Infertility being a multifactorial disorder, both genetic and environmental factors contribute to the etiology of infertile phenotype. Chromosomal anomalies and Y-microdeletion are the established genetic risk factors of male infertility. Y-haplotypes has been found as risk factor for male infertility in certain populations, though in certain others no association has been reported, suggesting a population-specific association of these variations with male infertility. In a case-control study, 165 azoo-/oligospermic patients and 200 controls were haplotyped for certain Y-haplogroups for a possible association with idiopathic male infertility in an Indian population. Analysed Y-haplogroups showed no association with infertile phenotype. Thus this genetic factor is not a risk for infertility in the studied Indian population but that does not rule out the possibility of any of them, to be a risk in other populations.

**Keywords:** Male infertility, single nucleotide polymorphism, Y-haplotypes

**Materials and Methods**

**Subjects**

In a case-control study, 165 patients classified as
idiopathic azoospermic/oligospermic (157 azoospermic and 8 severe oligospermic with sperm count less than 1 million/mL) and 200 fertile control individuals of comparable age group (30, SD ±3), belonging to same geographical region were haplotyped for the binary alleles on the Y-chromosome. The cases were referred from the outpatent Endocrinology Clinic at the University Hospital and an in vitro fertilization (IVF) and a Urology Clinic, all in the city of Varanasi, India. Atleast three seminal fluid examinations, carried out after 3-4 days of sexual abstinence, were performed to ascertain there infertility status. Informed consent was obtained from all the subjects to carry out molecular analyses. Institutional ethical committee approval was obtained.

Y Haplogroup analysis

Genotyping of 4 SNPs and a 12f2 indel marker for all the subjects and controls were carried out by PCR-RFLP method [Figures 1-3]. The SNPs comprising different haplogroups were C→G (M9),[11] C→T (92R7),[12] A→G→A (SRY1532),[13,14] C→T (RPS4Y).[15] 12f2 deletion was genotyped.[16]

Statistical analysis

Statistical analysis was carried out to assess the significance level of the distribution of haplogroups between the idiopathic azoospermic/oligospermic (n=165) and fertile male control population (n=200).

Results

For Y-haplotyping, SNPs M9, 92R7, SRY-1532, SRY1532 (A→G→A) was haplotyped by PCR-RFLP method.[13,14] 167 bp PCR product after digestion with Dra III yields 112 bp and 55 bp products for the wild type A allele and the mutant G allele was not cut by the enzyme (c). RPS4Y (C→T) was haplotyped by PCR-RFLP.[15] 528 bp PCR product was digested with Bsi I and after digestion for wild type C allele 234 bp, 154 bp and 140 bp products were obtained whereas for mutant T allele 388bp and 140 bp products were obtained.

RPS4Y and the Indel marker (12f2) were analysed. A parsimony network illustrating the relationships between the analysed haplogroups is shown in Figure 4. The haplogroup frequencies observed in azoo-/oligospermic
Table 1: Haplogroup (hg) distribution in azoo-/oligospermic cases and control population

<table>
<thead>
<tr>
<th></th>
<th>hg1 (%)</th>
<th>hg2 (%)</th>
<th>hg3 (%)</th>
<th>hg26 (%)</th>
<th>hg9 (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoo-/oligospermic</td>
<td>42(25.5)</td>
<td>42(25.5)</td>
<td>30(18.2)</td>
<td>30(18.2)</td>
<td>21(12.7)</td>
<td>165</td>
</tr>
<tr>
<td>Control Population</td>
<td>38(19)</td>
<td>52(26)</td>
<td>42(21)</td>
<td>49(24.5)</td>
<td>19(9.5)</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>94</td>
<td>72</td>
<td>79</td>
<td>40</td>
<td>365</td>
</tr>
</tbody>
</table>

χ² = 4.275, Df= 4, P-value>0.05

Figure 4: Phylogenetic tree of SNPs’ and Indel marker used in the study and the haplogroup they define

infertile and fertile control men are summarized in Table 1. Out of the 6 haplogroup (Hg) markers used, only five were present in the studied population, Hg10, defined by RPS4Y711 C→T transition, being absent.[17] The association data as well as the frequency of alleles of different markers between the fertile and the azoo-/oligospermic subjects were subjected to χ² statistical analysis. The test results revealed no statistically significant difference at the 5% level for both the groups. Similarly, the analysed haplogroups revealed no difference between the case and control samples [Table 1]. Also, there was no significant association in the distribution of any of the markers (M9, 92R7 and SRY-1532) with the case or the control populations.

Discussion

Compared with autosomal or mitochondrial DNA, the Y-chromosome shows a relatively higher level of geographic specificity. In the context of male infertility and Y-haplogroup, Hg 26+ shows a risk for infertility in a Danish population.[1] In another report, an association of Hg4 with low sperm count and infertility was found in a Japanese population.[6] However, a reassessment of Kuroki’s Hg 4 data along with other Y chromosome haplogroups (Hg20, 5, 2) from the same Japanese population did not find any association with infertility.[17] Similarly, a study on Italian subpopulations of infertile and control individuals initially indicated an association with specific haplogroups and idiopathic infertile males, but when samples were subdivided according to their origins the putative association between the two groups disappeared.[18] The present report too does not find any haplogroup’s association with infertility. Hg26+, which showed association in the Danish population, had only a minor presence in both groups in the present study. The fact that so far only Hg26+ has shown a clear association suggests that there is need for exploring more haplogroups in more populations, and that a more rigorous analysis of multiple haplogroups may be necessary to derive meaningful conclusions.

In this paper we have found no association of infertility with the Y-chromosome haplogroups in an Indian population. Nevertheless, we maintain that such association studies need to be extended in their scope and in diverse populations to get a better perspective of the genotype-environment interaction in this multifactorial disorder.

Acknowledgments

We are thankful to all the patients and volunteers for providing blood samples and tissue material. Thanks are also due to Dr. S. K. Singh for his help in patient recruitment.

Ethical Approval: Written consent of all the participants was obtained and approved by the “Research Ethical Committee” of the Institute of Medical Sciences, Banaras Hindu University, India. Funding: This work was supported by a grant from the Department of Biotechnology, New Delhi, to R. R. and a SRF to Kiran Singh from the CSIR.
References


Source of Support: Nil Conflict of Interest: None declared.