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THE RELATIVE FREQUENCY, PATTERN OF FIVE COMMON CFTR MUTATION AND IMPORTANCE OF GENETIC COUNSELING IN PATIENTS WITH CLINICAL SUSPICION OF CYSTIC FIBROSIS: A METROPOLIS EXPERIENCE

Genetics

Dr. Shailesh S Pande* MBBS,MS,FDBT(Medical Genetics), Senior Medical Geneticist & Genetic Counselor, Consultant-HOD, Dept. of Medical Genetics, Metropolis Healthcare Ltd, India *Corresponding Author

Pratiksha Chheda Sr. Manager | Molecular Pathology/ R&D, Metropolis Healthcare Ltd, India

Shweta Limaye Scientific officer | / R&D, Metropolis Healthcare Ltd, India.

Dollar Goradia Scientific Officer - / R&D, Metropolis Healthcare Ltd, India.

Dr. Sushant Vinarkar Head-Molecular Pathology and C - Molecular Biology, Metropolis Healthcare Ltd, India.

ABSTRACT

Cystic fibrosis is one of the most common autosomal recessive disorders and it is now observed to be far more common than was thought in India. As the number of genetic testing centres is increasing and thus the availability of testing facility which has shown that presence of CF cases is significant in Indian population. It has been reported that CFTR gene analysis in Indian population shows most common mutation in the form the delta F508. Mutations in CFTR gene additionally are associated with male infertility in cases with obstructive azoospermia. IVS8-T5 and delta F508 are most commonly seen mutations in individuals having obstructive azoospermia secondary to congenital absence of vas deference. Mutation studies of common five mutations can help to detect the condition in majority of the patient with clinical suspicion of CF. Appropriate genetic counseling can help the patient and family for estimation of risk to the individual and family members and opens the options of reproduction.

KEYWORDS

Cystic fibrosis (CF), CFTR, Genetic Counseling, molecular diagnosis, PCR, Congenital absence of vas deferens (CAVD)

Introduction:

Cystic fibrosis (CF) is a condition which is inherited in an autosomal recessive pattern affecting mainly lungs and also liver, pancreas, kidneys and intestine [2,5]. It is caused by mutation in the gene named as Cystic fibrosis transmembrane conductance regulator (CFTR) gene. The affected individual should have two mutated copy of CFTR gene from each parent .If the children have only one mutated copy inherited they won't develop the condition. Mutations in CFTR genes affects the cells that produces the mucus, sweat, and digestive juices[11,13]. The cystic fibrosis is the most commonly seen in northern Europe where the frequency is around 1 in 2500 and the carrier frequency around 1 in 25 [14]. The estimated incidence of CF in Asians migrated to united kingdom is about 1:10,000 to 1:12,000. Unfortunately the extensive data for Indian population is not available [7,8,11,12].

The signs and symptoms vary based on severity of the disease ,usually such individuals have higher level of salt in there sweat ,recurrent respiratory tract infection ,coughing or shortness of breath, inflamed nasal passage ,exercise intolerance ,bowel obstruction, foul smelling stools, severe constipation, meconium ileus [1,3,4] .The poor growth of the affected individual could be because of chronic lung infections ,poor absorption in the gastrointestinal tract and increased metabolic demands secondary to chronic illness especially in paediatric age group .However some individuals may not experience symptoms till adulthood. The improvement in the screening and treatment has led to comparative increase in the life expectancy and is between 42 to 52 years in developed countries and majority of deaths in individuals with CF are due to lung problems almost in 80 % of the cases.

In adults cystic fibrosis is one of the common causes of infertility. Almost 95 % of men with CF may have infertility and needs assisted reproduction. Congenital absence of vas deference (CAVD) leading to no sperm is the main cause of male infertility in individual having CF. Around 20-25 % of females with CF experience difficulties in conceiving due to thickened cervical mucus or malnutrition.

The most common mutation observed in two-third (66-70%) of CF cases worldwide is delta 508. It's in the form of deletion of three nucleotides which leads to loss of amino acid phenylalanine at 508th position on the protein.G542X, G551D, R553X and Intron 9 - PolyT/Poly TG mutation of CFTR gene including delta F508 are most common mutations .Almost more than 1900 different mutations in the CFTR gene have been reported till date and around 1500 are considered pathogenic as their association with the symptoms of patients with CF is observed.

Genetic screening for common five mutations can be very helpful in cases with clinical suspicion of CF.

Materials and Methods:

We studied 148 unrelated Indian patients (93 children and 55 adults) with clinical suspicion of cystic fibrosis (age 1 month to 45 years). A peripheral blood sample was collected from each patient and DNA was extracted using *QIAamp*® Blood Mini Kit (*Qiagen*). We screened for 5 common mutations - Delta F508, G542X, G551D, R553X and Intron 9 - PolyT/Poly TG mutations in exons 9, 10 and 11 of CFTR gene by PCR followed by Sanger sequencing using primers sets described earlier [16]. The capillary electrophoresis was carried out on Applied Biosystems 3500Dx Genetic Analyser and sequence was manually checked for the presence of specific variants [16]. Patients were recruited from different centres all over India after evaluation by Clinical Geneticists.

Results:

A total of 148 patients with clinical suspicion of cystic fibrosis were referred during the period of June 2015 to May 2018 for five common mutations namely Delta F508, G542X, G551D, R553X and Intron 9 - PolyT/Poly TG mutation of CFTR gene. Out of 148 patients, 92 patients (62.16%) were male and 56(37.83%) were females. Out of 92 males, 60 (66.66%) were below 15 years and 32(34.78%) were above 15 years. Out of 56 females, 33(58.92%) were below age of 15years and 23(41.07%) above the age of 15 years. In total of 148 cases 22(14.86%) cases have shown mutations in CFTR gene. Of the 22 cases showing mutations, 12 (54.54%) are males and 10(45.45%) are females. Also 12 (54.54%) cases were below 15years of age and 10 (45.45%) were above 15 years of age.

Out of 12 cases below 15 years of age, homozygous mutation was seen in 6(50%) cases, of these 6 cases 3(50%) were male and 3(50%) were females. Heterozygous mutation was seen in 3 cases, of these 3 cases, one (33.33%) was seen male and 2 (66.66%) in females. Compound heterozygous mutation was seen in 3 cases, out of which one (33.33%)

was male and 2 (66.66%) were females.

Total numbers of cases of above 15 years were 55 of which 32 (58.18%) were males and 23 (41.81%) were females. Out of these mutations in CFTR were seen in 10(18.18%) cases , 8 (80%) cases showed heterozygous mutation of which 6(75%) were males and 2(25%) were females .Homozygous mutation was detected in one (10%) female patient and compound heterozygous mutation was seen in one(10%) male.

Out of 22 cases detected with CFTR mutation 16(72.72%) cases showed **Delta** F508 deletion, 6 (27.27%) cases showed Intron 9 - PolyT/Poly TG mutation. However no G542X, G551D and R553X mutation were detected in any of the case The positivity observed is approximately same with other studies done on Indian patients with slight variations as the sample received at Metropolis Healthcare Ltd a Global Reference Laboratory are from different parts of India and also because of the selection bias since only the highly suspected samples were referred to our laboratory for studies.

Table 1: Number of CFTR mutation below 15 years of age

Gender	Homozygous	Compound Heterozygous	Heterozygous
Male	03	01	05
Female	03	02	00

Table 2: Number of CFTR mutation above 15 years of age

Gender	Homozygous	Compound Heterozygous	Heterozygous
Male	00	01	06
Female	01	00	02

Discussion:

Cystic fibrosis is one of the most common serious autosomal recessive disorder in northern Europe and it is now observed that far more common than was thought in India as well. There are around 1500 reported pathogenic mutation seen in CFTR gene however 5 common mutations included in our study are most commonly seen. Almost 66-70 % of the cases with CF shows delta 508F mutation .The variation of prevalence of type of CFTR mutation is well documented and a huge heterogeneity between the countries is reported [20]. Since CF is autosomal recessive consanguinity in parents can increase the risk of having CF. Genetic counseling in CF can be more accurate and useful with appropriate genetic testing which helps to detect the mutations in particular family[14]. If a couple has a previous child with CF and mutation analysis confirms the same possibility of having CF in next pregnancy is 25% as usually parents of such affected baby are carrier for the condition. Early prenatal testing in such couples can help to detect such mutations in the fetus and can assist the couple in taking the informed discussion. In patients showing no mutations in the 5 common mutation studies ideally should be correlated with clinical symptoms, family history and other laboratory findings and in symptomatic Patient, complete gene sequencing of CFTR gene should be always recommended to establish a definite diagnosis and same was suggested in counseling session at our centre. In our study for five common mutations, 22(14.86%) patients have shown mutations in CFTR gene. Out of 148 cases referred, homozygous mutation was seen in 7(4.72%) cases, heterozygous mutation in 11(7.43%) ,compound heterozygous mutation in 4(2.70%) cases this is to an extent matching with the other studies done in India.

Conclusion:

CF, an autosomal recessive genetic condition was once thought to be very rare in India, however recent studies from different part of India had shown that CF is not far uncommon in India than previously thought and could be under diagnosed [2,4,5,6]. The precise prevalence of CF in India is unknown still studies have shown that consanguineous marriage and high genetic heterogeneity in our population accompanies higher carrier frequency than expected. It is observed and believed that prevalence of CF is very rare the disease is rarely suspected and as very few genetic counselling and testing centres were available the condition was not confirmed due to non-availability of testing facility. However recently it is observed that the scenario is changing and awareness about the genetic conditions, genetic testing and importance of genetic counseling is comparatively increasing. Screening for common five mutations of CFTR gene followed by complete gene sequencing if needed along with appropriate genetic counseling can be very helpful for the patients for management of the condition and also for the couples with history of infertility it opens up options of reproduction. Prenatal diagnosis in such carrier couples can help to detect the mutation status in the foetus to make an informed choice [14].

Ethical statement

Our study has been done on live subjects and written and informed consent was not necessary in our diagnostic setting. The informed consent was obtained by clinician when this material was collected for diagnosis. This is an amalgamation of our data of testing of these results. All these tests referred were part of the routine diagnostic procedure and clinical details were part of the Test Requisition Forms which were analysed anonymously and the need for ethics committee approval was ruled out by The Institutional review board.

Conflict of Interest: No

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