Chapter V

CFTR gene polymorphism and male infertility
5.1. Introduction

The CFTR gene comprises a ~250 kb sequence of DNA located on the long arm of chromosome 7q31-32. A total of 27 coding regions have been sequenced (Figure 5.1). The normal CFTR gene encodes a 1480 amino acid glycoprotein known as cystic fibrosis transmembrane regulator protein (CFTR) [1]. The CFTR is a membrane protein, functions as a cAMP-regulator of chloride (Cl−) channel at the apical membrane of epithelial cells. If a cell is at rest, channels are closed and they do not secrete Cl− ions; however, when the channel opens to diffuse out of the cell and secretion ensues, which is an energy dependent process, regulated by the cAMP. Congenital bilateral absence of vas deferens (CBAVD) occurs in 1–2% of infertile men [2] and accounts for 25% of infertile men with obstructive azoospermia [3]. Studies have shown a high frequency of CFTR gene mutations in CBAVD patients [4, 5, 6, 7, 8, 9, 10, 11]. Types of mutations distributed throughout the entire gene were reported to be missense, frame shift, nonsense, splice, small and large in-frame deletions or insertions. It has recently been proposed that in absence of a well-defined CFTR mutation, frequent polymorphic CFTR variants may

![Figure 5.1 Genes located on chromosome 7](image-url)
predispose to obstructive azoospermia and perhaps other CFTR-associated diseases. Two such variants are the variable (TG)m repeat in front of the polythymidine tract in intron 8 and M470V polymorphisms in exon 10, are implicated as modulators of CFTR function. Indeed, genotype association with the M470V variant, producing less functional CFTR was found to be a risk factor for developing obstructive azoospermia, oligospermia and testicular failure. Although CFTR defect has not been generally implicated in spermatogenesis, a recent report suggested that the sperm maturation process can be delayed in CBAVD patients. Overall, it seems that the male reproductive tract is the most sensitive system of all CFTR-affected tissues, to even minor defects in CFTR function [12]. Therefore, the present study was extended to investigate on M470V polymorphism of CFTR gene on exon 10 and its association with male infertility.

5.2. Materials and methods

High molecular weight genomic DNA was isolated from PBL of controls \( n = 100 \) and cases \( n = 157 \) as mentioned in Chapter-2 (Section 2.4). The primer sequence for missense polymorphism M470V was adopted from the published literature [1] (Table 2.5) and the PCR program used for amplification is shown in Table 2.7.

5.3. Results

The PCR product was digested by \( HphI \) restriction enzyme and studied with PCR-RFLP. The amplified PCR product showed a band size corresponding to 491 bp. The genotype was interpreted as M/M homozygous (491 bp), V/V homozygous mutant 300 and 191 bp and M/V heterozygous (491, 300 and 191 bp) respectively based on the restriction digestion pattern (Figure 2.9 and 5.2). The allele and genotype frequency of M470V polymorphism in the exon-10 of CFTR gene, in controls \( n = 100 \) and infertile men \( n = 157 \) was investigated in the present study. In the control subjects analyzed, frequency of M and V alleles was found to be 0.55 and 0.45; the distribution of the M/M genotype was 36%, M/V genotype 18% and V/V genotype 46%. Whereas, in the infertile men the frequency of the M/M, M/V and V/V genotypes are 43%, 71% and 43% respectively (Figure 5.3). The frequency of M and V alleles was found to
be 0.5 each and the alleles were in Hardy-Weinberg equilibrium. The obtained results showed that M/V genotype was found to be significantly higher in cases than controls (OR 3.3; 95% CI 1.67-6.52 P < 0.001) suggesting an association of CFTR gene M470V polymorphism for infertility.

Table 5.1. Distribution of allele and genotype frequency of M470V polymorphism in controls and infertile men

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Genotype frequencies (%)</th>
<th>Allele frequency</th>
<th>P value</th>
<th>Chi square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M470V polymorphism</td>
<td>MM MM MV VV MV M V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n=100)</td>
<td>Observed 36 18 46 0.45 0.55 NS 40.495</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Expected 22.3 49.5 30.3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Infertile men (n=157)</td>
<td>Observed 43 71 43 0.55 0.50 0.0231 1.433</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expected 39.3 78.5 39.3</td>
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</tbody>
</table>

Table 5.2. Association of M470V polymorphism of CFTR gene and infertility

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotype frequency (%)</th>
<th>OR 95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n= 100</td>
<td>Infertile men n=157</td>
<td></td>
<td></td>
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<tr>
<td>M470V polymorphism</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MM MM MV VV VV</td>
<td>36 18 46 43 1 3.3 1.27</td>
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<tr>
<td></td>
<td>43 71 46 43 3.3 1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.27</td>
<td>0.43-1.44</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: Non Significant
Figure 5.2. Electrophoresis on a 2.5% agarose gel - PCR-RFLP of M470V polymorphism

Lane 1-2: M/M homozygous wild type allele at 491 bp; Lane 3-5: V/V homozygous mutant allele at 300 and 191 bp; Lane 6-9: M/V heterozygous allele shows restriction sites at 491, 300 and 191 bp; Lane 10: Molecular weight DNA ladder (100 bp)

Figure 5.3. Allele and genotype frequency distribution of M470V polymorphism
5.4. Discussion
Chromosomal aberrations in the entire genome and microdeletions on Y-chromosome are well recognized genetic causes for male infertility. In the absence of those well-defined genetic causes, recent literature has suggested variation or polymorphisms in many genes that have been associated with male infertility. In about 97-98% of men with CF, a CBAVD/CUAVD blocks the transport of spermatozoa from testicular or epididymal structures to the outer genital tract, resulting in azoospermia [13, 14, 15, 16]. In a study it was shown that 12 CFTR gene mutations were on 48% of CAVD patients L69H, F87I, G126S, F157C, E543A, Y852F and D1270E and F508del. In addition to reported mutations, CFTR gene polymorphisms have also been to influence male fertility [17]. Over 200 DNA polymorphisms that are not disease-associated have been identified within the CFTR gene; one half of these polymorphisms occur in CFTR coding regions and the other half result in amino acid substitutions. DNA polymorphisms have also been detected that could potentially result in alterations in CFTR expression [18], and an effect on the severity of the phenotype. Several reports have suggested that the most frequent CFTR polymorphism, M470V, plays a role in modulating CFTR protein at transcriptional and translational levels in infertile men [19, 20].

The present study displayed a 3.42% of chromosomal abnormalities, 12.56% of microdeletion on Y-chromosome, M470V polymorphism of CFTR gene in the infertile men with unknown etiology. The V allele (0.55%) was found to be frequent in controls (Table 5.1); the obtained results are consistent with a Chinese population [21] and other populations [22]. The control samples did not follow Hardy–Weinberg equilibrium; having excluded errors from genotyping methodology, the present study has assumed that a deviation of this sort is due to inbreeding, leading to enrichment of homozygosity as suggested earlier [23, 24].

Infertile men of the present study, M is found to be the frequent allele (0.55%) followed by V allele (0.50%). The heterozygous genotype MV is predominant with 71% (Table 5.3); this genotype showed an association (CI-1.67-6.52; P < 0.001) with the male infertility when
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compared to that obtained in control subjects. A total of 132 healthy unrelated subjects were randomly selected from the population of Jingsu Province of China showed dominant genotype M/V followed by V/V and M/M. M470V polymorphisms were distributed in other East Asians, and have marked differences from the frequencies of single haplotype polymorphisms or linkage haplotypes in Caucasians [21]. This has been demonstrated that infertile men with CBAVD and CUAVD showed higher risk in addition to mutations in many CFTR loci [17]. Previous studies have demonstrated that polymorphisms outside the CFTR gene, as well as within the gene, may affect transcription or function of the CFTR protein. Reports have shown that M470 allele causes a delay in CFTR protein maturation and gives raise an increased probability of chloride channel opening when compared with the V470 CFTR protein [19].

Homozygous wild type allele M or variant allele V did not show any association for male infertility, the reason being the allelic heterogeneity. Similar results have provided evidence for an extensive allelic heterogeneity in Indian patients with CFTR gene polymorphisms, for a larger number of CFTR [17, 25]. Alternatively, modifier genes can alter clinical expression through differences in the other relevant biochemical pathways implicated in the pathogenesis of organ manifestations. Several polymorphisms or the presence of a combination of particular alleles at different loci affects the quantity/quality of CFTR gene are known to influence disease severity in subjects with CFTR-related disorders [19]. The molecular heterogeneity of CFTR polymorphism in infertile men emphasizes the importance of extensive CFTR analysis in Indian populations. According to this view, there are basically no monogenetic disorders, i.e., phenotypic manifestations of the disease are influenced by other genes and potentially by environmental factors. To better understand the etiology of male infertility, future studies will need to be conducted on more subjects to obtain a statistical significance and to focus on identifying and studying new candidate genes in order to obtain a deeper understanding of the complex gene-to-gene interactions which has a profound effect on studied pathology and confirm this observation. It is suggested that studying genome-wide data substantiated with complete analysis of CFTR gene can provide convincing correlation and better counseling of
the patients. The study also requires further investigation into the functional aspects of novel variants in the cell-based system to predict their pathological significance.

5.5. Conclusion

Despite a significant improvement of the research in genetic work-up of infertile men in about 50% of cases the cause of abnormal spermatogenesis remains unknown and is called ‘idiopathic infertility’. Considering the involvement of a high number of genes in mutations or polymorphisms in spermatogenesis it is identified that the candidate genes are responsible for majority of ‘idiopathic’ forms of spermatogenic disturbances. The screening for these polymorphisms may be studied by the application of new technologies like whole-genome microarrays and a large number of well-selected controls and cases of different ethnic origins enable to increase efficiency of the screening and reliability of the results.


References


