbo E, Mors O, Mortensen PB, Pedersen CB. A comprehensive assessment of parental age and psychiatric disorders. JAMA Psychiatry 2014; 71:301–9.

80. Frans EM, Sandin S, Reichenberg A, Långström N, Lichtenstein P, McGrath JJ, et al. Autism Risk Across Generations: A Population-Based Study of Advancing Grandpaternal and Paternal Age. JAMA Psychiatry 2013; 70:516–21.

81. Hultman CM, Sandin S, Levine SZ, Lichtenstein P, Reichenberg A. Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. Mol Psychiatry 2011; 16:1203–12.

82. Pedersen CB, McGrath J, Mortensen PB, Petersen L. The importance of father’s age to schizophrenia risk. Mol Psychiatry 2014; 19:530–530.

83. Gratten J, Wray NR, Peyrot WJ, McGrath JJ, Visscher PM, Goddard ME. Risk of psychiatric illness from advanced paternal age is not predominantly from de novo mutations. Nat Genet 2016; 48:718–24.

84. Escher J, Yan W, Rissman EF, Wang H-LV, Hernandez A, Corces VG. Beyond Genes: Germline Disruption in the Etiology of Autism Spectrum Disorders. J Autism Dev Disord 2021 available online ahead of print at 10.1007/s10803-021-05304-1.

85. Antaki D, Guevara J, Maihofer AX, Klein M, Gujral M, Grove J, et al. A phenotypic spectrum of autism is attributable to the combined effects of rare variants, polygenic risk and sex. Nat Genet 2022 available online ahead of print at 10.1038/s41588-022-01064-5.

86. Taylor JL, Debost J-CPG, Morton SU, Wigdor EM, Heyne HO, Lal D, et al. Paternal-age-related de novo mutations and risk for five disorders. Nat Commun 2019; 10:3043.

87. Yoon S, Munoz A, Yamrom B, Lee Y, Andrews P, Marks S, et al. Rates of contributory de novo mutation in high and low-risk autism families. Commun Biol 2021; 4:1–10.

88. Weiser M, Fenchel D, Frenkel O, Fruchter E, Burshtein S, Yehuda AB, et al. Understanding the association between advanced paternal age and schizophrenia and bipolar disorder. Psychol Med 2020; 50:431–7.

89. Khachadourian V, Zaks N, Lin E, Reichenberg A, Janecka M. Advanced paternal age and risk of schizophrenia in offspring - Review of epidemiological findings and potential mechanisms. Schizophr Res 2021; 233:72–9.

90. Goriely A, McGrath JJ, Hultman CM, Wilkie AOM, Malaspina D. "Selfish spermatogonial selection": a novel mechanism for the association between advanced paternal age and neurodevelopmental disorders. Am J Psychiatry 2013; 170:599–608.

91. Wang T, Zhao PA, Eichler EE. Rare variants and the oligogenic architecture of autism. Trends Genet 2022; 38:895–903.

92. Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, et al. Convergence of genes and cellular pathways dysregulated in autism spectrum disorders. Am J Hum Genet 2014; 94:677–94.

93. Krishnan A, Zhang R, Yao V, Theesfeld CL, Wong AK, Tadych A, et al. Genome-wide prediction and functional characterization of the genetic basis of autism spectrum disorder. Nat Neurosci 2016; 19:1454–62.

94. Frans EM, McGrath JJ, Sandin S, Lichtenstein P, Reichenberg A, Långström N, et al. Advanced paternal and grandpaternal age and schizophrenia: a three-generation perspective. Schizophr Res 2011; 133:120–4.

95. Wilkie AOM, Goriely A. Gonadal mosaicism and non-invasive prenatal diagnosis for 'reassurance' in sporadic paternal age effect (PAE) disorders. Prenat Diagn 2017; 37:946–8.

96. Kaplanis J, Samocha KE, Wiel L, Zhang Z, Arvai KJ, Eberhardt RY, et al. Evidence for 28 genetic disorders discovered by combining healthcare and research data. Nature 2020; 586:757–62.

97. Abascal F, Harvey LMR, Mitchell E, Lawson ARJ, Lensing SV, Ellis P, et al. Somatic mutation landscapes at single-molecule resolution. Nature 2021; 593:405–10.

98. Maher GJ, Bernkopf M, Koelling N, Wilkie AOM, Meistrich ML, Goriely A. The impact of chemo- and radiotherapy treatments on selfish de novo FGFR2 mutations in sperm of cancer survivors. Hum Reprod 2019; 34:1404–15.

99. Moorhouse AJ, Scholze M, Sylvius N, Gillham C, Rake C, Peto J, et al. No evidence of increased mutations in the germline of a group of British nuclear test veterans. Sci Rep 2022; 12:10830.

100. Nakamura N. Genetic Effects of Radiation in Atomic-bomb Survivors and Their Children: Past, Present and Future. J Radiat Res (Tokyo) 2006; 47:B67–73.

101. Kryukov GV, Bielski CM, Samocha K, Fromer M, Seepo S, Gentry C, et al. Genetic Effect of Chemotherapy Exposure in Children of Testicular Cancer Survivors. Clin Cancer Res 2016; 22:2183–9.

102. Ozasa K, Cullings HM, Ohishi W, Hida A, Grant EJ. Epidemiological studies of atomic bomb radiation at the Radiation Effects Research Foundation. Int J Radiat Biol 2019; 95:879–91.

103. Yeager M, Machiela MJ, Kothiyal P, Dean M, Bodelon C, Sumannson S, et al. Lack of transgenerational effects of ionizing radiation exposure from the Chernobyl accident Science 2021; 372:725-729

104. Smits RM, Xavier MJ, Oud MS, Astuti GDN, Meijerink AM, de Vries PF, et al. De novo mutations in children born after medical assisted reproduction. Hum Reprod 2022; 37:1360–9.

105. Caperton L, Murphey P, Yamazaki Y, McMahan CA, Walter CA, Yanagimachi R, et al. Assisted reproductive technologies do not alter mutation frequency or spectrum. Proc Natl Acad Sci USA 2007; 104:5085–90.

106. Pinson A, Xing L, Namba T, Kalebic N, Peters J, Christina Eugster Oegema C, et al. Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals. Science 2022; 377:eabl6422

Figure legends

Figure 1: Origins of *de novo* mutations (DNMs): **(A)** Sequencing of the whole genome or whole exome of both biological parents and a child (trio sequencing) allows identification of new mutations only present in the child. Such studies have shown that each newborn acquires ~30–90 DNMs, depending on parental age at conception. **(B) Determination of the parental origin of a DNM by haplotype phasing**. When an informative heterozygous single nucleotide polymorphism (SNP) - for example, the SNP is AA (purple) in the mother and BB (blue) in the father - is present in the vicinity of a DNM (green star) in the child, it can be used to distinguish the maternally- and paternally-derived alleles and determine the parent of origin of the DNM. **(C) Gametogenesis and origins of DNMs**. In humans, segregation of primordial germ cells (PGCs) from somatic lineages occurs after ~10 mitoses, just before gastrulation takes place. Embryonic germ cells then undergo a few more replications (~22 in females and ~30 in males). After birth, oocytes do not undergo any further mitotic divisions. However, throughout adulthood, spermatogonial stem cells (SSCs) actively replicate to sustain sperm production, dividing every ~16 days (~23 divisions per year). It can be estimated that the sperm produced from a 25-year old male has undergone ~350 replications, while ~750 SSC replications would have taken place to sustain sperm production in a 45-year old male. These differences in germ cell biology likely account for the observed 80:20 ratio of paternal to maternal DNMs observed in offspring, the majority of which arise from copying errors during SSC cycling, with the number of DNMs doubling with every additional 20 years of paternal age. Lightning bold represents a mutational event. Figures created with BioRender.com.

Figure 2:
Selfish spermatogonial selection (A) In selfish selection, rare specific mutations occur in genes involved in the homeostatic regulation of spermatogonial stem cells (SSCs), conferring gain-of-function properties to the encoded protein. This provides the SSCs with a selective advantage over the wild-type neighbours and results in their clonal expansion within individual seminiferous tubules. As a consequence of clonal growth, the relative proportion of mutant sperm increases over the course of time. Fertilization of an oocyte by a sperm carrying a selfish mutation results in a genetic disorder in the offspring. This process is akin to tumorigenesis but occurs in the germline with consequences for the next generation. **(B)** Single-cell transcriptomics has allowed the key signaling pathways active in SSCs to be identified. To date, all known selfishly selected genes (highlighted in red) cluster within the Receptor Tyrosine Kinase (RTK)-RAS-MAPK pathway (red box). Deciphering the role of these pathways/genes in controlling proliferation, growth and survival of SSCs allows us to focus on new promising candidates for selfishly selected genes within the testes. Note that most of these genes cause genetic disease when mutated. Adapted from refs 74-75. Figures created with BioRender.com.