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Analysis Variants of the CFTR Gene in Iranian Cystic Fibrosis Patients

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Abstract

Background: Cystic fibrosis (CF) is known as one of the most common autosomal recessive disorders, which is caused by mucosal glands. A deficiency in the Cystic fibrosis transmembrane conductance regulator gene (CFTR), which encodes a chloride channel, triggers damage to epithelial cells in respiratory ducts, pancreas, intestine, genital ducts in males, liver system, and sweat glands.
Objective: This study aimed to conduct the sequencing of 27 exons of the CFTR gene to screen the spectrum of the variants in patients from all over Iran from different ethnicities.
Methods: This study is a descriptive-analytical, that was performed for eleven years from 2010 to 2021. Totally 345 patients were referred to Tehran Medical Genetics Laboratory by specialists. These patients were categorized into four groups. The first group included clinically confirmed patients of CF having clinical features and biochemical abnormalities, plus a positive sweat chloride test. The second group included couples with an alive or deceased child affected. The third group included CBAVD (Congenital bilateral absence of the vas deferens) cases, and the fourth group included prenatal diagnoses who were looking for carrier detection, or her spouse is affected with CBAVD.
Results: Fifty-four variants and five deletions were found from 345 patients, the most common frequent variant were c.1521_1523delCTT ([delta]F508) (47 (6.81%)), c.1000C>T (R334W, 31 (4.45%)), c.1911delG (2043delG, 25 (3.62%)), c.2051_2052delAAinsG (2183AA>G, 15 (2.17%)), c.1624G>T (G542X, 12 (1.74%)), c.1697C>A (A566D, 12 (1.74%)), c.1210-12T [5] (9(1.30%)) and c.3196C>T (R1066C,7 (1.01%)) respectively and frequency of other variants were less than 1%. Deletion in some exons was established by MLPA assay, the most common deletion was c. (53+1_54-1) _ (164+1_165-1) del (CFTRdele2, 7 (1.01%)).
Conclusions: This study improves our knowledge concerning carrier analysis and genetic counseling. Also, it helps to develop a cost-effective newborn screening program.

Keywords: CFTR Gene, Variant, CBAVD, Sequencing, Allele

1. Background

Cystic fibrosis (CF) is known as one of the most common autosomal recessive disorders, which is caused by mucosal glands. A deficiency in the Cystic fibrosis transmembrane conductance regulator gene (CFTR), which encodes a chloride channel, triggers damage to epithelial cells in respiratory ducts, pancreas, intestine, genital ducts in males, liver system, and sweat glands (1). The CFTR gene is located at chromosomal region 7q31.2 and contains 27 exons. More than 2000 variants have been reported in the CFTR gene. It is believed, that 382 of these variants are CF-causing (https://cftr2.org/mutations_history). In some cases, whole or partial deletion occurs, which can be detected by the MLPA assay (2). The Variants of the CFTR gene are correlated with a vast spectrum of features (phenotypes). These phenotypes contain intense classical cystic fibrosis, CFTR-related conditions such as male infertility by the congenital bilateral lack of the vas deferens (CBAVD) (3), disseminated bronchiectasis, and chronic pancreatitis (4). The F508del is the most rampant variant. The distribution and frequency of the F508del are various among different geographic and ethnics (5). Molecular genetic tests are quite essential to detect affected people, carriers, and prenatal cases. Also, genetic tests are necessary to confirm the biochemical and clinical findings in CF disease. Few studies have been evaluated the CF prevalence in Asia in particular among Iranian.

2. Objectives

In this study, we conducted the complete sequencing of the 27 exons of the CFTR gene to screen the spectrum of the variants in patients who had been referred to Tehran Medical Genetic Laboratory from all over Iran from different ethnicities.

3. Methods

From 2010 to 2021, 345 patients were referred to Tehran Medical Genetics Laboratory by specialists. These patients were categorized into four groups. The first group included clinically confirmed patients of CF, having clinical features and biochemical abnormalities, plus positive sweat chloride test. The second group included couples with an alive or deceased child affected. The third group included CBAVD (Congenital bilateral absence of the vas deferens) cases, and the fourth group included prenatal diagnosis who looking for carrier detection or her spouse is affected with CBAVD.

Family history, race, birth, symptoms, and sex information were recorded per participant.

Consent letters were signed by all participants or their caretakers.

Five mL of peripheral blood was collected in EDTA tubes. Genomic DNA has been extracted utilizing the salting-out procedure. Primers of all exons of the CFTR gene were designed, and PCR was set up for sequencing all candidate exons (Table 1). For detecting duplications and deletions SALSA MLPA® Kit P091-D1 CFTR-MRC-Holland was used based on the manufacturer's instructions. The comparison of the candidate loci with the Cambridge reference sequences, which is published on the NCBI website, was conducted after replicating the target loci. Kinds of the candidate variants were characterized in the CF Database.

4. Results

All of the patients were sequenced in all coding regions and exon-intron boundaries of the CFTR gene.

In group one, which includes 62 patients with CF, 42 of 62 patients had two pathogenic variants, and 20 of 62 showed no pathogenic variants. Their age range was between 45 days to 17 years old. 35 (56.45%) of them were men, and 27 (43.55%) of them were women (Table 2). Most of the patients were suffering from pancreatic complications, developmental delay, Pulmonary and digestive difficulties, severe coughs, and respiratory problems, and their sweat tests were positive on two occasions.

In group two, they had either one affected offspring or siblings affected with CF. Some of the cases in this group were ladies whose spouses had CBAVD, who had no symptoms of cystic fibrosis.

143 persons were identified as carriers, and 69 were normal for pathogenic variants. Their age range was between 20 to 42 years. Participants in this study included 90 (42.45%) men and 122 (57.55%) women (Table 2). In group three, the subjects with CBAVD had infertility caused by the absence of somniferous vas deferens. Out of 25 patients, seven were affected, 6 were carriers, and 12 people had no pathogenic variant. Their age range was between 20 to 47 years (Table 2).

In group 4, the couples were carriers of known pathogenic variants in the CFTR gene. Therefore, their fetus was screened for the mutant allele. Out of 46 cases, 8 fetuses inherited two mutant alleles, 26 inherited one mutant allele, and 12 inherited two normal alleles (Table 2). All fetal specimens were examined for maternal and paternal variants.

The allelic frequency and genotype frequencies of variants identified in all coding regions and exon-intron boundaries of the CFTR gene of this study are shown in Tables 3 and 4.

Fifty-four variants and five deletions were found from 345 patients, the most common frequent variant were c.1521_1523delCTT ([delta]F508) (47 (6.81%)), c.1000C>T (R334W, 31 (4.45%)), c.1911delG (2043delG, 25 (3.62%)), c.2051_2052delAAinsG (2183AA->G, 15 (2.17%)), c.1624G>T (G542X, 12 (1.74%)), c.1697C>A (A566D, 12 (1.74%)), c.1210-12T [5] (9(1.30%)) and c.3196C>T (R1066C,7 (1.01%)) respectively and frequency of other variants were less than 1%. Deletion in some exons was established by MLPA assay, the most common deletion was c. (53+1_54-1) _ (164+1_165-1) del (CFTRdele2, 7 (1.01%)).

5. Discussion

Iranians are a racial admixture population with a very complex genetic structure. Iranian have a broad spectrum of CFTR mutations. Therefore, the detection of CFTR variants in Iranian patients is necessary to get a prenatal diagnosis. In our study, all coding region and exon-intron boundaries of the CFTR gene were sequenced among patients, and the MLPA assay was used to improve the detection rate of CFTR variants among 345 patients. In this study, fifty-four variants and five deletions were detected.

The variant c.1521_1523delCTT ([delta]F508) was present in 47 (6.81%) patients and has been reported in many studies among different countries (6-10). The variant c.1521_1523delCTT is the second most frequent variant in Western Iran (11). The variant c.1521_1523delCTT is the second most frequent variant in Western Iran (11). This variant is a prevalent CF causing mutation among CF patients of east Mediterranean countries such as Crete, Spain, France, Cuba, and Mediterranean France (12-15). Moreover, the variant c.1000C>T has been reported in Brazil, Latin America, Po-land, Greece, Romania, Germany, Czechs, and Ukraine (16-20).

Furthermore, we have detected the variant c.1911delG (2043delG). This variant is the third most common muta-

Table 1. Sequence of Primers (5'-3') Used to Sequence All Exons of CFTR Gene

PCR.P (bp)	Reverse	Forward	Exon
905	CAGCTTCAGTTCATTCTTCCATC	TGCACCTTGCAAACGTAACAG	1
473	TTAGGCACCATACTTGGCTCC	TCCCTCCCAATCCCTTTGAC	2
309	ATTCACCAGATTTCGTAGTC	CTTGGGTTAATCTCCTTGGA	3
458	TCAGCATTTATCCCTTACTTG	TTTCACATATGGTATGACCCTC	4
493	GCTCTGCTATACAATTGACCTTTC	ATTATTTCTGCCTAGATGCTGG	5
402	TGACACTCAAGATCACTGTTCTATG	GTGTGCTCAGAACCACGAAGTG	6 (6a)
531	CAAACATCAAATATGAGGTGGAAG	TTAGTCAAGCCACTTCACCTCAC	7 (6b)
410	GCAAAGTTCATTAGAACTGATC	AGACCATGCTCAGATCTTCCAT	8 (7)
540	AGTGATCCTTCCTTCCAGTTCTAC	CAGGGTTGCTCAGATCTTCCAT	9 (8)
507	AGACACTACACCCATACATTCTCC	CATGTCCTCTAGAAACCGTATGC	10 (9)
450	TGTAGACTAACCGATTGAATATGG	TGTGCATAGCAGAGTACCTGAAAC	11 (10)
425	GCACAGATTCTGAGTAACCATAAT	CAACTGTGGTTAAAGCAATAGTGT	12 (11)
401	GAAACTGGTTTAGCATGAGGC	GCATGTAGTGAACTGTTTAAGGC	13 (12)
1023	AAGATACACCTTATCCTAATCCTATG	TGCTATCAGAATTCACAAGGTAC	14 (13)
514	TATGTATACATCCCCAAACTATC	AAAAGGTATGCCACTGTTAAG	15 (14a)
302	TAATGCTTGGGAGAAATGAAAC	CAGGAACACAAAGCAAAGGAAG	16 (14b)
732	CTGAGACATGTGCATGCCAG	AAATTGTGAGCATGTGCAGC	17 (15)
436	CAAATCACTCCCAAGTAGACAGC	TGAATGCGTCTACTGTGATCC	18 (16)
773	GGGTGTCTGAAGACAACAAGC	GACGAGTTAGTGGGTGCAGTG	19 (17a)
466	ATAACCTATAGAATGCAGTGT	TTCAAAGAATGGCACCAGTGT	20 (17b)
494	ATTTAATGACAGATACACAGTGACC	CACTTTCCTAATATTCAATCGCTC	21 (18)
454	GCTAACACATTGCTTCAGGCT	GCCCGACAAATAACCAAGTGA	22 (19)
471	CTATGAGAAAACTGCACTGGA	GGTCAGGATTGAAAGTGTGCA	23 (20)
712	CAGTTAGGGGTAGGTCCAGTC	AGGCTTATTCAGAGAAATCCAAG	24 (21)
410	CCACTGGGCAATTATTTCATATC	ACATAAGCTTTCAGAACTCCTGTG	25 (22)
598	CTATTTTGAGTAAAGCTGGATGG	TACATGGGCCTAATCTGATCC	26 (23)
1205	AACTTCTTCATCAAGGGAACCATC	GAATCTTCCAGCTGCTGAGTAG	27 (24)

Table 2. Basic Demographic in Group 1 - 4 [a]

Groups	No. of Patient	Carrier	Affected	No. of Variant	Gender, No. (%)		Range Age at Presentation
					Male	Female	
Group 1	62	-	42	20	35 (56.45)	27 (43.55)	35 day to 17 years
Group 2	212	143	-	69	90 (42.45)	122 (57.55)	20 to 42 years
Group 3	25	6	7	12	25 (100)	-	20 to 47 years
Group 4	46	26	8	12	-	-	10 to 18 weeks

[a] Group 1: Clinically confirmed patients of CF; Group 2: Carrier detection and her spouse is affected with CBAVD; Group 3: CBAVD; Group 4: Prenatal diagnosis.

Table 4. Genotype Frequency of Deletion Found in CFTR Gene in (Four Groups) Patients by MLPA Assay

Deletion Gene Location	Variant c.DNA Name	Variant Legacy Name	No. of Alleles		Total Alleles (N = 690) [a]
			Homo	Hetero	
Exon 2	c.(53+1_54-1)_(164+1_165-1)del	CFTRdele2	2	5	7 (1.01)
Exon 2 - 4	c.(53+1_54-1)_(489+1_490-1)del	CFTRdele2-4	2	2	4 (0.58)
Exon 4 - 10	c.(273+1_274-1)_(1584+1_1585-1)del	CFTRdele4-10	2	-	2 (0.29)
Exon 4 - 11	c.(273+1_274-1)_(1679+1_1680-1)del	CFTRdele4-11	2	2	4 (0.58)
Exon 12 - 18	-	CFTRdele12-18	2	2	4 (0.58)

[a] Values are expressed as No. (%).

tion in this study, with a mean frequency of 3.62%. This variant is reported in Iranian Azeri Turkish ethnic patients (21).

The variant c.1624G>T (G542X) accounts for 2.4% of the CFTR mutations worldwide (22). In this study, the frequency of the variant c.1624G>T was 1.74%. However, this frequency is less than the result, which has been reported by Alibakhshi et al. in Iranian cystic fibrosis patients (23).

For the first time, the variant c.1697C>A (A566D) was found as a novel mutation in a homozygous state in one consanguineous patient in Iran who has originated from Esfahan province in the center of Iran (23). Our result concerning this variant confirmed the Alibakhshi et al. (23) result study, and all patients with this variant originated from the Esfahan province from unrelated families.

Moreover, in our study, the frequency of c.1210-12T [5] is reported 1.30%. The polymorphic c.1210-12T [5_9] includes three typical variants, called 5T, 7T, and 9T. The locus c.1210-12T acts as an acceptor site in the alternative splicing of CFTR intron 9 (24). The existence of some variants of this locus is not disease-causing, whereas some SNPs of this locus are correlated with the existence of specific splice mutations. The variant 5T (activity correlated with the most elevated amount of preliminary transcripts. The mRNA without exon 9 outcomes in unripe CFTR proteins with inappropriate activity (25).

In our study, variant c.3196C>T (R1066C) was observed with 1.01% frequency, and also, it has been seen in Portugal too. It is a severe mutation, similar to that observed in patients homozygous for F508del (26).

In conclusion, the present study improves the knowledge of CF carrier analysis and genetic counseling. Also, it helps to develop a cost-effective newborn screening program.

Footnotes

Authors' Contribution: H. N.F contributed to the development of the protocol, abstracted data, and prepared the manuscript and wrote the manuscript, collected the clinical data, interpreted them, MT. A. developed the original idea and analyzed data and revised the manuscript.

Conflict of Interests: One of the authors of this article is a member of the committee board of the Journal of Human Genetics and Genomics.

Data Reproducibility: The data presented in this study will be available to the reviewers or the EIC upon their request without any limitation as a part of the review process.

Ethical Approval: Information regarding of birth, race, sex, symptoms, and family history were mentioned in the questionnaires prepared for each patient. Written consent was obtained from patients or their companions at the time of sampling.

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Informed Consent: Written consent was obtained from patients or their companions at the time of sampling.

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Table 3. Genotype Frequency of Variants Found in CFTR Gene in (Four Groups) Patients by Sequencing Technique

Gene Location	Variant c.DNA Name	Variant Legacy Name	No. of Alleles		Total Alleles (N = 690) [a]
			Homo	Hetero	
Exon 1	c.234 C>T	F78L	-	1	1 (0.14)
Exon 3	c.174_177delTAGA	306delTAGA	-	2	2 (0.29)
Exon 3	c.254G>A	G85E	-	2	2 (0.29)
Intron3	IVS3-6T>C	L159W	-	2	2 (0.29)
Exon 4	c.274C>T	E92K	-	2	2 (0.29)
Exon 4	c.349C>T	R117C	-	1	1 (0.14)
Exon 4	c.350G>A	R117H	2	2	4 (0.58)
Exon 4	c.443T>C	I148T	-	3	3 (0.43)
Exon 5	c.523A>G	I175V	-	2	2 (0.29)
Exon 5	c.532G>A	G178R	2	2	4 (0.58)
Exon 5	c.547C>A	L183I	-	1	1 (0.14)
Exon 6 (6a)	c.601G>A	V201M	-	1	1 (0.14)
Exon 8 (7)	c.1000C>T	R334W	14	17	31 (4.45)
Exon 8 (7)	c.1029delC	1161delC	2	4	6 (0.89)
Exon 8 (7)	c.1040G>A	R347H	-	1	1 (0.14)
Exon 8 (7)	c.1043T>A	M348K	-	1	1 (0.14)
Exon 9 (8)	c.1163C>T	T388M	-	1	1 (0.14)
Intron 9 (8)	c.1210-12T [5]	5T	-	9	9 (1.30)
Exon 10 (9)	c.1262delA	-	2	2	4 (0.58)
Exon 10 (9)	c.1331T>C	I444T	-	1	1 (0.14)
Exon 11 (10)	c.1540G>T	E514*	4	4	8 (1.16)
Exon 11 (10)	c.1521_1523delCTT	[delta]F508	10	37	47 (6.81)
Exon 11 (10)	c.1542_1543delAT	Y515*	-	1	1 (0.14)
Exon 11 (10)	c.1545_1546delTA	1677delTA	-	4	4 (0.58)
Exon 11 (10)	c.1418delG	1548delG	-	2	2 (0.29)
Exon 11 (10)	c.1584G>A	1716G/A	-	1	1 (0.14)
Exon 12 (11)	c.1624G>T	G542X	2	10	12 (1.74)
Exon 12 (11)	c.1646G>T	S549I	-	1	1 (0.14)
Exon 12 (11)	c.1647T>G	S549R	-	1	1 (0.14)
Exon 13 (12)	c.1696G>C	A566P	2	2	4 (0.58)
Exon 13 (12)	c.1697C>A	A566D	4	8	12 (1.74)
Exon 14 (13)	c.1823A>G	E608G	-	2	2 (0.29)
Exon 14 (13)	c.1897C>A	L633I	2	1	3 (0.43)
Exon 14 (13)	c.1911delG	2043delG	14	11	25 (3.62)
Exon 14 (13)	c.2051_2052delAAinsG	2183AA->G	8	7	15 (2.17)
Exon 14 (13)	c.2052delA	2184delA	2	2	4 (0.58)
Exon 14 (13)	c.2195T>G	L732X	-	2	2 (0.29)
Exon 14 (13)	c.2374C>T	R792X	-	1	1 (0.14)

Exon 15 (14a)	c.2562T>G	T854T	-	2	2 (0.29)
Exon 17 (15)	c.2729C>T	T910I	-	1	1 (0.14)
Exon 17 (15)	c.2834C>T	S945L	2	3	5 (0.72)
Intron 17 (15)	c.2909-15T>G	3041-15T->G	-	3	3 (0.43)
Exon 18 (16)	c.2950G >A	D984N	-	1	1 (0.14)
Intron 18 (16)	c.2988+1G>A	3120+1G->A	-	3	3 (0.43)
Exon 19 (17a)	c.2991G>C	L997F	-	1	1 (0.14)
Exon 20 (17b)	c.3154T>G	F1052V	-	1	1 (0.14)
Exon 20 (17b)	c.3196C>T	R1066C	2	5	7 (1.01)
Exon 21 (18)	c.3419T>A	M1140K	-	1	1 (0.14)
Exon 21 (18)	c.3454G>C	D1152H	-	3	3 (0.43)
Exon 22 (19)	c.3472C>T	R1158X	-	5	5 (0.72)
Exon 22 (19)	c.3484C>T	R1162X	-	2	2 (0.29)
Exon 23 (20)	c.3846G>A	W1282X	-	6	6 (0.87)
Exon 24 (21)	c.3909C>G	N1303K	-	2	2 (0.29)
Exon 27 (24)	c.4249G>A	E1417K	-	1	1 (0.14)
No variant			226	175	401 (58.11)

[a] Values are expressed as No. (%).