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Genetic Factors in Male Infertility and their Implications

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ABSTRACT Infertility in 20% of involuntary childless couples is attributable to purely male factors. In about 30% of such males the underlying cause is genetic. Male infertility problems tend to aggregate in the families and a decline of ~ 50% in the sperm concentration has occurred in the past 50 years. The mutations and microdeletions in the MSY region, especially those involving AZF, can cause oligospermia or azoospermia. The use of various assisted reproductive techniques by infertile men with microdeletions would significantly increase the incidence of male infertility.

INTRODUCTION

The prevalence of infertile couples is reported to be 10-15% (WHO 1987). It is usually accepted that ~90% of fertile couples conceive successfully within one year (Tietze 1956, 1968). Therefore, a couple that fails to conceive after 12 months of regular intercourse, in the absence of contraceptive use, is defined as infertile couple (Krausz and Forti 2000). The infertility in 20% of involuntary childless couples is attributable to pure male factors (WHO 1987). In ~70% cases of male infertility the underlying etiological cause can be found (Krausz and Forti 2000) but in the remaining 30% cases, the cause(s) remain unknown and these are referred to as idiopathic. In most of these cases there is an underlying genetic abnormality especially at the molecular level (Huynh et al. 2002). The reports indicate that the use of various assisted reproductive techniques can cause increase in the incidence of male infertility.

Trends in Geographical Variation of Male Infertility

Carlsen and co-workers (1992) reported that there has been almost 50% decline in the sperm concentration, from 113 to 60 million/ml, in the past 50 years. According to Joffe (2001) the sperm density has decreased at some places (e.g., Paris, Edinburg, Gent) but not at others (e.g., Toulouse, Finland and five US cities). The reported deterioration is not only in the sperm concentration but also in their morphology and motility. Such decline could have started much earlier than 1970s, but the data and the evidence to support this trend is not available. Kuroki et al. (1999) observed variations in the sperm counts among different populations and substantiated the genetic contributions of the normal Y chromosome to male fertility.

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Inheritance of Male Infertility

Male infertility problems tend to aggregate in the families and infertile males tend to have fewer siblings and their brothers also have inferior semen quality (Lilford et al. 1994; Meschede et al. 2000). It has been hypothesized that genetic damage in the germ line is responsible for impairment of male reproductive system. This damage may include chromosomal abnormalities, genetic anomalies, and may also cause cancer in the offspring as well as in the future generations (Joffe 1999; Wyrobek 1993). Heritibility of subfertility appears to be impossible, at first sight, as the presence of a single 'gene for infertility' is not possible as it would be quickly eliminated (Joffe 2003). Theoretically, this may not apply if the recessive gene carried some advantage but there is no evidence about it (Ober et al. 1999). However, an alternative explanation may be possible. In a steady state, a balance exists between the selection against polymorphisms that reduce fertility and the new polymorphisms originating due to genetic damage. As there is relation between these two processes, the new damage will occur at a greater rate than the

elimination, which would result in increased incidence of infertility (Joffe 2003). Moreover, since the end of last century, the assisted reproduction techniques have been helping subferitle and infertile couples to conceive. This increases the proportion of births to clinically subfertile couples at the population level. These two tendencies would decrease the rate of removal of subfertile polymorphisms from the population and it might also predispose to increase in testicular cancer (Joffe 2003) as there is a relationship between the infertility genes of the Y chromosome and the gonadoblastoma.

Chromosomal Abnormalities and Male Infertility

Kjessler (1974) and Chandley (1979), after a large karyotypic survey of subfertile males, proposed the first possible association between the chromosomal abnormalities and male infertility. The 47,XXY karyotype variant of Klinefelter's syndrome, is the most common chromosomal disorder associated with male infertility. 11% of the azoospermic and 0.7% of the oligospermic men have this condition (Bielanska et al. 2000; Bhasin et al. 2000). Cytogenetic abnormalities have been observed to be more frequent among infertile men than in the general population (Shi and Martin 2001). More specifically, the incidences of sex chromosomal and autosomal abnormalities in infertile men were observed by Bhasin et al. (2000) to be 15 times and 6 times higher respectively, than in the general population. They also showed that ~4% of infertile males had sex-chromosomal abnormalities, while ~1% had autosomal abnormalities.

Translocations or inversions have been frequently observed in infertile men but their contribution to infertility has not been properly understood (Huynh et al. 2002). The heterozygotes of autosomal translocations were seen seven times more often among infertile men as compared to the newborns (Krausz and Forti 2000). De Braekeleer and Dao (1991) reported that marker chromosomes were eight times more prevalent in infertile men. Chromosomal inversions were reported to be 13 times more frequent in infertile men as compared to the normal controls by Krausz and Forti (2000), who suggested that paracentric inversions in chromosomes 1, 3, 5, 6 and 10 might be interfereing with meiosis, resulting in decreased capacity to produce sperms and thus causing infertility in men.

DNA Variation and Male Infertility

Kuroki and co-workers in 1999 detected DNA variation in the Y chromosome among their cases and divided these cases into four groups according to their Y haplotypes (I, II, III and IV). They observed that haplotype II men had a lower sperm count than men in the other categories, and men with haplotype III were more likely to have azoospermia than others; thus implying that males with a certain specific haplotype were at a greater disadvantage for fathering children. However, Paracchini and co-workers (2000) failed to find any such correlation between the haplotypes and different sperm counts in Italian population.

Joffe (2003) observed that single nucleotide polymorphisms (SNPs) could lead to poor sperm quality as well as concentration and morphology as also reported by Wyrobek (1993). Krausz et al. (2001) reported that the relatively low sperm count noted in Denmark was not attributable to microdeletions, which normally lead to severe spermatogenic failure.

Y Chromosome Mutations and Deficiency of Males

The Y chromosome is a likely target for increased genetic damage. It has been reported that the probability of mutational damage in Y chromosome is increased due to the rapid division of germ cells, both during fetal and adult life. In the former case the exposure would be to the pregnant mother and in the latter case to the father before conception; but both cases would affect the birth cohort effect (Joffe 2003). The very high number of divisions in spermatogenesis as compared to the oogenesis led to the hypothesis that evolution is 'male driven' (Ellegren and Fridolfsson 1997). Furthermore, as all genes on the Y chromosome are "haploid", the defects in a single gene are more likely to produce effects, even though it is complicated due to the presence of multi-copy genes on the Y chromosome (Hargreave 2000). According to Davis et al. (1998) the selective effects on the Y-chromosome-carrying spermatozoa, leading to selective loss

of male embryos or fetuses, or mutations in the SRY gene on the Y chromosome can cause a deficit in the male births.

Y Microdeletions and Infertility

The important infertility related regions within the "Non-Recombining region of the Y chromosome" (NRY) or the "Male Specific region on Y" (MSY) include AZF (AZoospermic Factor), SRY (Sex determing Region on Y chromosome), and TSPY (Testes Specific Protein, Y-encoded). The mutations and microdeletions in these regions, especially those involving AZF, can cause either oligospermia or azoospermia. Though the exact frequency of occurrence of microdeletions is not known, it has been estimated that 1 in 4000 newborn boys have Y micro-deletions (Kuroda-Kawaguchi et al. 2001). It is also not known whether these deletions occur at a higher frequency in the testes of patients with impaired spermatogenesis (Le Bourhis et al. 2000). The observations of Tiepolo and Zuffardi (1976) about the presence of microdeletions in the distal part of Yq in six azoospermic men, with no other phenotypic anomaly, suggested that the factors influencing human spermatogenesis might be located on the distal region of Y (Yq11.23), which later on came to be know as 'azoospermia factor' (AZF). Disteche and co workers (1986) reported two infertile cases of 46 XY females, with small deletions in the short arm of the Y chromosome. The DNA analysis showed that the deletions in both the patients were different but included a common overlapping region that likely contained the testis-determining factor (TDF) gene.

The AZF region consists of three distinct region component viz., AZFa, AZFb and AZFc that are associated with male infertility (Vogt et al. 1996). AZFa is located in the proximal portion of deletion interval 5 (subinterval 5C). AZFb extends from distal deletion interval 5 to proximal end of deletion interval 6 (subinterval 5O-6B). The size of these two regions is expected to be between 1-3 Mb, but the definite size is not know due to the presence of repeating sequences in-between (McElreavey et al. 2000). AZFc lies immediately proximal to heterochromatin region of the Y chromosome, between deletion interval 6C and 6E. This region is ~1.4 Mb in size but the exact size of this region is also not kown due to the presence of repeating sequences (McElreavey et al. 2000).

Microdeletions of AZF region have been linked with various abnormalities like, Sertoli cell syndrome, spermatogenic arrest, morphological abnormalities of post-meiotic germ cells, etc., (Vogt et al. 1996). Hargreave (2000) also observed that severe infertility is a consequence of microdeletions in AZFa, AZFb and AZFc regions of Y chromosome. Foresta et al. (1999) and Dada et al. (2004) have reported that microdeletions in Yq may be responsible for severe bilateral testicular damage that might be phenotypically expressed by unilateral cryptorchidism, as well as by idiopathic infertility.

Repping et al. (2002) have reported that deletions of the palindromic sequences on the Y chromosome cause spermatogenic failure. They observed that the P5/proximal-P1 deletion had up to 6.2 Mb sequences and 32 genes and transcripts whereas; P5/distal-P1 deletion had up to 7.7 Mb and 42 genes and transcripts. Extensive STS based analysis of these palindromic complexes showed that *AZFb* and *AZFc* regions were not independent of each other as reported earlier by Vogt and co-workers (1996), but these overlapped. However, the AZFa region, which spans about 0.8 Mb sequences, was independent of both *AZFb* and *AZFc* regions (Sun et al. 1999). Ali and Hasnain (2003) have observed that in spite of exhaustive studies, no single gene responsible for male infertility in this region has been identified. The analysis of some still undetected small deletion will probably uncover the individual gene or gene families involved in spermatogenesis

Ferlin et al. (1999) reported that deletions in the AZFc region involving the DAZ gene were the most frequent and that larger deletions involving more than one AZF-candidate genes are associated with a more severe testicular phenotype. McElreavey et al. (2000) also observed that the deletions of AZFc region are the most common known cause of spermatogenic failure and account for more than 80% of all Y chromosome microdeletions. On the other hand, Kent-First et al. (1999) reported that Y-deletions are also present in 0.87% of normal fertile males

Cause of AZF deletions

The microdeletions in the Y chromosome that arise in the testes of a fertile father are usually due to accidental recombination between large sites having sequence identity and these result

in the loss of intervening sequences. Micro-deletions in the AZFa, AZFb and AZFc regions usually arise due to common de novo events (Katz et al. 2002).

Microdeletions in the AZFa region are thought to be the result of intrachromosomal homologous recombinations between highly homologous sequences present on both sides of AZFa region. These are human endogenous retrovirus (HERV) sequences (Lower et al. 1996 and Patience et al. 1997) that are 10 kb long and are separated by 700-800 kb (Blanco et al. 2000; Kamp et al. 2000; Sun et al. 2000). Such intrachromosomal recombination between two HERV sequences can result either in homogenizing sequence conversion or in a microdeletion in the AZFa region; causing the male infertility. This mechanism is believed to be responsible for most of the AZFa deletions (Blanco et al. 2000; Kamp et al. 2000; Sun et al. 2000). However, the deletion of any or all of the three azoospermia factors, *AZFa*, *AZFb* or *AZFc* disrupts spermatogenesis (Sun et al. 1999; Sun et al. 2000).

Kuroda-Kawaguchi and co-workers (2001) observed that a similar mechanism exists for AZFc reigion. However, the homologous sequences bounding the AZFc region are much bigger than the ones for AZFa region. A complex of three palindromes, the largest spanning 3 Mb with 99.97% identity between its arms, surrounds the AZFc region. These workers also reported that the deletions of the AZFc that cause infertility are remarkably uniform, and usually result from intrachromosomal recombinations between homologous 229-kb direct repeats that bound this region. Furthermore, Silber and Repping (2002) observed that the frequency with which the deletions occur seems to correspond to the length of stretch homology. As the stretch homology in AZFc region is 229 kb while in AZFa it is 10 kb, thus the AZFc deletions are much more common than AZFa deletions.

Transmission of AZF Deletions

Majority of the deletions of Y chromosome are believed to arise de novo or these have been transmitted by the father through intra-cytoplasmic sperm injection (ICSI) (Kent-First et al. 1996; Mulhall et al. 1997; Jiang et al. 1999). No natural transmission of microdeletions of AZFa region has been reported (Silber and Repping 2002). Rolf and co-workers (2002) reported the transmission of a partial AZFb deletion in three generations and they suggested that there might be an age related decline in the fertility of patients with partial deletions.

Natural transmission of the AZFc microdeletions from fathers to sons has been reported in some cases (Chang et al. 1999; Patsalis et al. 2002; Saut et al. 2000). Vertical transmission of the AZFc deletions is also possible through the use of ICSI and these have been reported but with reduced fertilization rate and poor embryo quality (van Golde et al. 2001). Stuppia and co-workers (1996 a) reported a case showing widening of AZFc deletion after being passed on from father to son; thus suggesting that some deletions may not lead to infertility but make the Y chromosome more vulnerable to second deletions that result in infertility (Rolf et al. 2002).

45X/46XY chromosomal mosaicism, in peripheral blood lymphocytes, has been reported among a subgroup of infertile men with AZFc deletions. Siffroi et al. (2000) believe that such mosaicism aggravates the spermatogenic failure. They further suggested that sex chromosome mosaicism could arise when AZFc-deleted Y chromosomes are transmitted from one generation to another through in-vitro fertilization. Patsalis and co-workers (2002) believe that men with AZFc microdeletions, who are going for ICSI, should be offered preimplantation diagnosis to reduce the chances of above mentioned anomalies.

Role of Y Microdeletions in Infertility Treatment

The detection of a microdeletion in an infertile man essentially provides a proper diagnosis. It also allows the clinician to avoid empirical, unnecessary, and often expensive hormonal treatments for improving fertility, and has important ethical consequences if the patient would be opting for assisted reproduction techniques (Foresta et al. 2001). Until the introduction of ICSI, there were no treatment options for infertile men (Palermo et al. 1992; Van Steirteghem et al. 1993). With ICSI even the most severe forms of male infertility i.e., the males having 100% morphologically abnormal sperms or those having just a rare spermatozoa in ejaculate, have been able to conceive (Liu et al.

1994; Nagy et al. 1995). In 1993, techniques like, TESE (testicular sperm extraction) and MESA (microsurgical epididymal sperm aspiration) were introduced in conjugation with ICSI for the treatment of obstructive azoospermia (Schoysman et al. 1993; Devroey et al. 1994). Later it was observed that TESE was also effective in many cases of non-obstructive azoospermia (Devroey et al. 1995; Silber et al. 1995; Silber et al. 1996). The rate of success of TESE-ICSI is so high that men with no sperms in their ejaculate can reproduce; the success being dependent upon the age of female and the quality of her ovarian reserve rather than on the quality of sperms (Silber et al. 1997; Silber et al. 1998). However, Hopps and co-workers (2003) observed that if there are microdeletion of the entire AZFa or AZFb regions then these portend an exceptionally poor prognosis for sperm retrieval, whereas majority of the men with AZFc deletions would have sperm within the semen or testes available for use in the IVF/ICSI.

Need for Genetic Ascertainment Before ICSI

As many cases of non-obstructive azoospermia are of genetic origin, there has been a concern that these genetic defects will be transmitted to the next generations. Moreover, many authors (Simoni et al. 1997; Chang et al. 1999; Page et al. 1999; Dada et al. 2004) have observed that Yq microdeletions could result in progressive worsening of sperm production and that with time the oligozoospermic men may become azoospermic. Therefore, it has been recommended that all males having AZF deletions, either through natural transmission or through ICSI should undergo andrological examination at puberty and if sperms are present; these should be cryopreserved in early adulthood before their possible decline with age (Rolf et al. 2002). It has been estimated that if half of all the azoospermic men undergo ICSI, the incidence of male infertility will double in seven generations (Faddy et al. 2001).

Patsalis et al. (2002) recommend that men with AZFc microdeletions, opting for ICSI, should be genetically counselled about the possibility of sexual ambiguities and other anomalies in their offspring. The genetic consequences of transmission of Y microdeletions warrant that the precise estimation of the relative function of various Y chromosome microdeletions in different Y chromosome genes, especially those involving AZF region, in the causation of male infertility, be precisely ascertained.

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