

# Y chromosome microdeletions: implications for assisted conception

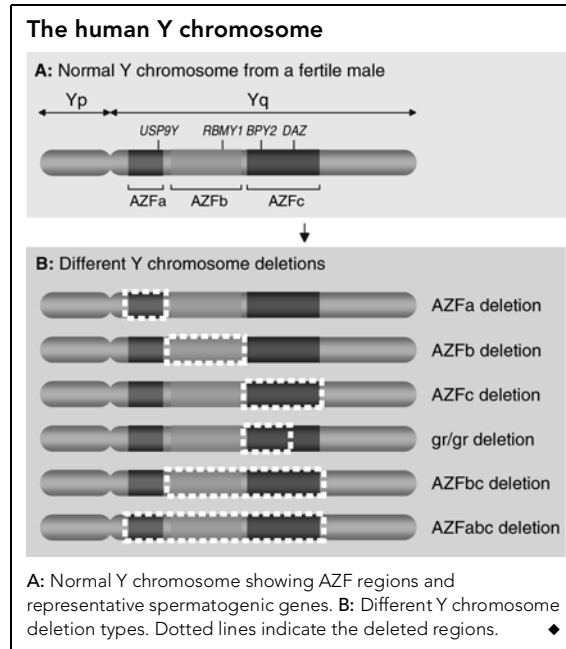
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*Some boys conceived through artificial techniques may inherit their fathers' subfertility*

Clinical assessment of couples unable to conceive naturally often identifies causative or contributory factors associated with the male partner. Male infertility affects one in 20 men, accounts for a third of all infertility, and is a cofactor in over half of assisted reproductive technology (ART) treatments worldwide.<sup>1</sup> Primary spermatogenic failure (SgF, also termed idiopathic infertility) accounts for more than half the cases, yet, in most of these cases, its cause is unknown.<sup>1</sup> In clinical practice, classification of SgF is based on semen parameters (describing combinations of poor sperm number, motility or function) and reflects an ignorance of the pathogenesis.<sup>2</sup> However, recent research has determined that up to 15% of SgF is related to at least six known Y chromosomal deletions, with implications for genetic testing, counselling, assisted reproduction and even subsequent male offspring conceived by ART.

Spermatogenesis is a complex process of cell division and structural modification involving the coordinated expression and interplay of many gene products. Recent data point increasingly towards a genetic basis for SgF. In particular, deletions of the Y chromosome — called microdeletions — are the most significant recognised cause of SgF in otherwise healthy men.<sup>3</sup> The Y chromosome is 60 megabases (Mb) in size, and comprises a short arm (Yp) that encodes the male sex-determining gene *Sry*, and a long arm (Yq) (Box). Of the 27 Y chromosome genes identified, nine are located on Yp and the remaining 18 on Yq.<sup>4</sup> Twelve of the 18 Yq genes are expressed in a testes-specific manner and are vital for normal sperm production.<sup>4</sup> Y chromosome microdeletions in fact involve substantial DNA deletions within the Yq region, ranging from 1.6 to 14.5 Mb and, depending on the deletion type, result in the loss of specific combinations of spermatogenic genes. Accordingly, men with Yq microdeletions are often (but not always) infertile, but many can still father children through intracytoplasmic sperm injection (ICSI),<sup>5</sup> using the few viable sperm present in semen or mature spermatids isolated directly from the testis.<sup>6</sup>

An association between Y chromosomal deletions and infertility was first reported in 1976 by Tiepolo and Zuffardi,<sup>7</sup> who detected large Yq deletions in six azoospermic men by routine karyotyping involving chromosomal banding. They proposed that an azoospermia factor (AZF) region was associated with spermatogenesis. Extensive physical, functional and genetic analyses of the Y chromosome<sup>8,9</sup> have now identified three AZF regions (AZFa, AZFb and AZFc), which encode spermatogenic genes such as *USP9Y*, *RBM1*, and *BPY2* and *DAZ* (Deleted in AZoospermia) (Box). DNA sequencing of the Y chromosome has identified unique structural features such as large palindromes (DNA sequences that read the same in both directions) that



encompass highly repetitive DNA elements.<sup>4</sup> Homologous recombination, involving elimination of one repetitive sequence at the expense of another, is believed to be the underlying mechanism that accounts for the random appearance of *de novo* AZF microdeletions in men. Interestingly, the fathers and brothers of men with Yq microdeletions usually have non-deleted Y chromosomes and normal sperm counts, indicating that these deletions are spontaneous events. The reason for the appearance of Yq microdeletions in some men is not known. We speculate that these deletion events occur during gametogenesis or early preimplantation development and may involve a deficiency in enzymes responsible for normal DNA repair.

Up to 15% of men with SgF and sperm densities below 5 million/mL have AZF deletions.<sup>3</sup> Variation in the reported incidence of Yq deletions in infertile men

has been attributed to factors such as patient selection criteria, the molecular test format, and the propagation of specific Y chromosome types (called haplotypes) within population groups that have different susceptibilities to deletion events. The three identified AZF regions contribute to six different Yq deletion types: AZFa, AZFb, AZFc, AZFbc, AZFabc,<sup>8</sup> and the *gr/gr* subdeletion<sup>9</sup> within AZFc (Box). There is no clear relationship between genotype and spermatogenic phenotype, but some generalisations can be made.

Most microdeletions (59.6%) involve the AZFc region<sup>3</sup> and are associated with the histological appearance of hypospermatogenesis, which is characterised by a reduction in germ cell number, mature elongated spermatids in some or all tubules, and low sperm densities ranging from 5 million/mL to azoospermia.<sup>10</sup> Less common Yq deletions involve AZFb (15.8%), AZFbc (13.6%), AZFa (4.9%) and AZFabc (<1%) regions; such men are often azoospermic and have more severe spermatogenic pathologies, such as arrested germ cell development or the Sertoli-cell-only syndrome. In 6% of cases, Yq deletions that involve regions outside of the three AZF regions have been identified in men with spermatogenic failure.<sup>3</sup>

Recent investigations of the Y chromosome have identified several smaller deletions within the AZFc region. For example, we have found that one such deletion, called *gr/gr*,<sup>9,11</sup> is more prevalent than AZFc deletions in severely oligospermic or azoospermic men (4.7% v 2.2%, respectively).<sup>12</sup> However, we also found *gr/gr* deletions at a similar frequency in oligospermic men (sperm densities, 5–40 million/mL). Interestingly, *gr/gr* deletions have also been found in some fertile men who have a particular Y haplotype.<sup>13</sup> Thus, *gr/gr* deletions are relatively independent of sperm density, but significantly associated with infertility. At this stage, the *gr/gr* deletion appears to be a risk factor for infertility rather than a definitive cause of SgF. The variable

sperm parameters observed in men with AZFc and gr/gr deletions could be due to a number of factors, including molecular heterogeneity of the deletion or functional compensation of the lost DAZ gene by its gene homologue DAZLA on chromosome 3. We are currently involved in an international effort to define more precise correlations between sequence variants of these deletions and semen parameters.

Vertical transmission of AZFc Yq deletions from infertile men to their sons via ICSI<sup>14</sup> and natural conception<sup>13</sup> has been reported, the latter underscoring that male fertility is possible even at low sperm output. We have generated a DNA database of more than 150 infertile men and their ICSI-conceived sons, and have identified and mapped the AZFc deletions in three Y-deleted men: in all cases, the same deletion was transmitted to the sons without expansion to other AZF regions.<sup>14</sup> Furthermore, analysis of a larger panel of Y chromosomal markers located throughout the AZF and surrounding regions has not identified *de novo* Yq deletions in 100 ICSI-conceived sons tested to date, indicating that ICSI treatment is not a risk factor for the generation of Yq deletions. However, it seems very likely that these ICSI-conceived boys with AZFc deletions will be subfertile and will need close review as they reach sexual maturation and proceed into adulthood with aspirations for fatherhood.

The European Molecular Genetics Quality Network (<http://www.emqn.org>) has established guidelines for Yq deletion testing and provides an important quality assurance function.<sup>15</sup> These laboratory guidelines have been widely adopted and have led to standardisation of Yq testing. The test is based on the analysis of a large panel of conserved molecular markers or genes located within and outside the AZF regions, using multiplex polymerase chain reaction (PCR) on peripheral blood genomic DNA. The pattern of these markers determines the Yq deletion type. Because of the inherent instability of the Y chromosome, it is likely that new Yq microdeletions will be identified and associated with SgF. High-resolution microarrays for chromosome screening will enable further investigation of the Y chromosome in fertile and infertile men, and microarray-based testing may replace multiplex PCR as the gold standard for Y chromosome testing in the future.

Given the relatively high prevalence of Yq deletions, most andrology and infertility centres now routinely offer Y chromosome testing to men with severe SgF, especially before ART treatment. Yq deletions have important implications for infertile couples, and genetic counselling following testing is recommended.

There are several considerations that support routine assessment of Yq deletions. Firstly, a positive test will provide a firm diagnosis of the man's problem, which, for some couples with longstanding infertility, can help resolve stress, blame or feelings of guilt. Secondly, knowledge of the type of Yq deletion may assist the clinician in determining the best ART treatment. For example, a Yq microdeletion involving the AZFa or AZFb regions carries a poor prospect of sperm retrieval, even with testicular biopsy,<sup>16</sup> thereby questioning the value of this approach and raising donor sperm treatment for discussion. Thirdly, couples should be offered this information, as they must understand that their male offspring will almost certainly be subfertile and require reproductive monitoring from the time of sexual maturation. As the natural history of SgF is poorly understood, it seems wise that consideration be given to sperm storage, as these young men born by ICSI may move from oligospermia to azoospermia before seeking fatherhood. Lastly, infertile men are known to be at increased risk of androgen deficiency<sup>17</sup> and testicular neoplasia,<sup>18</sup> however, whether the subgroup with Yq deletions have a greater risk also requires careful monitoring.

Following genetic counselling about their Yq deletion, most couples still proceed with in-vitro fertilisation using either the male partner's

sperm or donor sperm.<sup>19</sup> In a small number of cases, couples have used preimplantation genetic diagnosis to select female embryos for transfer,<sup>19</sup> in an attempt to avoid passing on the genetic abnormality to their children.

The relationship of Y chromosome deletions and other genetic lesions to male infertility will continue to be an active area of interest. Given the widespread use of ICSI to resolve male infertility, it is important that this research is translated rapidly and appropriately into clinical practice, and that prospective couples are provided with essential information that allows them to be fully informed when making this crucial life decision.

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