

## Identification of Unique Pattern of CFTR Gene Mutations in Cystic Fibrosis in an Ethnic Kashmiri Population (North India)

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### Abstract

**Background:** Cystic Fibrosis (CF) one of the most common severe autosomal recessive disorders is caused by mutations in CFTR gene. The mutation distributions vary widely between different geographical and ethnic groups. In view of ethnic nature of Kashmiri population (North India), we aim at looking for the 3 common mutations  $\Delta 508$ , 3849+10 kb, C>T and W1282X in CF suspected cases.

**Method:** The mutations were evaluated in 150 highly suspected children with CF, proven by clinical features. ARMS-PCR was used for mutation detection of  $\Delta 508$  and W1282X while as 3849+10 kb, C>T was assessed by indigenously developed ARMS-PCR and results were confirmed by RFLP.

**Results:** Of the 150 suspected CF cases, one of the three mutations was found in 60 out of the 300 alleles genotyped.  $\Delta 508$  mutation was found in 36 of 150 (24%) cases, 3849+10 kb, C>T in 24 of 150 (16%) cases while as no mutation was observed in W1282X. Interestingly 08 of 09 samples with normal sweat chloride were detected positive for 3849+10 kb, C>T mutation.

**Conclusion:** In this report, frequency of the  $\Delta 508$  mutation in Kashmiri children with CF is less as compared to the Western Countries. Interestingly, we identified 3849+10 kb, C>T mutation as unique in population under study with much higher frequency as compared to rest of the world. Further we found intron 19, 3849+10 kb, C>T mutation serves as marker in those CF cases having sweat chloride negative.

**Keywords:** Cystic Fibrosis; ARMS-PCR; Sweat Chloride; Kashmiri; Intron 19

### Introduction

Cystic Fibrosis (CF; MIM# 219700) is one of the most common and life-shortening autosomal recessive disorders. Since the identification in 1989 of the Cystic Fibrosis Trans membrane Conductance Regulator (CFTR) gene >2000 CF mutations and polymorphisms have been documented [1-4]. CF is usually estimated to affect as many as 1 in 2,000–3,000 Caucasian newborns, with a carrier frequency that averages out to a striking 1 in 26 individuals in such populations [5,6]. CF is still the most prevalent hereditary metabolic disorder detected through newborn screening [7]. The overall mutation spectrum and the common mutations found in specific geographic or ethnic background varied significantly among different population. CF is normally rare in Asians and there are few reports of CF affected people of Asian origin. The exact incidence is not known but the predicted incidence for Asians in the United Kingdom (mainly Indian/Pakistani) is 1 in 10,000 and 1 in 40,000 in the USA [8,9]. In India, the CF incidence is estimated to be 1 in 40,000 to 100,000 live births (S. Kabra, 2002, unpublished).

The mutations in CFTR disrupt the CAMP regulated chloride channel formed by CFTR and also interfere with its regulation of other ion channels such as the amiloride-sensitive sodium channel and the outwards rectifying chloride channel [10]. Thus specific mutations of the CFTR gene may lead to variable clinical changes and altered phenotype [11-14]. These mutations have been classified into five categories depending on their functional effects vis-à-vis the CFTR protein in patients with CF, Class 1 mutations are defined as loss of function, and thus are expected to result in no functional CFTR protein and to be associated with a severe phenotype [15]. Class 2 mutations produce misprocessed/misfolded proteins (eg,  $\Delta 508\text{del}$ ), class 3

mutations affect activation or gating of the CFTR channel (eg; G551D), class 4 mutations reduce ion conductance through the channel pore (eg; R117H, R334W, R347P), and class 5 mutations reduce the amount of functional CFTR either by partially aberrant splicing (eg; 3849 + 10kb, C>T, 5T) or by inefficient trafficking (eg; A455E). Class 6 includes nonsense and frame shift mutations (eg, Q1412X, 4326delTC, 4279insA). Whilst the CFTR protein is expressed in many internal organs, the major effect of such mutations is on the respiratory, gastrointestinal, and reproductive tracts, causing, in each of these sites, obstruction by thick, viscous secretions. Pulmonary disease leads to most of the morbidity associated with CF and is the cause of death in more than 90% of patients.

Nearly 2,000 CFTR alleles have been identified to date (Cystic Fibrosis Genetic Analysis Consortium (CFGAC, 2000) ([www.genet.sickkids.on.ca/cftr](http://www.genet.sickkids.on.ca/cftr)) [16]. Although these mutations vary greatly in their frequency and distribution, the vast majority are either private or limited to a small number of individuals. A group of CFTR alleles also exists in non-Caucasian populations [17]. There are about 10 to 20 less common mutations that exist at over 0.1% worldwide. Some mutations

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can reach higher frequencies in particular populations due to founder effect (e.g., M1101K in Hutterites) [18].

The most common defect causing CF is the  $\Delta 508$  mutation (c.1521\_1523delCTT; p.Phe508del according to the current standard nomenclature), a 3 bp deletion in exon 10 causing loss of the amino acid phenylalanine at position 508 of the protein. Worldwide, this allele accounts for ~60% of all CF chromosomes (range 27%–87.5%), with considerably variable frequencies depending on populations and geographic locations [19]. There are another 23 relatively common mutations (frequency 0.5%) worldwide and a few mutations with an unusually high frequency in specific populations, indicating a founder effect and genetic drift. The remaining mutations represent rare or even individual alleles that are distributed throughout the entire gene. Mutations (missense, nonsense, frameshift, splice, small and large in-frame deletions and insertions) contribute to the phenotype by their nature and position in the gene and can be classified by their molecular effects on CFTR [20, 21].

The worldwide frequency of 3849+10kb C>T is 0.2%, but it has a higher incidence in North Carolina (1.8%) and in the Jewish population (4.5% of eastern European Jewish individuals and 6.3% of Ashkenazi Jewish individuals) (Cystic Fibrosis Genetic Analysis Consortium, in press) [22]. Microsatellite analysis of chromosomes of Czech and German origin has shown that four different haplotypes are associated with this mutation (Table 1). Haplotype analysis with other CFTR markers suggests that 3849+10kbC>T most probably originated in different genetic backgrounds and that independent recombination events have occurred. This C>T in 3849+10kb mutation in intron 19 is found in patients with sufficient sweat-duct epithelial absorption to produce normal sweat and further patients appear to have milder pulmonary disease than with  $\Delta 508$  [23].

W1282X is a mutation of single origin that has historically been associated with the Ashkenazi Jews. This mutation frequency has been amplified to almost double that of the  $\Delta 508$  mutation in this distinct

population [24]. As with all other population specific mutations, the W1282X mutation is seen within the mutational arrays of the multitude of countries that have had a significant Ashkenazi Jewish influence.

As the CFTR mutations have considerable geographical and ethnic variations, there is always a need to confirm the mutations for evaluating CF as per the order of their frequencies in other parts of the world. Keeping in view of non-migratory ethnic nature of Kashmiri population (North India) and prevalence of CF here, we aim at finding in preliminary phase the spectrum of three common mutations like  $\Delta 508$  (3 bp deletion), 3849+10 kb, C>T and W1282X.

## Materials and Methods

### Patients and Sampling

A total of 150 highly suspected cases of patients affected with CF from related/unrelated families were referred to department of Advanced Centre for Human Genetics (ACHG), Sheri-Kashmir Institute of Medical Sciences (SKIMS) Srinagar (J&K, India). No ethical clearance was needed as the this Centre has been approved by department of Science and Technology (J&K, India) and subsequently by SKIMS to conduct the molecular diagnostics of various diseases including CF. All the details, including clinical and family history, were collected through a detailed questionnaire. The patients particularly belonged to ethnic Kashmiri from at least four generations and represented all major ethnic groups. Patients considered were highly suspected for CF on a particular diagnostic criteria based on typical radiological findings of pulmonary /gastrointestinal disorder and sweat chloride tests (>60 mmol/L). Some patients had borderline sweat chloride status and some strong suspected cases with normal values.

5 ml blood from each patient was collected in EDTA vials. Genomic DNA was isolated using standard proteinase-K digestion, phenol/chloroform extraction, and ethanol precipitation method from whole-blood samples.

### Mutation Detection by ARMS-PCR FOR $\Delta 508$ and W1282X

The mutations for  $\Delta 508$  and W1282X were detected using the Amplification Refractory Mutation System (ARMS-PCR). The standard ARMS reaction mixture was carried out in a final volume of 25 ul two tube reaction, one with wild primer and second tube containing mutant primer. The reaction mixture contained 500 ng genomic DNA template, 1X PCR buffer (Biotools, B & M Labs, Madrid, Spain) with 2 mmol/L  $MgCl_2$ , 0.4 mmol/L of each primer (Genscript, Piscataway, NJ), 50 mmol/L dNTPs (Biotools, B & M Labs), and 1 U Taq polymerase (Biotools, B & M Labs). For PCR amplification, the standard protocol was used as follows: one initial denaturation step at 94°C for 7 minutes, followed by 40 denaturation cycles of 40 seconds at 94°C, 30 seconds of annealing at 54°C, and 40 seconds of extension at 72°C, followed by a final elongation cycle at 72°C for 5 minutes. Electrophoresis of the products of the ARMS reaction was carried out in a 3% (3:1 agarose: Nusieve) gel containing ethidium bromide and was visualized under UV light. Both the tests developed included an internal control PCR reaction, a failure of PCR in one reaction tub would not result in misdiagnosis.

### Mutation detection of 3849+10 kb, C>T by indigenously developed ARMS-PCR:

Owing to the presence of good percentage of 3849+10 kb, C>T in our preliminary screening by sequencing in CF cases, we developed

Variable	Cases (n=150)	Percentage (%)
<b>Age group</b>		
≤20	94	62.7
>20	56	37.3
<b>Gender</b>		
Female	90	60.0
Male	60	40.0
<b>Dwelling</b>		
Rural	85	53.4
Urban	65	46.6
<b>Clinical status</b>		
Recurrent pulmonary infection	67	44.7
Bronchioectasis	30	20.0
Failure to thrive	15	10.0
Meconium Ileus	10	0.67
Pulmonary TB	7	0.47
Pancreatitis	9	0.60
Others	12	0.80
<b>Consanguinity</b>		
Yes	80	53.4
No	70	46.7
<b>Sweat Chloride*</b>		
Positive (≤60 mmol/L)	32	44.5
Negative (>60 mmol/L)	40	55.5

**Table 1:** Demographic detailing of study subjects for various genetic disorders. \*Sweat chloride values were available for 72 patients only

a cost effective ARMS-PCR to screen this mutation. Normal and mutant-specific ARMS primers were designed such that their terminal 3' base was homologous to either the normal or altered nucleotide of the mutation under study. Thus a two tube reaction protocol was designed, one specific for the wild type (C) and another tube specific for the mutant allele of 3849+10 kb, C>T. Under the standard ARMS conditions, an additional deliberate mismatches were introduced just two nucleotides before into the commonly inserted 84 bp sequence in most CFTR transcripts in order to improve discrimination between normal and mutant alleles. The standard ARMS reaction mixture thus contained 10pmol of each of the two primers with one for wild 5'-CAT CTG TTG CAG TAT TAA AAT GGC -3' and one for mutant 5'-CAT CTG TTG CAG TAT TAA AAG GGT -3' (Genscript, Piscataway, NJ) with common reverse primer (4713) 5'-GAA TCA TTC AGT GGG TAT AAG CAG-3' [Figure 1]. One internal control primer was used within the sequencing region of intron 19 to amplify 437 bp genomic DNA PCR products with forward primer as (4712) 5'-AGG CTT CTC AGT GAT CTG TTG-3' and reverse primer (4713) 5'-GAA TCA TTC AGT GGG TAT AAG CAG-3' [22]. The reaction was put in a total of 25 ul mixture containing 500 ng genomic DNA template, 1X PCR buffer (Bio tools, B & M Labs, Madrid, Spain) with 2 mmol/L MgCl<sub>2</sub>, 0.4 mmol/L of each primer (Genscript, Piscataway, NJ), 50 mmol/L dNTPs (Bio tools, B & M Labs), and 1 U Taq polymerase (Bio tools, B & M Labs). For PCR amplification, the standard protocol was used as follows: one initial denaturation step at 94°C for 7 minutes, followed by 35 denaturation cycles of 30 seconds at 94°C, 30 seconds of annealing at 64°C, and 30 seconds of extension at 72°C, followed by a final elongation cycle at 72°C for 5 minutes. Electrophoresis of the products of the ARMS reaction was carried out in a 1.5% (3:1 agarose: Nusieve) gel containing ethidium bromide and was visualized under UV light incubation for a further 10 min. The presence of 257 bp product in both the tubes showed heterozygous nature of the mutation (CT) while presence of this band in only wild tube indicates normal allele (CC) and in mutant tube, it depicts homozygous mutation (TT).

### Validation of 3849+10 kb, C>T by indigenously developed ARMS-PCR by RFLP and Sequencing

The positive samples obtained by the in-house developed ARMS PCR were validated by testing the same samples by PCR-RFLP already established by Edward High Smith et al [22]. In this method, 3849+10 kb, C>T mutant creates a restriction site and is digested by HphI enzyme. The normal allele produces only one HphI restriction site and displays two bands 349 bp and 88 bp while homozygous mutant creates another restriction site and gives 222, 127 and 88 bp bands. There was 100% concordance and no differences were observed in the genotype derived by either method. Though the findings from both methods were in complete agreement, but evaluation of this mutation

by our method was time-efficient. Our method was more cost-effective (70%) compared to conventional RFLP method. Further previously genotyped samples were also analyzed in a blind trial, again with no errors where one researcher performed RFLP and other one ARMS-PCR but were unaware of each other's results. The reproducibility of the technique was investigated by the repeated testing of all heterozygotes and homozygote samples by both techniques. The performance of the test was not affected by a 10 fold dilution in the amount of sample DNA (25ng to 250ng).

Further, we validated our results by direct DNA sequencing of the both normal and mutated samples to verify the exact locus (Figure 1) and further results obtained were in total agreement with ARMS-PCR and RFLP.

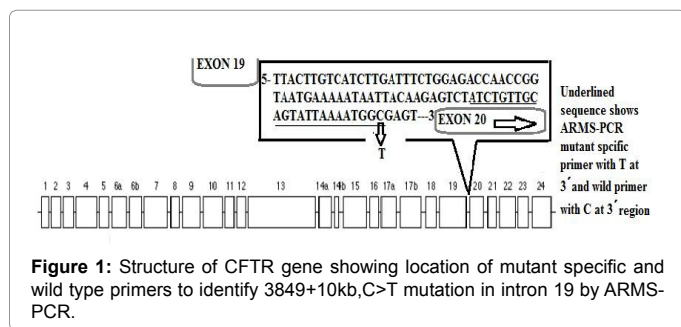
### Results

A total of 150 highly suspected cases of CF patients that belonged to related/unrelated families between 01 months and 30 years. Among these 40% were males 60% females with male: female ratio 3:2. The most frequent clinical problem in patients were recurrent pulmonary disease comprising 67 cases (44.7%), followed by Bronchioectasis in 30(20%), Failure to thrive in 15 cases (10%), Meconium ileus in 10(6.7%) and pancreatitis in 09 cases (6.0%) (Table1). In some cases two or more clinical conditions overlapped (supplementary data). In the present study 60 of 150 cases (53.4%) of patients were as a result of consanguine marriage and the degree of relationship between parents of these CF patients was mostly second degree. Positive sweat chloride tests (>60 mmol/L) were observed repeatedly in 32 (44.5%) subjects while as 40(55.5%) had negative sweat chloride values (>60 mmol/L) despite being clinically highly suspected cases of CF on typical findings of pulmonary/ gastrointestinal disorders.

Among these patients with negative sweat chloride, 20 cases had a broad spectrum of pulmonary diseases of low intensity with mucoid cough or had undefined pancreatic and borderline (40-60mmol/L) sweat chloride values.

Of the three mutations studied, the Δ508 mutation was found to be the most common mutation in population under study, accounting for 24.0% of the total cases screened followed by 3849+10kb, C>T in 16% cases while as W1282X mutation was not found in any case. A total of 60 mutations among 150 highly suspected cases were detected in CFTR gene (Table 2). 05 cases (3.4%) had a heterozygous compound mutation of Δ508 and 3849+10kb, C>T. When these two mutations were stratified, we found Δ508 accounting for 60% (36 of 60) of total mutations with heterozygous mutation in 27 (75%) and homozygous mutants in 09 cases (25%). 3849+10kb, C>T was found in 24 of 60 positive cases (40%) with a frequency of heterozygotes as 79.1% (19 of 24) and homozygous mutation in 05 cases (20.9%) (Table 2).

When both the mutations were evaluated in various clinical groups of CF patients, in females Δ508 was found, with a frequency of 23.4% (21 of 90) (with 16 heterozygous and 05 homozygous mutations) while in males 15(25%) cases harbored this mutation (11 heterozygotes and 04 homozygotes). In 3849, C>T, 17 mutations were found in females (14 heterozygotes and 03 homozygotes), in comparison to 07 mutations in males (05 heterozygous and 02 homozygous). Patients with recurrent pulmonary disease had 19 mutations (28.4%) in Δ508 (15 heterozygotes and 04 homozygotes) while as 10 mutations (15%) were seen in 3849, C>T (07 heterozygotes and 03 homozygotes) and in aggregate for both, total 29 mutations (43.3%) were found in this condition. It was followed by Bronchioectasis having 09 mutations in



Δ 508 (30%: 07 heterozygous and 02 homozygous) and 05 mutations in 3849, C>T (16.6%: 04 heterozygous and 01 homozygous). For other conditions mutation frequency is given in Table 3. The group with consanguineous marriage had 26 mutations (32.5%) in Δ508 (19 heterozygotes and 07 homozygotes) while as 3849, C>T was found in 16 cases (20%: 07 heterozygous and 01 homozygous) and in aggregate both mutations were found in 52.5 % cases (42/80).

In non-consanguineous groups, 10 mutations (14.2%: 08 heterozygous and 02 homozygous) were found in Δ508 and 8 mutations (11.4 %: 7 heterozygous and 1 homozygous) were in 3849, C>T with aggregate frequency for both mutations as 25.7% (18 of 70). Patients with positive sweat chloride had 21 mutations (65.6 %) in Δ508 (12 heterozygotes and 09 homozygotes) as compared to 3849, C>T in 06 cases only (20.0%) (05 heterozygotes and 10 homozygotes) and in aggregate for both mutations 27 cases (86.6%) were positive. In sweat chloride negative cases, 13 heterozygous mutations (32.5 %) were detected in Δ508 and interestingly we found 18 mutations (45%) in 3849 C>T for such cases. Among these negative sweat chloride cases, 04 were homozygous mutations and 14 as heterozygous (Table 1).

## Discussion

Evaluation of CFTR mutations is now vital to early diagnosis, genetic counseling, patient-specific treatment, and the understanding of Cystic Fibrosis (CF) pathogenesis. CFTR mutations are reported to show a lot of variations in several ethnic groups and geographic area. Population under study (Kashmir) remains conserved through generations as we do not prefer to marry outside our ethnic group and

majority of the population are consanguine marriage. The mutation spectrum in Kashmiri populations, however, remains poorly defined as there has been no study till this date. Lack of information about the scope and prevalence of mutations that contribute to CF in Kashmiri population had limited the appropriate genetic counseling in this population. Keeping this in view, we performed preliminary CFTR gene analysis in a group of patients suspected with CF for three common mutations Δ508, 3849 +10kb, C>T and W1282X by ARMS PCR and RFLP.

We applied the common ARMS PCR for detection of Δ508. In our group of patients we found Δ508 present in frequency of 24% (36 of 150) which is consistent with certain regions like Turkey, Tunisia and Iran [24]. The frequency of Δ508 mutation in Turkish population is in complete agreement with population under study (24.5% Turkey versus 24% population under study) but in slight agreement with Iranian population (17.8% in Iran versus 24% ours population [17, 25,26]. In contrast to our report, a high frequency of Δ508 mutation is found in European and other populations exceeding more than 50% [27-29]. Worldwide, the Δ508 allele, which presumably arose from a single origin, ranks as the most common CFTR mutation [30]. Its frequency varies between ethnic groups, accounting for 70 per cent of all cystic fibrosis mutations in the white populations of Britain and US but fewer than 50 per cent in southern European populations. CFTR mutations are correlated with disease severity [31].

The most common defect causing CF is the Δ508del mutation (c.1521\_1523delCTT; p.Phe508del according to the current standard

Type of mutation	No. of patients	Frequency %	Wild type	Heterozygous	Homozygous
Δ508	150	36 (24)	114	27	9
3849,10kb C>T	150	24 (16)	126	21	3
W1282X	150	(0)00	0	0	0
	Mutations 60	Frequency %	Wild type	Heterozygous	Homozygous
Δ508	36	60%	24	27	9
3849,10kb C>T	24	40%	36	19	5

Table 2: Frequency of different mutations studied in CFTR gene.

Variable	Cases n=150	Mutation type Δ508		Nature of Mutation 3849+10kb		Compound mutation
		Het	Hom	Het	Hom	
Age group						
≤20	94					
>20	56					
Gender						
Female	90 (60.0)	16	5	14	3	3
Male	60 (40.0)	11	4	5	2	2
Clinical status						
Recurrent pulmonary infection	67(44.7)	15	4	7	3	2
Bronchioectasis	30(20.0)	7	2	4	1	0
Failure to thrive	15(10.0)	1	2	3	0	1
Meconium Ileus	10(0.67)	1	1	1	1	0
Pulmonary TB	07(0.47)	1	0	1	0	0
Pancreatitis	9(0.60)	2	0	1	0	1
Others	12(0.80)	1	0	2	0	0
Consanguinity						
Yes	80(53.4)	19	7	12	4	4
No	70(56.7)	8	2	7	1	1
Sweat Chloride						
Positive (>60 mmol/L)	32 (44.5)	12	9	5	1	4
Negative (≤60 mmol/L)	40 (55.5)	13	0	14	4	1

Table 3: Distribution of CFTR mutations in various clinico-pathological characteristic.

nomenclature), a 3 bp deletion in exon 10 causing loss of the amino acid phenylalanine at position 508 of the protein. Worldwide, this allele accounts for ~60% of all CF chromosomes (range 27%–87.5%), with considerably variable frequencies depending on populations and geographic locations [2].

Although  $\Delta 508$  is the most prevalent mutation for most populations, there are some, such as certain Jewish groups and many Middle Eastern populations, in which other mutations show a higher frequency. Examining the  $\Delta 508$  allele across European countries illustrates how variable the CFTR mutation frequencies are as ancestry varies. Indeed,  $\Delta 508$  frequencies vary from a maximum of 100% in the isolated Faroe Islands of Denmark. There is a clear northwest to southeast gradient in  $\Delta 508$  frequency across Europe [32,33].

Because of complexity and patient exposure to a multitude of endogenous and exogenous factors, the pulmonary outcome is clinically the most variable as well as the most unpredictable component of the CF phenotype. Studies focusing on pulmonary status as a function of the  $\Delta 508$ del allele have reported a wide range of effects, from a detectable impact of CFTR genotype to no or statistically insignificant influences [34,35]. Other studies using more refined assessment of CFTR mutations have shown statistically significant correlations between CFTR genotypes and pulmonary status, whereas still others have failed to detect a significant association [36]. Overall we found  $\Delta 508$  heterozygous mutation in 27 (75%) cases and homozygous mutants in 09(25%). 05 cases (3.4%) had a heterozygous compound mutation in  $\Delta 508/3849+10\text{kb}$ , C>T.

$\Delta 508$  mutation in our group of patients was highly observed in bronchioectasis harboring 09 mutations (30%) followed by recurrent pulmonary disease with 19 mutations (28.4%).

In this report one compound mutation in  $\Delta 508/3848$ , C>T was observed in a case of pancreatitis; There is a close relationship between the CFTR genotype and the pancreatic phenotype, revealing “severe” mutations (eg,  $\Delta 508$ del, all class 1 mutations) to be associated with pancreatic insufficiency and “mild” mutations, such as a series of missense and alternative splice mutations, to be associated with pancreatic sufficiency [37,38]. Interestingly, pancreatic-sufficient patients carrying two mild CFTR mutations are at significantly higher risk of developing pancreatitis than patients with moderate or moderate/severe genotypes correlating with the residual CFTR function and the pancreatic acinar reserve [39]. The impact of CFTR genotypes has also been investigated in other organs, such as the sweat glands and the reproductive tract in men [22,40]. One major conclusion resulting from these studies was that expression of CFTR in different organ systems of the same individual is highly variable, and that therefore each organ may be variably affected, making diagnostic and therapeutic approaches much more complicated.

The second mutation we evaluated was 3849+10 kb, C>T by PCR-RFLP and an in-house developed ARMS-PCR. The results obtained by our method were 100% in accordance with RFLP. The need to develop this method was owing to high frequency of this mutation in population under study, therefore a simple and cost effective method was the need of the hour. The C>T mutation in intron19 leads to novel alternative splicing through partial activation of a cryptic site and insertion into the most CFTR transcripts of a new 84 bp exon complete with an in-frame stop codon between exons 19 and 20 and C residue, a part of CpG is the frequent point mutation.

We found 3849+10kb, C>T mutations in 24 of 150 (16%) highly

suspected cases of CF and overall 24 of 60 positive cases (40%) with a frequency of heterozygotes as 79.1% (19 of 24) and homozygous mutation in 05 cases (20.9%). This is the first report depicting high frequency of 16% of this mutation anywhere in the world. The worldwide frequency of 3849+10kbC>T is 0.2%, but it has a higher incidence in North Carolina (1.8%) and in the Jewish population (4.5% of eastern European Jewish individuals and 6.3% of Ashkenazi Jewish individuals) [22, 41]. Most of the European countries have frequency of around 0.5% to 1% of 3849+10kb, C>T mutation in CFTR gene, but it goes on increasing towards eastern parts in Jewish regions where it ranges from 4% to 6.5% [2]. Our report shows a frequency of 16% for 3849+10kb, C>T which is only followed by 8.3% in Latvian population [28].

$\Delta 508$  was detected positive in 21 cases (65.6 %) with positive sweat chloride (>60mm/L) but in contrary 3849, C>T mutation was found in 06 cases only (20.0%). Interestingly we found 18(45%) cases positive for 3849 C>T mutation in patients with sweat chloride negative (>60mm/L). Among these, 04 cases were homozygous mutants and 05 as compound heterozygotes for 3849, C>T/  $\Delta 508$  and rest 09 cases as heterozygotes. Thus 3849, serves as a marker mutation for the highly suspected CF cases for a selected group of patients having normal sweat chloride values and becomes more important to be included in the screening panel to avoid misdiagnosis. A delay in such a diagnosis on the genetic level could result in significant lung involvement by the time the diagnosis is made clinically. Further we observed 03 of the 04 cases homozygous for 3849,C>T intron 19 mutation with milder pulmonary disease as compared to those patients homozygous for  $\Delta 508$  having severe lung disease compounded with other clinical conditions like Failure to thrive. The reason that 3849, C>T intron 19 mutation causes milder pulmonary disease and normal sweat chloride is due to sufficient sweat-duct epithelial absorption to produce normal sweat chloride and thus less mucous in the lung epithelium. The need to probe for the 3849+10 kb, C>T mutation in screening programs becomes even more important than including other minor CFTR alleles in population under study and those who belong to our ethnic groups ranging from Azad Kashmir region (Pakistan) to Gilgat (North Pakistan). Kashmir's are well spread in various parts of the State but their major concentration lies in the Valley of Kashmir, Kishtwar, Bhadarwah, Doda and Ramban tehsils of the Jammu Division. ‘Kashmiri’ is a wide term which has loosely been applied for several streams of immigrated mainly from Turkey, Iran, Central Asia and Afghanistan, and settled in the valley. Although we have found high frequency of 3849 C>T, in population under study, it is difficult to trace its ethnic roots in other countries we may belong to as it has not been reported from these countries yet like Turkey, Iran and Central Asia).

W1282X mutation was not found in any case in our study group. W1282X is a mutation of single origin that has historically been associated with the Ashkenazi Jews where it is found to be almost double than the most common mutation  $\Delta 508$  [23]. This mutation is found in a range of 0.5 to 3% in most European countries but higher in the areas or countries that have had a significant Ashkenazi Jewish influence.

## Conclusion

In this report, frequency of the  $\Delta 508$  mutation in Kashmiri children with CF is less as compared to the Western Countries. Interestingly, we identified 3849+10 kb, C>T mutation as the unique in population under study with much higher frequency as compared to the rest of the world. Besides, this mutation serves a marker for the selected group of CF patients having normal sweat chloride.

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