Chapter 29
How do paternal factors such as diet, smoking, stress, and environmental chemical exposures affect germ cell mutations?

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What are mutations and why do they matter?

Mutations are changes in the DNA sequence affecting one or several complementary base-pairs. Point mutations, or single nucleotide variants (SNV), are changes in a single base, whereas insertions or deletions of base-pairs (INDELS) can range in size from a single base-pair to tens of thousands of base-pairs.

In general, most mutations are silent (i.e., have no observable effect on the organism's phenotype), but they can also cause devastating genetic disorders or even, in very rare instances, lead to a beneficial adaptation. A mutation in protein-coding genes or their regulatory regions can alter the amino acid sequence of the protein or gene expression levels resulting in a manifested genetic disease.

Mutations that occur in somatic cells can cause diseases such as cancer. However, mutations can also occur in germ cells that serve as the progenitors for the offspring. These inherited germline mutations will be present in the entire organism and have a higher chance to result in a variety of adverse phenotypes. Therefore, the faithful transmission of DNA through the germ cells from parents to offspring is critical to individual and population health. Thus, highly accurate DNA repair mechanisms have evolved to protect the integrity of the genome and prevent mutations. Indeed, germ cell mutation frequencies are approximately one order of magnitude lower than somatic mutation frequencies.

How do paternal factors affect germ cell mutations?

What are the consequences of germ cell mutations?

Germ cell mutations can have pre- and postnatal effects on the embryo or child, respectively. Mutations affecting prenatal development can lead to pregnancy loss, whereas mutations compatible with life can be associated with postnatal effects including malformations, cancer predisposition syndromes and neurodevelopmental disorders such as autism, epilepsy and intellectual disability. Moreover, inherited germline mutations can be transmitted from one generation to the next and spread through families and populations.

What is the difference between *de novo* mutation and germline mosaicism?

A mutation in an individual that is not found in the parents is known as a *de novo* mutation. *De novo* mutations are a major cause of severe diseases that can arise in early childhood or later in life and account for almost 30% of rare diseases. The rate of *de novo* SNV mutations in humans is about 1.2×10^{-8} mutations per nucleotide per generation (which corresponds to approximately 60-80 mutations per genome). Around 80% of *de novo* mutations are of paternal origin and may result from (a) a mutation in the germ cell itself, or (b) DNA damage in the male germ cell that was not repaired correctly in the fertilized egg. However, mutations may also appear during early embryonic cell divisions, leading to a mixture of cells in the fetus with and without the mutation – a state called mosaicism. These mosaic mutations can be distributed to both somatic and germ cells of the developing organism, or only affect a specific population of cells.

What are the general causes of germ cell mutations?

Mutations may occur spontaneously but can also be induced by exogenous factors that damage the germ cell DNA. Damage introduced during DNA replication can be converted into mutations if not properly repaired. Thus, mutations primarily arise during phases of spermatogenesis with active replication and cell division (Fig. 1). There is evidence to suggest that germ cell susceptibility to mutation may vary across life stages but more research is needed in this area.

Similarly, chromosomal damage can occur during mitosis and meiosis in germ cells (Fig. 1). DNA recombination can be a source of

mutations and abnormalities in chromosome number or structure, with important reproductive and clinical consequences. Finally, DNA damage can accumulate in post-meiotic phases of spermatogenesis when DNA repair mechanisms decline; this DNA damage can be converted into mutations after fertilization.

A large number of exogenous agents (*e.g.*, ionizing radiation and many chemicals) are known to induce mutations in animal germ cells by various mechanisms. These agents are collectively called mutagens. Mutagens can occur naturally or be man-made and include acrylamide, benzo(a)pyrene (a combustion by-product), and chemotherapeutic agents, among others. For example, both tobacco smoke and air pollution cause mutations in mouse sperm and paternal exposure to mutagenic compounds present in tobacco smoke cause genome-wide *de novo* mutations in the offspring of the exposed male mice. These studies demonstrate a dose-dependent association between mutagenic exposure and mutations in both sperm and offspring.

What are the known and suspected causes of germ cell mutations in humans?

Paternal age is strongly associated with the number *de novo* mutations in human offspring. On average, ~1.5 additional *de novo* mutations are transmitted to offspring with every year that a man delays fatherhood. Interestingly, this paternal age effect varies between different families, suggesting that environmental factors (such as diet, smoking, stress, and environmental exposures) may play a role. In line with this, a reduced number of *de novo* mutations has been reported in an Amish population, who have different lifestyles and exposures than people living in urban environments.

Despite the strong evidence in animals, there is still no consensus on whether human germ cell mutagens exist. The strongest evidence for an association between an exposure and germ cell mutations in humans at present is smoking. Tobacco smoke contains many established mutagens that cause DNA adducts, strand breaks and oxidative damage, and is the largest cause of cancer in the world (with cancer being largely dependent on mutagenesis). It is well established that the sperm of smokers have elevated levels of DNA damage. Paternal smoking is also associated with malformations and childhood cancer in offspring. However, although the children of smoking fathers appear to have

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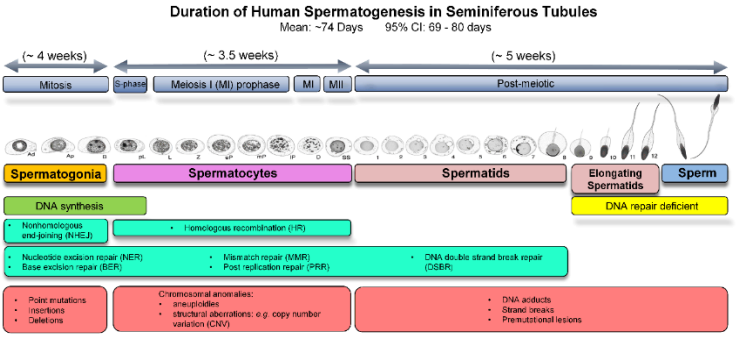

Figure 1: Schematic diagram of the developmental sequence of germ cell types present in the seminiferous tubules during human spermatogenesis. The approximate periods of DNA synthesis, active DNA repair (with specific types of repair indicated), inactive DNA repair, as well as the most common types of genetic damage that are induced in each germ cell type are shown. Ad, A-dark spermatogonium; Ap, A-pale spermatogonium; B, B spermatogonium; pL, preleptotene spermatocytes; L, leptotene spermatocytes; Z, zygotene spermatocytes; eP, early pachytene spermatocytes; mP, mid-pachytene spermatocytes; lP, late pachytene spermatocytes; D, diplotene spermatocytes; SS, secondary spermatocytes; 1–12, first 12 steps in the development of spermatids. The drawings of the germ cell types are taken from *Biology of Reproduction (2013) 89(3):60, 1–10.*

an elevated number of mutations in a region of non-coding repetitive DNA, a significant increase in other types of mutations such as SNVs in the children of smoking fathers has yet to be reported. Additional potential germ cell mutagens in humans are ionizing radiation, chemotherapeutic drugs, dioxin, and air pollution.

Exposures that have been associated with numerical and structural chromosome abnormalities in human spermatozoa include smoking, alcohol consumption and occupational exposure to benzene. Air pollution and ionizing radiation are also associated with sperm DNA fragmentation.

Studies in humans on the influence of diet on germ cell mutagenesis are limited. However, the vitamin folate (for which preconception supplementation is arguably the most effective intervention against human congenital disorders) appears to protect against aneuploidy in human sperm. At present, studies on

the impacts of social, psychological, and physiological stresses on human germ cell mutations are lacking.

What role do mutations play in male reproductive function?

As much as 20% of unexplained male infertility is associated with *de novo* mutations in genes. This may be related to the higher rate of structural mutations in the Y chromosome (in which microdeletions can cause infertility), as compared to the overall rate of point mutations in the genome. *De novo* structural mutations, including copy number variants, affect about 100 times more nucleotides per generation than single base substitutions and, as with SNVs, most often arise in the paternal gamete.

Certain mutations in germ cells that show a paternal age effect, such as the gene responsible for achondroplasia, a common form of dwarfism, appear to give an advantage to the germ cell clone (the selfish spermatogonial selection hypothesis) despite causing severe disease in the offspring. These findings support the UK's guideline recommending an upper limit of < 46 years old age for sperm donors. Taken together, the data suggest that, as with women, men also have a 'biological clock'.

Couples who are carriers of disease-causing genes can undergo *in vitro* fertilization (IVF) and preimplantation genetic testing (PGT) to achieve a healthy pregnancy. During this process, embryos are screened for the particular mutation, after which embryos without the targeted mutation can be transferred to the mother. However, genetic screening for *de novo* mutations, which could occur anywhere in the genome, is not yet being applied in PGT. Advances in assisted reproductive techniques (ART) are also allowing men with poor semen quality to become fathers. A recent study reported genome-wide *de novo* SNV mutations (most of which were paternal) to be higher in children conceived after ART than in children from natural conceptions. This is an area that merits additional investigation.

Conclusions

Animal studies have repeatedly shown that *de novo* mutations in offspring can be caused by a wide range of paternal exposures including tobacco smoke and air pollution. It seems hard to believe that no exposure would be able to cause such mutations in humans. Despite the lack of consensus within the expert community, the

weight of evidence supporting the existence of human germ cell mutagens continues to grow. Exposures to ionizing radiation, several chemotherapeutic agents, and tobacco smoking have been associated with genetic changes in human sperm. What remains to be established is a significant increase in *de novo* mutations in the children of exposed fathers. In this context, a recent study found a few cases of increased *de novo* SNV mutations in children of men who had been treated with chemotherapeutic agents. In addition, it is now firmly established that paternal age at the time of conception is strongly and positively correlated with the number of *de novo* mutations occurring in their children. A major impediment to the study of human germ cell mutagenesis has been the lack of sensitive and accurate methodologies to quantify *de novo* mutations. Emerging genomic technologies, including highly accurate next-generation sequencing approaches, are anticipated to address this methodological gap in the future and provide proof of the existence of human germ cell mutagens.

Suggested reading

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