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Spectrum and distribution of CFTR gene mutations in asthma and chronic pancreatitis cases of North Indian population

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ABSTRACT

Background: Cystic fibrosis transmembrane regulator (CFTR) gene accounts for an autosomal recessive condition called cystic fibrosis (CF). In the Indian subcontinent, CF and its related diseases are under-diagnosed by the medical community due to poor knowledge of the disease and its confounding diagnosis, and also due to poor medical facilities available for these patients, thus causing an increased infant mortality rate with a low life expectancy in general. The aim of the study was to document the spectrum and distribution of CFTR mutations in controls, asthma and chronic pancreatitis cases of North India.

Methods: A total of 800 subjects including 400 controls, 250 asthma cases and 150 chronic pancreatitis cases were analyzed for 6 mutations (F508del, G542X, G551D, R117H, W1282X, and S549N) and IVS8 Tn polymorphism. **Results:** Out of 800 subjects, 18% [asthma – 24% (n = 250), CP – 29.33% (n = 150) cases and controls – 9.3% (n = 400)] were positive for heterozygous mutation, 0.8% of the (n = 250) asthmatic cases (n = 250) were homozygous for IVS8 T5 polymorphism while no subjects were found positive for W1282X mutation. T5 polymorphism was more common in asthmatic cases while F508del mutation in chronic pancreatitis cases. The carrier frequency of F508del, G542X, G551D, R117H, S549N and T5 was 0.015, 0.025, 0.02, 0.005, 0.005, and 0.022 respectively. The cumulative carrier frequency was 0.093.

Conclusion: CFTR mutations were underestimated in Indian population. The present study will serve in establishment of genetic screening and prenatal setup for Indian population.

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1. Introduction

Mutation in both the alleles of the cystic fibrosis transmembrane regulator (CFTR) gene accounts for an autosomal recessive condition called cystic fibrosis (CF) (Mickle and Cutting, 1998). More than 1910 mutations have been reported in the CFTR mutation database since the identification of this gene in 1989 (Riordan et al., 1989), and most of which are either point mutations or small (1–84 bp) deletions. However, the predominately observed mutation is F508del (Phe508del), which accounts to 30%–80% of the mutant alleles, depending on the ethnic groups (Watson et al., 2004).

Mutations in the CFTR gene are also involved in diseases that share part of the CF symptoms, such as congenital bilateral absence of vas deferens (CBAVD), obstructive azoospermia, disseminated bronchiectasis, diffuse panbronchiolitis, pulmonary emphysema, allergic bronchopulmonary aspergillosis, asthma, chronic pancreatitis (CP) and neonatal hypertrypsinemia. Among all the conditions: pulmonary diseases are the major cause of morbidity and mortality in CF (Moskowitz et al., 1993).

Asthma is the most common disease associated with the CFTR. Although there have been numerous studies that have documented a positive association of this gene with asthma, there also other studies that claim there is either a protective or a lack of association between the two. The diagnosis of asthma in CF patients is mainly clinical with several suggestive factors involved (Maurya et al., 2012).

Several recent studies have shown a positive association of CFTR gene with asthma (Dahl et al., 1998, 2005; Lazaro et al., 1999; Ngiam et al., 2006; Tzetis et al., 2001), while other studies found either protective (Schroeder et al., 1995) or lack of association (De et al., 2001; Douros et al., 2008; Kauffmann et al., 1998; Kim et al., 2010; Lowenfels et al., 1998; Mennie et al., 1995; Munthe-Kaas et al., 2006).

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane regulator gene; CP, chronic pancreatitis; CBAVD, congenital bilateral absence of vas deferens; dNTPs, deoxyribonucleotide triphosphates; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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In CP, CFTR gene involvement is defined more clearly (Shwachman et al., 1975). However, the reports are contradictory, where a group of studies observed the CFTR association with CP (Audrezet et al., 2002; Cohn et al., 1998; Noone et al., 2001; Sharer et al., 1998) while other studies found no association (Monaghan et al., 2000; Sharer et al., 1998). Recent studies have reported a higher frequency of CFTR mutations in patients with alcohol-related CP suggesting that mutations in the CFTR gene could be a risk factor for alcohol-related CP (Truninger et al., 2001).

It is likely that cystic fibrosis and CF-related diseases are under-diagnosed in Indian continent due to the medical community's lack of knowledge of the disease, poor access to medical facilities and health care for CF patients, confounding diagnosis, a high infant mortality rate, and low life expectancy in general. Respiratory and gastro-intestinal problems associated with malnutrition are very common in developing countries, and the diagnosis of CF can therefore be missed due to a low index of suspicion.

For this reason, we aimed to document the spectrum and distribution of CFTR mutations in controls, asthma and chronic pancreatitis cases of North India.

2. Materials and methods

2.1. Study subjects

Clinically confirmed asthma cases ($n = 250$) with exclusion criteria of increased sweat chloride level of >60 mmol/L were recruited from the Department of Pediatrics, King George's Medical University (KGMU), Lucknow. From the Department of Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow clinically confirmed CP cases ($n = 150$) were recruited with exclusion criteria of alcohol intake and familial history of pancreatic cancer. Altogether 800 subjects were enrolled; it includes 400 cases and 400 controls. Controls were healthy individuals unrelated to the cases. Informed consent was obtained from the subjects before enrolling them in our study. The study was ethically approved by Institutional Ethics Committee.

DNA extraction was carried out in all the samples by the standard phenol chloroform method (Poncz et al., 1982).

2.2. Molecular analysis

We have chosen 6 most common mutations and 1 polymorphism that has been well reported in literature. For the present study the

following methodologies were used (<http://www.genet.sickkids.on.ca/cftr/Home.html>).

2.3. ARMS PCR

Identification of F508del, G551D, G542X, R117H and W1282X mutations were carried out by ARMS PCR (Ferrie et al., 1992). The structures of primers and their fragment sizes are given in Table 1. For each mutation two separate PCR reactions were set, each one specific for wildtype and other for mutant allele. Experiment was performed in a 25 μ L reaction volume containing 25 ng of genomic DNA, 5 pmol of forward and reverse primers, 200 μ M dNTPs and 1 U of Taq polymerase. Amplified products were analyzed on 1.5% agarose gel.

2.4. PCR RFLP

S549N mutation was analyzed by PCR RFLP (Kerem et al., 1990). PCR reaction contained 25 ng of genomic DNA, 10 pmol of forward and reverse primers, 200 μ M dNTPs, and 1 U of Taq polymerase and the final volume was made up to 25 μ L. 10 μ L of amplified product (425 bp) was incubated with 10 U of DdeI restriction enzyme overnight at 37 $^{\circ}$ C. Digested products were analyzed on 2% agarose gel. Wildtype allele shows three fragments of 13 + 238 + 174 bp, and mutant allele shows two fragments of 13 + 412 bp.

2.5. Nested PCR

For IVS8 polyTn polymorphism nested PCR method was used as described by Chillon et al. (1995). The analysis of the product was carried out on 8% non-denaturing polyacrylamide gel electrophoresis. The allele T9/T7 is considered as major type, and T5 as minor allele.

2.6. Statistical analysis

Genotypes were analyzed for deviation from the Hardy Weinberg equilibrium by chi-square test, where $p < 0.05$ was considered to be deviation from equilibrium and similarly chi-square was applied for analyzing categorical variable where p value < 0.05 was also considered significant.

Table 1

Primers used in detection of mutations.

C—common, N—Normal, M—Mutant.

Mutation	Primer	Method	Amplicon (size in bp)	Reference
DF508	C GACTTCACCTCTAATGATGATTATGGGAG N GTATCTATATTCATCATAGGAAACACCAC M GTATCTATATTCATCATAGGAAACACCAT	ARMS	160 157	Ferrie et al. (1992)
G551D	C TAAATTTTCAGCAATGTTGTTTTTGACC N GCTAAAGAAATCTTGCTCGTTGCC M AGCTAAAGAAATCTTGCTCGTTGCT		285 286	
G542X	C TAAATTTTCAGCAATGTTGTTTTTGACC N ACTCAGTGTGATCCACCTTCTAC M CACTCAGTGTGATCCACCTTCTCA		256 257	
R117H	C CACATATGGTATGACCTCTATATAAACT N CCTATGCCTAGATAAATCGCGATAGAAC M CCTATGCCTAGATAAATCGCGATAGAAAT		237 237	
S549N	For TTCAGCAATGTTGTTTTGACCAAC Rev CACAGATTCTGAGTAACCAATAC	RFLP (DdeI)	N: 13 + 238 + 174 H: 13 + 238 + 174 + 412 M: 13 + 412	Kerem et al. (1990)
IVS8 Tn	For1 TAATGGATCATGGGCCATGT Rev1 ACAGTGTGTAATGTGGTGCA For2 CCGCCGCTGTGTGTGTGTGTGTGTTTT Rev2 GGATCCAGCAACCGCAACA	Nested PCR (XmnI)	5T – 84 7T – 86 9T – 88	Chillon et al. (1995)

3. Results

We genotyped 800 subjects for 6 mutations and an intron 8 Tn polymorphism (splice site variant) of the CFTR gene. Altogether 17.6% (n = 800) of subjects were heterozygous [Asthma – 24% (n = 250), CP – 29.3% (n = 150) and controls – 9.3% (n = 400)] for the genotyped mutations. W1282X mutations, known to be the second most common among North Americans (<http://www.genet.sickkids.on.ca>), were not detected in the present study. Only 0.8% (n = 250) asthmatic cases were homozygous for IVS8 T5 polymorphism. All the genotyped mutations were found to be in the Hardy Weinberg equilibrium.

3.1. Spectrum of CFTR mutation

3.1.1. Controls (n = 400)

Minor allele frequency of F508del, G542X, G551D, R117H and S549N observed to be 0.0075, 0.0125, 0.01, 0.0025 and 0.0025 respectively (Table 2). The frequency of T5 allele, pathogenic allele of IVS8-Tn polymorphism, was 0.01125.

We further stratified data as per their prevalence and found G542X to be the most common mutation with the frequency of 2.49%, followed by G551D, F508del, R117H and S549N with 2.00%, 1.50%, 0.50% and 0.50% respectively and 2.24% for 5T allele (Fig. 1A).

3.1.2. Asthma cases (n = 250)

We observed a minor allele frequency of 0.008, 0.022, 0.028, 0.002, 0.01 and 0.058 for F508del, G452X, G551D, R117H, S549N and IVS8 T5 respectively (Table 2). The frequency of individuals falling in different mutations were 10%, 5.6%, 4.4%, 2.0%, 1.6% and 0.4% with respect to IVS8 T5, G551D, G542X, S549N, F508del and R117H (Fig. 1B).

3.1.3. CP cases (n = 150)

300 chromosomes belonging to this subgroup found to have the minor allele frequency of 0.04, 0.03, 0.03, 0.006, 0.013 and 0.02 for F508del, G452X, G551D, R117H, S549N and IVS8 T5 respectively (Table 2). The frequency of heterozygous individuals were 8.67%, 6.67%, 6.00%, 2.67%, 1.33% and 4.00% for F508del, G452X, G551D, R117H, S549N and IVS8 T5 respectively (Fig. 1C).

4. Deviations in spectrum

In order to assess whether the mutation identified in asthma patients differed from those observed in CP patients or appeared in different frequencies, we compared our results of asthma and CP patients (Table 3).

5. F508del mutation

This mutation falls under class II category of Sheppard and Welsh's (1993) classification. It gives rise to an improperly matured protein which is destroyed by endoplasmic reticulum associated with degradation pathway (Farinha and Amaral, 2005).

The predicted bias is supported by the relative frequency of F508del observed in 8.7% (13/150) of CP patients that is statistically significant compared to asthma patients 1.6% (4/250) and controls 1.3% (6/400).

6. G542X mutation

The conversion of glycine residue at 542 position to termination codon (G542X) interrupts mRNA processing. It is classified as class I mutation, half of all CFTR mutation falls under this class (Sheppard and Welsh, 1993).

Among CP patients, G542X mutation prevalence was 6.7% (10/150), followed by asthma patients and controls with 4.4% (11/250) and 2.5% (10/400) respectively. Its genotype frequency in CP patients showed a statistical significance with controls but not with asthmatics. However, in asthma patients G542X heterozygosity was slightly higher in relative to controls but statistically insignificant.

7. G551D mutation

A missense mutation arises by replacing glycine amino acid at 551 position to aspartic acid, and belongs to class III mutation. This class of mutation produces protein that is trafficked to the cell membrane but does not respond to cAMP stimulation (Sheppard and Welsh, 1993).

The prevalence of G551D mutation is more or less similar between CP and asthma patients with the frequency of 6.0% (9/150) and 5.6% (14/250) respectively, followed by controls with 2.0% (8/400). Statistical comparison showed a significant deviation of both CP and asthma patients compared with controls.

8. R117H and S549N mutations

The mutation falls in class IV and class III categories respectively. In class IV mutation, the CFTR gene encodes a protein that is correctly trafficked to the cell membrane and responds to stimuli but generates a reduced Cl[−] current (Sheppard and Welsh, 1993).

The frequency for R117H mutation among the CP patients was 1.3%/150, which was statically different compared to neither the group of asthma patients (0.4%/250) nor the controls (0.5%/400). Similarly, the genotype frequency of S549N mutation of CP (2.7%/150), asthma (2%/250) and controls (0.5%/400) showed no significance.

9. IVS8-5T polymorphism

The polymorphism is seen in intron 8 of the CFTR gene, with T5, T7, and T9 alleles varying in T nucleotide length. The shorter the polythymidine tract the more often exon-9 skipping occurs. CFTR protein without exon-9 results in a protein with no chloride channel activity (Strong et al., 1993). The shorter allele, 5T, is the most common among congenital bilateral absence of vas deferens and asthma patients (Kanavakis et al., 1998; Ngiam et al., 2006).

As the polymorphism is triallelic, the genotype T5/T5 is considered as mutant homozygous, 5T/7T & 5T/9T as heterozygous, and 7T/7T, 9T/9T and 9T/7T as wild-type. The incidence of the IVS8-5T polymorphism was observed to be higher in the asthma patients (10%), compared to that of CP patients (4%) and controls (2%) with statistical significance. Particularly, in asthma patients we identified 0.8% out of the 10% patients as homozygous for T5 allele.

Table 2

Minor allele frequency.

Q2	Sample no.	Mutation	General population (400)	Asthma (250)	Chronic pancreatitis (150)
t2.4	1	DF508	0.0075	0.0080	0.0433
t2.5	2	G542X	0.0125	0.0220	0.0333
t2.6	3	G551D	0.01	0.0280	0.03
t2.7	4	R117H	0.0025	0.0020	0.0066
t2.8	5	S549N	0.0025	0.0100	0.0133
t2.9	6	IVS8-5T	0.01125	0.0580	0.02

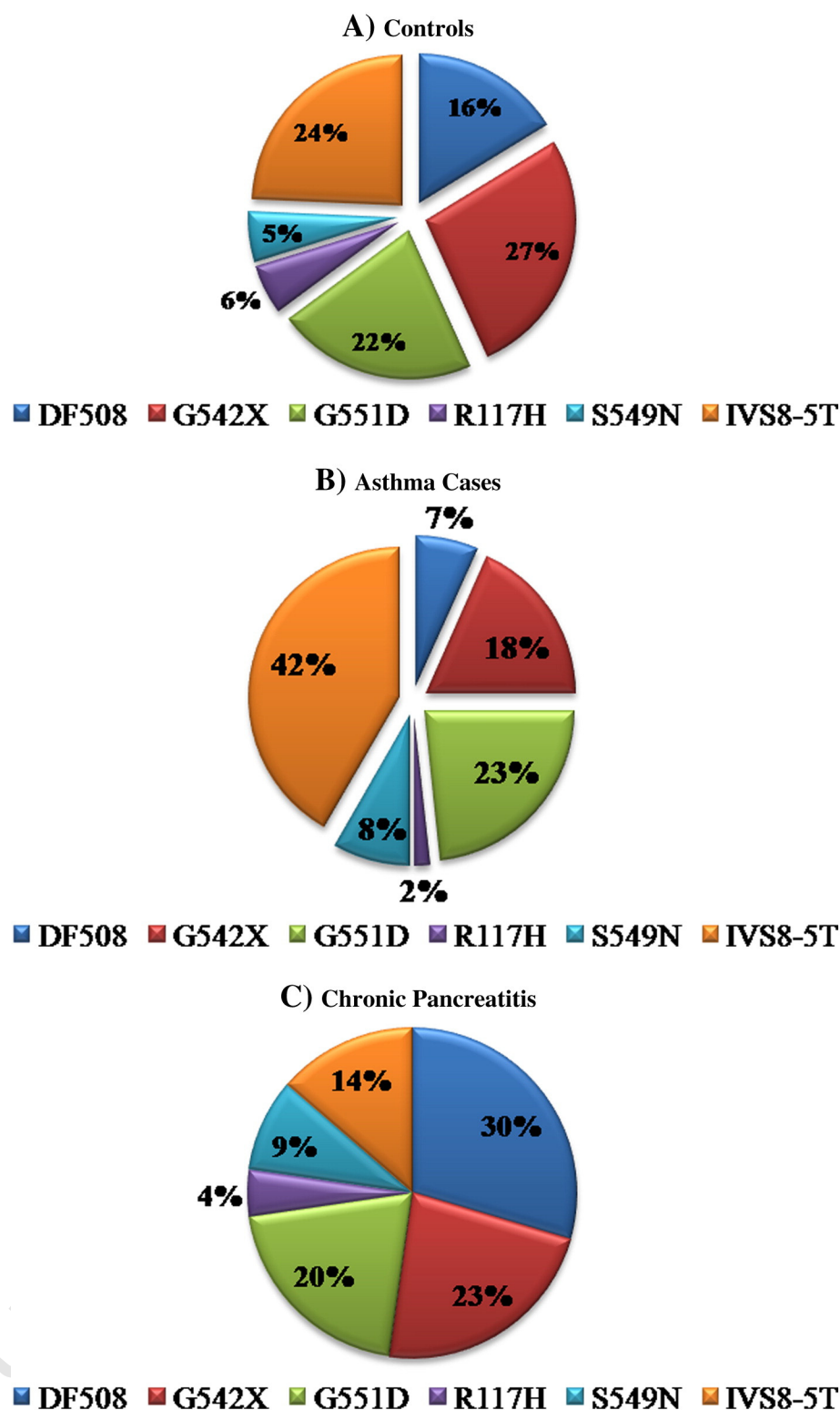


Fig. 1. Spectrum of CFTR gene mutations. A) Among the studied 5 mutation and 1 polymorphism, G542X mutation accounts for 27% (10 out of 37) in controls and the least one is S549N of 5% (2 out of 37). B) In asthma patients IVS8-T5 polymorphism is the common one accounting for 42% (25 out of 60) and the least one is R117H mutation of 2% (1 out of 60). C) In chronic pancreatitis cases the most common mutation is F508del account for 30% (13 out of 44) and the least one is R117H mutation of 4% (2 out of 44).

10. Discussion

Classic CF is characterized by the cluster of phenotypes like abnormal mucous secretions resulting in chronic pulmonary disease, failure to thrive, pancreatic insufficiency, elevated sweat electrolytes, late-stage diabetes and cardiac failure, besides male infertility due to congenital

bilateral absence of the vas deferens (CBAVD) (Schrijver et al., 2005). Although the underlying genotypes have been identified in many patients with CF-associated phenotypes, the mutation spectrum in Indians has not been well-characterized.

The estimated gene frequency of cystic fibrosis among Caucasians is approximately 1 in 2500; however, in other ethnic groups such as the

Table 3
Percentage of heterozygous individual.

Sample no.	Mutation	General population (n = 400)%	Asthma (n = 250)%	Chronic pancreatitis (n = 150)%	Chi square-test* P value		
					Asth vs ctrl	Cp vs ctrl	Asth vs CP
1	DF508	1.5	1.6	8.7	NS	0.0002	0.0013
2	G542X	2.5	4.4	6.7	NS	0.03	NS
3	G551D	2.0	5.6	6.0	0.02	0.02	NS
4	R117H	0.5	0.4	1.3	NS	NS	NS
5	S549N	0.5	2	2.7	NS	NS	NS
6	IVS8-5T	2.2	10	4.0	<0.0001	NS	0.03
7	Total	9.3	24	29.3	<0.0001	<0.0001	NS

* P < 0.05 is considered significant.

black population it is 1 in 17,000 and in the native American population with an approximate incidence of 1 in 80,000. The estimated incidence of CF in the migrated South Asians (Indians and Pakistanis) population in UK is about 1:10,000 to 1:12,000 (Prasad et al., 2010).

In Indian context there was a presumption that cystic fibrosis mutations are not hugely common. However, a review of all the reports from Indian subcontinent suggests that CF is probably far more common than previously assumed (Ashavaid et al., 2003, 2005; Kabra et al., 1996, 2000, 2003; Kapoor et al., 2006; Powers et al., 1996; Prasad et al., 2010). The first case of cystic fibrosis in Indian population was reported by Bhakoo et al. (1968); later in subsequent years several cases were recorded. Recently, Sachdeva K et al., reported that 35% (n = 225) of the cases suspected for CF have mutations in the CFTR gene (Sachdeva et al., 2012). However, the total number of studies from India is still countable (Garg et al., 2009; Kabra et al., 1996, 2000, 2003; Sharma et al., 2008, 2009a,b; Shastri et al., 2008).

In a study, 120 Indian children with CF found to have F508del mutation at the frequency of 19%; 16% were homozygous and 6% were

heterozygous, while the spectrum of other mutations is highly variable and some rare and new mutations have also been observed (Kabra et al., 2000). Consistent with above finding Sharma et al and Sachdeva K et al from Chandigarh and Bangalore, respectively, have also recorded F508del as the commonest mutation (Fig. 2) (Sachdeva et al., 2012; Sharma et al., 2009b).

It is remarkable that the frequency of CFTR mutation in the tested CP patients (29.3%) is relative and two fold higher than those in asthma patients (24%) and controls (9.3%), respectively. The most obvious difference between the study groups lies in the frequency of the F508del mutation, accounting for one third of the mutations in CP patients. Similarly, higher incidence of T5 polymorphism (10% out of 24%) possibly confirms their role in pathology of the diseases.

In CF patients, the presence of F508del mutation in homozygous status has been proved to be associated with pancreatic insufficiency (Kristidis et al., 1992; Kerem and Kerem, 1995). In CP patients, the presence of this mutation along with one or more additional environmental factors like alcoholism, smoking and metabolic disorders may

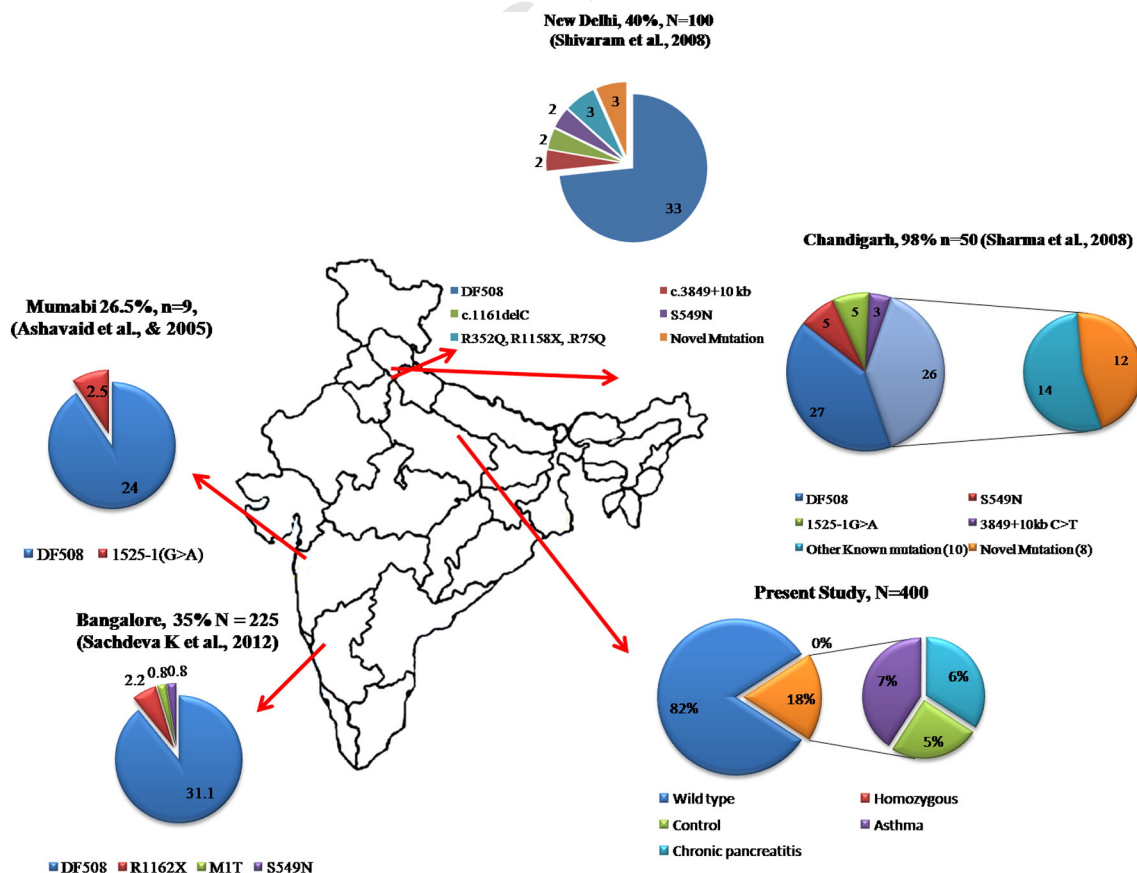


Fig. 2. Distribution of CFTR gene mutation in CF patients in India based on various literature available from India.

contribute to damaging the pancreas and subsequently precipitating the disease (Gaia et al., 2002; Griesenbach et al., 1999). In asthma patients, higher frequency of IVS8-T5 allele was observed that is contradictory to western studies where F508del was common (Dahl et al., 1998; Griesenbach et al., 1999); perhaps ethnic background could be a factor. The 5T allele increases the incomplete transcript, mRNA without exon 9, that decodes a protein with impaired chloride ion conductance and may increase susceptibility for obstructive lung diseases (Radpour et al., 2006). In support of our finding few studies have found an association with T5 allele and lung disease (Chu et al., 1993; Cuppens et al., 1998; Niksic et al., 1999).

Besides F508del mutation the impressing nature of CF alleles in Indian population is the absence of the common CF mutations related to other Mediterranean and European population (Kabra et al., 1996). Literature review confirmed that apart from F508del more than 25 other mutations are involved in CF cases, some of them are novel mutations (Table 4). Recently, Shastri and Kabra reported 1161delC, 3849 + 10kbC-T and S549N as other most common mutations in India (Shastri, 2012), which is in agreement with other reports (Sharma et al., 2009b); however in the control group of the present study 1161delC and 3849 + 10kbC-T mutations were not found (unpublished data) whereas G542X, W1282X, 621 + 1G > T that occur in lesser percentage among Hispanic and non-Hispanic Caucasians (Watson et al., 2004) were not found in earlier studies from India while the present study recorded that G542X mutation is quite common among our controls (2.5%), asthmatic (4.4%) and CP (6.7%) cases in heterozygous nature.

The present findings suggest a panel of mutation for genetic screening in related pathological conditions—asthma and chronic pancreatitis—rather than looking for mutations reported in classical CF cases. As per ACMG guideline, mutations with frequency of >0.1% should be included in the screening panel of CFTR mutation. Based on our data G551D, G542X, F508del, T5, R117H and S549N mutations may be considered for Indian subjects.

In conclusion, our study indicates that CFTR mutations had been overlooked in our population while the carrier frequency of mutations F508del, G542X, and G551D are 3/200, 1/50 and 1/40, respectively, that is more common with reference to 1/238 of F508del in an earlier

report by Kapoor et al. (2006). This requires a special attention of pediatricians, gastroenterologist and pulmonary medicine practitioner to include CFTR mutation screening in their diagnostic procedure and positive cases should be addressed to geneticist for further counseling.

Conflict of interest

The authors do not have any conflict of interest.

Uncited reference

World Health Organization [WHO], 2004

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Table 4
Novel mutation identified in Indian population.

	Sample no.	Mutation	No of cases	Reference
t.4 Q4	1	R80N11fs * 11	1	Sachdeva et al. (2012)
t.4.5	2	R75G	1	
t.4.6	3	Y577X	1	
t.4.7	4	Y808Yfs * 10	1	Sharma et al. (2009a,b)
t.4.8	5	I331X	1	
t.4.9	6	3986–3987delC	2	
t.4.10	7	876–6del4	2	Sharma et al. (2008)
t.4.11	8	1792InsA	2	
t.4.12	9	L69H	1	
t.4.13	10	S158N	1	Sachdeva et al. (2012)
t.4.14	11	Q493L	1	
t.4.15	12	I530L	2	
t.4.16	13	E1329Q	1	Sharma et al. (2009a,b)
t.4.17	14	1002–7_1002–5delTTT	1	
t.4.18	15	G149X	1	
t.4.19	16	L183I	1	Sachdeva et al. (2012)
t.4.20	17	E217Gfs * 11	–	
t.4.21	18	A1285V	–	
t.4.22	19	L69H	1	Sharma et al. (2009a,b)
t.4.23	20	F87I	1	
t.4.24	21	G126S	1	
t.4.25	22	F157C	1	Sachdeva et al. (2012)
t.4.26	23	E543A	1	
t.4.27	24	Y852F	1	
t.4.28	25	D1270E	1	

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