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Commentary

CFTR gene variants in Indian congenital bilateral absence of vas deferens & its relevance in genetic counselling

Cystic fibrosis is an autosomal recessive genetic disorder and common in populations of Northern European descents. The disease is caused by the mutation of cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that encodes a chloride-conducting transmembrane channel and regulates ion and water secretion as well as absorption in epithelial cells (anion transport and mucociliary clearance)[1]. More than 2000 *CFTR* gene variants are known with variable (asymptomatic to severe) clinical manifestation[2]. CFTR variants are classified into Class I to Class VI where variants of Classes I to III present as complete lack of function of *CFTR* gene with severe manifestations and Classes IV to VI variants present as mild phenotype due to some *CFTR* gene activity[3]. F508del (Phe508del/class II) variant is most frequently observed in north-western Europe; however, it is less frequent as well as associated with milder phenotype in Asians, including Indians[4-6]. *CFTR* gene variants (Class I-III) may present as classical cystic fibrosis (with severe pulmonary and pancreatic involvement) or as mild respiratory problem or as male infertility (Class IV-VI) associated with congenital absence of vas deferens (CAVD).

CAVDs are various types such as bilateral (CBAVD) or unilateral (CUAVD), complete or partial and with or without urogenital anomalies, including unilateral renal agenesis. The prevalence of CAVD in infertile men is 1-2 per cent[7]. Men with CAVD may present as cystic fibrosis or as CFTR-related disorders or as obstructive azoospermia or asymptomatic (discovered by chance). CBAVD is associated in almost all men with classical CF[8]. CAVD may be associated with two CFTR mutations (homozygous or compound heterozygous) with or without partial penetration or other mechanisms such as mutations in other related genes. Family discordant is also evident like siblings with identical CFTR variant-displaying different phenotypes, one with normal fertility whereas the other inferile[9]. This indicates involvement of additional genes in the aetiology of CBAVD. Majority of CAVD cases have at least one cystic fibrosis causing mutation, mostly compound heterozygotes[10]. Men with compound heterozygosity (one mild/variant type IV-VI and other severe mutation/variant type I-III) may have only CBAVD or with mild form of CF. Among these variants 5T, F508del and R117H are common. These mutations have also been found in Indian males with CAVD, although the frequency of 5T allele is more and F508del is less[11]. 5T is generally considered a mild mutation, but its association with (TG)m variants (12 or 13 TG repeats) in the intron 9 of *CFTR* gene enhances its severity and association with CBAVD[2].

Approximately 90 per cent men with CAVD as a monosymptomatic form of CF have at least one mutated CFTR allele, and those having mutations in both the alleles carry a severe mutation in one allele and a mild mutation in the other but never two severe mutations[12,13]. Meta-analysis studies also have shown hemizygous state/single variants in about 25-28 per cent cases of CAVD, in particular, with unilateral type[14,15]. CFTR variants in CAVD with or without renal agenesis are controversial, some found association[16,17] whereas others found no association[18,19]. It is also important to mention here that some CBAVD men do not have mutations in *CFTR* gene. This suggests that there might be other genetic or environmental factors that might also be responsible for CAVD. Other genetic associations with CAVD are mutations in adhesion G protein-coupled receptor G2 (ADGRG2)[20,21] and copy number variations in pantothenate kinase 2 (PANK2) as well as solute carrier family 9 sodium/hydrogen exchanger isoform 3 (SLC9A3) genes[22]. About two per cent of CAVD cases are associated with mutation in

ADGRG2 gene (X-linked hemizygous mutation). Some of these genes (ADGRG2/SLC9A3) are validated in animal-knockout experiments[23]. Other rare genes associated with CBAVD are sodium channel epithelial 1 subunit beta (SCNN1B) and carbonic anhydrase 12 (CA12)[24]. However, in about 25 per cent cases of CAVD, mostly with CUAVD with a solitary kidney, no genetic associations are detectable.

It is often found that men with CBAVD have normal production of spermatozoa, but due to agenesis of vas deferens, there are no sperms in the ejaculate. This suggests that men with CBAVD can attain fatherhood by using assisted reproductive techniques (ARTs) such as epididymal/testicular sperm aspiration followed by *in vitro* fertilization or intra-cytoplasmic sperm injection[25]. In this modality of achieving fertility, there is always a chance of transmission of CFTR/other pathogenic variants to the offspring, especially if the female partner is a carrier for CF. Therefore, genetic counselling should be offered to such couples undergoing ART, and both partners should be screened for *CFTR* and other genes mutations to discuss the probability of having offspring with similar problem. Further, parents must be investigated for CFTR mutations of cases with unknown or single mutation/variants associated with CAVD. This will help in proper prediction of pathogenicity/role of variant/s in causing phenotype and thus accurate reproductive genetics counselling.

Due to limited information available on the frequency and spectrum of *CFTR* and other related gene mutations in CAVD in the Indian population, it is difficult to provide accurate genetic counselling to couples, at present. From this point of view, the study of Gaikwad *et al*[26] published in this issue has a noticeable significance. The study was undertaken to investigate the spectrum and frequency of *CFTR* gene mutations in Indian men with CBAVD and to determine the female CF carrier status. Significant association was observed for *CFTR* gene variants in CBAVD men versus controls. Direct DNA sequencing of the *CFTR* gene in 80 CBAVD men, their female partners and 50 controls from general population, led to the identification of 20 *CFTR* gene variants (comprising of ten novel and ten known variants) in 53 CBAVD men with 66.3 per cent frequency. Pathological significance of the identified novel *CFTR* gene variants was carried out using *in silico* tools. Of the 10 novel sequence variants, eight were located in highly conserved regions, which could functionally alter CFTR protein organization. Two novel splice-site variants are predicted to induce splicing error and thus damage the translated CFTR protein, but further *in vitro* functional assays or case–parent trio studies will be required to provide significant insights regarding the pathogenicity of the novel (newly identified) variants.

An important aspect of this study is the genetic analysis of female partners, as only a few studies have attempted to screen for CF female carrier status in the Indian population. A total of 13 (16.2%) female partners were found to be CF carriers. The T5 allele was the most frequent variation found in both males and females. Appropriate genetic counselling was also provided to the couples before ICSI. Nine couples had a risk of transmitting mutant CFTR allele to the offspring, while six of them underwent ICSI, with two successful pregnancies resulting in live birth. It would have been interesting to know what the status of their offspring was, had the authors analyzed the *CFTR* gene status in those children as well. One more area of concern is that 33.7 per cent CBAVD men had no mutations in the *CFTR* gene and role of genes such as *ADGRG2, SLC9A3, PANK2, SCNN1B* and *CA12* was not evaluated. These genes are also to be evaluated for variants as well as copy number variation (in particular for *SLC9A3* and *PANK2* genes)[27] for associations and prevalence in Indian men with CAVD. Therefore, it is the need of the hour to discover new male infertility genetics in obstructive azoospermic men with CAVD by making use of next-generation sequencing techniques, in particular, the whole-exome sequencing along with copy number variation analysis as the first step. Finally, it is worth exploring CFTR/related genes involvement in conditions such as semen with high viscosity and high liquefaction time or conditions with thick/viscid/scant mucus (cervical, nasotracheal, salivary, *etc.*).

***Conflicts of Interest*:** None.

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