

·Short Communication·

Higher frequency of Yq microdeletions in sperm DNA as compared to DNA isolated from blood

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Abstract

Aim: To determine if Yq microdeletion frequency and loci of deletion are similar in two tissues (blood and sperm) of different embryological origin. **Methods:** The present study included 52 infertile oligozoospermic cases. In each case, DNA was isolated from blood and sperms and polymerase chain reaction (PCR) microdeletion analysis was done from genomic DNA isolated from both the tissues. The PCR products were analyzed on a 1.8% agarose gel. PCR amplifications found to be negative were repeated at least three times to confirm the deletion of a given marker. **Results:** Only 1 case harbored microdeletion in blood DNA, whereas 4 cases harbored microdeletion in sperm DNA. **Conclusion:** The frequency of Yq microdeletions is higher in germ cells as compared to blood. As the majority of infertile couples opt for assisted reproduction procreation techniques (ART), Yq microdeletion screening from germ cells is important to understand the genetic basis of infertility, to provide comprehensive counseling and most adapted therapeutics to the infertile couple. (*Asian J Androl* 2007 Sep; 9: 720–722)

Keywords: assisted reproductive techniques; infertility; semen; genomic DNA; Yq screening

Of men in the reproductive age group, 40%–50% have qualitative or quantitative defects in sperm production. Approximately 10%–15% of infertile men with idiopathic azoospermia or oligozoospermia have de-

letions on the long arm of the Y chromosome [1]. Sperm are produced by a complex differentiation process, which is controlled by many developmental genes. Spermatogenic alterations leading to infertility might be a consequence of systemic diseases, cryptorchidism, endocrinological diseases, outflow tract obstruction or infection or exposure to environmental endocrine disruptors, the xenoestrogens [2]. Genetic causes of infertility are an important aetiological factor leading to irreversible partial or complete spermatogenetic arrest. The genes critical for spermatogenesis and spermiogenesis are on the long arm of Y chromosome deletion interval 5 and 6.

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This locus is known as the azoospermia factor (AZF) as the most severe phenotype associated with its deletion is azoospermia. It has three subregions, AZFa, b and c, and deletion of each locus results in a characteristic testicular phenotype. To date, all studies from India and the majority of studies worldwide have analyzed Yq microdeletions from DNA isolated from blood [3–6]. However, DNA isolated from blood might not be representative of sperm DNA, which is of different embryological origin (enodermal) as compared to blood DNA, which is of mesodermal origin.

Sperm DNA might have higher rate of deletions and DNA damage as a result oxidative stress. Wang *et al.* [7] reported that reactive oxygen species levels are higher in the semen of infertile men. Therefore, sperm are susceptible to oxidative stress especially because of their unique structural composition. Also during spermiogenesis, when sperms shed their cytoplasm it leads to a deficiency in antioxidant enzymes. DNA damage in the Y chromosome can cause gene deletions in the Y chromosome [8]. The Y chromosome has the highest spontaneous loss of genetic material [9]. This is because of aberrant recombination events between areas of homologous or similar sequence repeats or gene families between the X and the Y chromosome or within the Y chromosome itself by unbalanced sister chromatid exchange [1]. The instability of the Y chromosome might also be a result of a high frequency of repetitive elements clustered along its length. Deletion interval 6 is rich in both direct and inverted repeats. This results in genomic instability of the Y chromosome. However, the main reason for deletion formation is the inability of the Y chromosome to undergo recombination repair. Deletions are preceded by double strand breaks, which are repaired by homologous recombination. However, because 95% of the Y chromosome is the male specific Y or the non recombining region it does not undergo recombination repair and, therefore, is the favored site for deletion formation [9].

Assisted reproduction procreation techniques (ART) have revolutionized the management of severe male factor infertility. In all cases opting for ART it is better to screen sperm for Yq deletions rather than blood. As germ cells genome is iatrogenically transmitted via ART, knowing the Yq microdeletion status of germ cells is more important.

The present study was undertaken to determine whether the blood DNA microdeletion picture matches the semen DNA Yq microdeletion profile. Semen analysis was done in each case to determine the sperm count,

viability, morphology and volume of semen according to World Health Organization (WHO) guidelines [10]. In the present study, blood and semen DNA microdeletion analysis was performed according to guidelines prescribed by the European Academy of Andrology in 52 oligozoospermic men and 40 fertile controls [11]. Polymerase chain reaction (PCR) screening was done for Yq microdeletions. Genomic DNA was isolated from blood and sperms of oligozoospermic men using an organic method. The semen samples were processed to extract DNA from germ cells [12]. Each of these patients was examined for six AZF loci that mapped to interval 5 and 6 of the long arm of the Y chromosome. The STS primers used were: for AZFa, sY84, sY86; for AZFb, sY127, sY134; and for AZFc, sY254, sY255. The internal control used was the sex determining region of the Y: sY14. PCR was set up in duplex. Fertile male and female samples were used as positive and negative controls and water was used as blank. This primer set was suggested by Simoni *et al.* [11] and prescribed by the European Academy of Andrology. It enables the detection of over 90% deletions in the AZF loci and allows for minimal standardization and comparison of the data on AZF deletions from different laboratories.

Of the 52 oligozoospermic cases analyzed, only one case harbored microdeletion in the blood DNA, whereas four cases harbored microdeletion in DNA isolated from germ cells. The deletions that were detected in sperm DNA were confirmed thrice to rule out amplification failure. No deletions were detected in blood or semen DNA of the control subjects.

One infertile case had AZFc microdeletion in blood and semen DNA and testicular fine needle aspiration cytology (FNAC) of this case showed hypospermatogenesis [13, 14]. The deletion was in the same loci. In addition in three sperm DNA samples, there was deletion of AZFb ($n = 1$) and AZFc ($n = 2$) loci, respectively, which were not detected in blood. FNAC in two cases showed hypospermatogenesis and in one case with AZFc deletion had maturation arrest. The DNA from the blood samples from these three cases showed amplification of all seven loci. The Yq deletion analysis was repeated thrice in sperm DNA samples by lowering the annealing temperature and increasing the amount of template DNA, but there was no amplification of DNA in these samples. Therefore, sperm DNA had a much higher frequency (7.6%) of Yq microdeletion as compared to that in blood DNA (1.9%).

The results of this preliminary study highlight the need for larger more extensive studies to determine the frequency and loci of deletions in blood and germ cells. Sperm Yq microdeletions aids in determining genetic aetiology in infertile men. It also helps in determining the prognosis and providing better management to couples should they opt for ART. Because the frequency of Yq microdeletions is higher in germ cell DNA, in all cases opting for ART it is more relevant and essential to analyze germ cell DNA than DNA isolated from blood for Yq microdeletions.

The Y chromosome is very prone to deletion because it is the only haploid component of the human genome and does not undergo recombination in the male specific region or non-recombining region of the Y chromosome. Therefore, deleterious mutations tend to accumulate on this chromosome. These mutations are promutagenic and once the paternal genome is expressed from day 3 (during cleavage) it can have deleterious effect on the developing embryo.

The results of the present study indicate the need for Yq microdeletion analysis from germ cells to understand the aetiology of infertility and to precisely define the genes involved in germ cell development and differentiation. In all couples opting for ART, sperm DNA Yq screening should take place to provide the most adapted therapeutics and counseling to the couple.

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