Missing heritability: paternal age effect mutations and selfish spermatogonia

Anne Goriely and Andrew O.M. Wilkie

In a recent Viewpoint on missing heritability (Missing heritability and strategies for finding the underlying causes of complex disease. *Nature Rev. Genet.* **11**, 446–450 (2010))¹, several commentators mentioned the expected importance of rare variants. Except for copy number polymorphisms (CNPs), contemporary technologies do not enable the systematic identification of rare variants

that, given moderate effect size, could contribute significantly to missing heritability². However strategies to identify such sequence changes will soon become available³.

In this context we wish to highlight an emerging mechanism contributing to the introduction of new disease alleles into the population, which we term paternal age effect (PAE) mutation. The

best-characterized PAE mutations encode mutant proteins with gain-of-function properties and share other distinctive features: near-exclusive paternal origin, high apparent germline mutation rate (up to 1,000-fold above background), and elevated paternal age (by 2–5 years, compared to the population average). Well-documented examples are in the fibroblast growth factor receptor 2 (*FGFR2*), *FGFR3*, *RET*, protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*) and *HRAS* genes, heterozygous substitutions of which cause congenital skeletal disorders, sometimes with additional predisposition to cancer⁴.

Recent evidence, based on quantification of mutation levels in sperm^{4,5} and testes⁶, indicates that the spermatogonial (pre-meiotic) cells in which PAE mutations originally arose are positively selected and expand clonally (FIG. 1). This clonal growth, which is likely to take place in the testes of all men, leads to the relative enrichment of mutant sperm over time — accounting for the distinctive paternal age effect of these mutations — and in extreme cases, to the formation of testicular tumours⁴.

All well-established PAE mutations activate one pathway, involving growth factor receptor–RAS signalling^{4,7}. This is likely to reflect a crucial role of this pathway in controlling the fate choice (self-renewal versus differentiation to produce mature sperm) of spermatogonial cells; additional pathways involved in the proliferation and survival of spermatogonia might also be vulnerable to such ‘selfish’ mutations. Given the importance of RAS in many contexts (for example, neurogenesis, organ homeostasis and tumorigenesis) the consequences of similar but more weakly acting spermatogonial mutations (which might be CNPs, regulatory mutations or coding substitutions) could affect a wide range of phenotypes, including neurocognitive disorders and cancer predisposition^{7–9}. Intriguingly, paternal age effects have been reported in several of these disorders^{10–12}. Although *de novo* mutations do not themselves contribute to heritability, they will do so if the phenotypic effect is mild enough to permit transmission of the new mutation to subsequent generations.

How might the overall contribution of PAE mutations to disease be quantified? Direct measurements^{4–6} in normal sperm and testes are laborious, and segregation of normal and mutant alleles in sperm of heterozygotes for PAE mutations is not expected to deviate from 50:50. Recently, the first direct study of new mutations in the human germline, obtained by whole-genome sequencing

Figure 1 | Illustrative depiction of how enrichment of functionally significant paternal age effect mutations could occur. The cellular processes taking place in two different regions of a normal testis (top) are shown. In the normal situation (left), the spermatogonial cell (large unfilled circle) has undergone ten asymmetric mitotic divisions, in each case yielding one daughter spermatogonial cell and one cell (small black circle) committed to producing sperm. Note that there is no net change in the number of spermatogonial cells over time. On the right, a chance mutation (asterisk), which arose during the second mitotic division, has yielded a spermatogonial cell with altered signalling and growth properties (orange circle). Three of 17 subsequent cell divisions have occurred symmetrically, yielding two daughter spermatogonial cells. At the end of the time frame there are four spermatogonial cells producing the identical mutation in half their sperm. This process provides a constant supply of new, functionally significant mutations in the next generation. In practice, fewer than 2% of cell divisions would need to occur symmetrically to account for up to 1,000-fold enrichment in sperm associated with observed paternal age effect (PAE) mutation^{6,15}.

of a family quartet, estimated ~70 new mutations per diploid genome¹³. Impressive as this work is, it did not mention the age of the parents at the birth of each child, nor was the parental origin of each mutation (which could be established using physically linked SNPs)¹⁴ analysed. As further studies are undertaken, we urge investigators to include this information, so that an overall picture of the contribution of PAE mutations to disease burden can be established.

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