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Characteristics of the L138ins (p.Leu138dup) mutation in Russian cystic fibrosis patients

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ABSTRACT

The L138ins mutation, found in Russian cystic fibrosis (CF) patients, is a duplication of three nucleotides (CTA) in exon 4 of the *CFTR* gene and is categorised as a small in-frame insertion/deletion. As a result, the CFTR protein molecule elongates by one amino acid residue, leucine, at position 138 (codon 138 (CTA)). In accordance with the new nomenclature, it should be called c.411_412insCTA (p.Leu138dup). The c.411_412insCTA (p.Leu138dup, L138ins) mutation is found in CF patients of Slavic origin (Russians, Ukrainians) and has been linked to a single haplotype of the intragenic DNA markers IVS1CA-IVS6aGATT-IVS8CA-IVS17bCA — 22-7-16-13.

Keywords: cystic fibrosis, L138ins (c.411_412insCTA, p.Leu138dup) mutation, haplotype.

Introduction

Cystic fibrosis (CF; OMIM # 219700) is an autosomal recessive disease caused by a mutation in the *CFTR* gene (OMIM * 602421), characterised by a variable clinical picture ranging from a relatively mild disease course with monosymptomatic manifestations to severe multiorgan lesions [1]. The prevalence of CF in European countries is about 1 in 2500–4500 newborns and in the Russian Federation, it is 1 in 10,000 newborns [1]. The spectrum and frequencies of *CFTR* mutations vary widely among different populations and ethnic groups.

The spectrum of *CFTR* mutations specific to Russian CF patients has been recently identified. Moreover, the creation of the Russian Cystic Fibrosis Patient Registry (RCF-PR) and the provisions of the National Consensus documents on CF care have made it possible to combine data from clinical trials and researches conducted in different centres to better clarify the frequency of mutations both in the Russian Federation itself and in specific regions within the Federa-

tion [2, 3]. According to the RCF-PR 2016, the ten most common mutations (frequency) are: F508del (52.06%), CFTRdele2.3 (5.71%), E92K (2.67%), 2143delT (2.06%), 3849+10kbC>T (2.04%), 2184insA (1.87%), W1282X (1.82%), N1303K (1.47%), 1677delTA (1.44%), and G542X (1.35%). Mutation c.411_412insCTA (p.Leu138dup, L138ins) can be considered common in Russian patients since its frequency exceeds 1% (1.15%) of the total number of identified mutant alleles (ranging from 0.29–2.89% across different regions) [2, 3]. The frequency of this mutation in Europe has not been determined due to the rarity of this particular pathological allele in European populations [1]. Indeed, the L138ins mutation is not included in the panel of routinely analysed *CFTR* mutations in European countries. The purpose of this study is to describe the genotypic features of the L138ins mutation in Russian CF patients.

Material and Methods

DNA was isolated from whole blood samples of CF patients using a DNA extraction kit (Promega, USA). Molecular genetic testing for the c.411_412insCTA (p.Leu138dup, L138ins) mutation was performed using amplified fragment length polymorphism (AFLP) analysis as part of the testing for frequent *CFTR* mutations in 1700 CF patients and was conducted in the Laboratory of Genetic Epidemiology at the Research Centre for Medical Genetics, Moscow [4]. Sanger sequencing to confirm the c.411_412insCTA (p.Leu138dup, L138ins) mutation presence was performed in 37 CF patients. Analysis of DNA markers IVS1CA, IVS6aGATT, IVS8CA, and IVS17bCA was performed in 24 CF patients carrying the c.411_412insCTA (p.Leu138dup, L138ins) mutation and their parents using a previously described procedure [5]. This research project was approved by the Ethics committee of the Research Center for Medical Genetics (Moscow). CF patients, or their fiduciaries, provided written informed consent.

Results and discussion

In 2000, while testing for the 621+1G>T mutation in a Russian CF patient using RFLP test, the abnormal mobility of the exon 4 fragment of the *CFTR* gene was first observed (**Figure 1**). Sequencing confirmed the presence of a *CFTR* mutation (L138ins) not previously found in the Russian population (**Figure 2**). By 2006, the L138ins mutation was detected in six unrelated CF patients from Moscow and the Moscow region [4]. Sub-

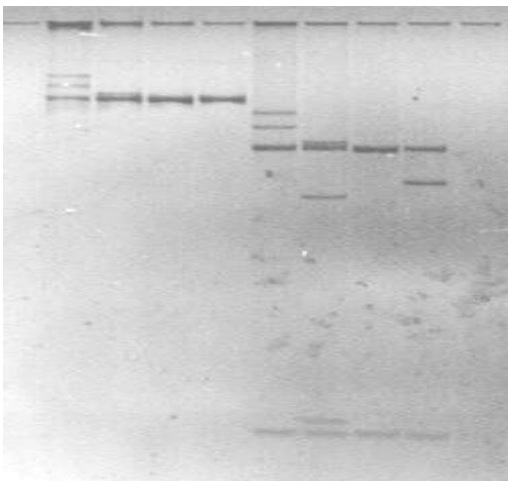
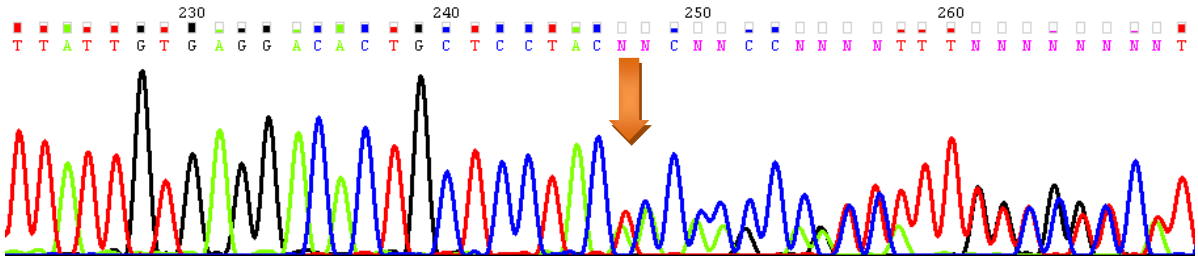


Figure 1. Detection of mutations in exon 4 of the *CFTR* gene in patients with CF. Lanes 1–4 are amplification products of exon 4: lanes 1 and 2 are samples with abnormal mobility of amplified fragments. Lanes 5 and 6 are restriction products of amplicons 1–4 using TruII endonuclease: 5 — L138insA / normal; 6 — 604insA / normal; 7 — normal / normal; 8 — 621 + 1 G>T / normal

a) **from the forward primer**



b) **from the reverse primer**

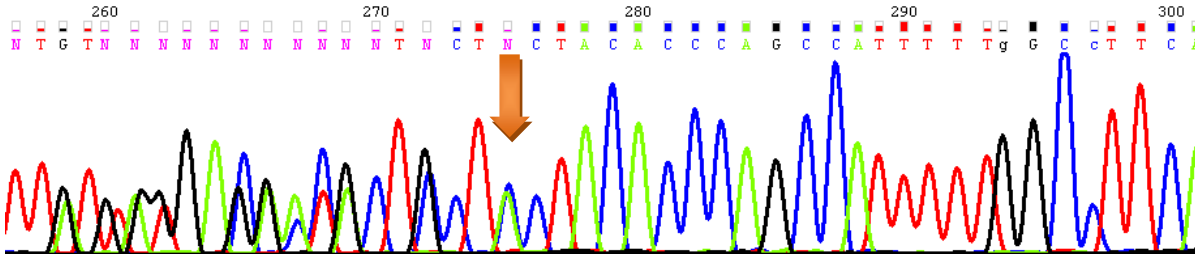


Figure 2. Chromatogram of sequencing results of DNA fragment containing exon 4 of the *CFTR* gene with the L138ins (p.Leu138dup) mutation

sequently, the L138ins mutation was also identified in two patients from the Krasnodar region [6]. Currently, the L138ins mutation is included in the panel of common *CFTR* mutations routinely tested for in the Russian population [3].

Analysis of the chromatogram (**Figure 1**) shows that the initial sequence of 5'-CACTGCTC**CTA**CACCCAGCC is changed to 5'-CACTGCTC**CTACTA**CACCCAGCC. Formally, four different events can lead to such a rearrangement (**Table 1**): insertion of the CTA triplet between 411 and 412 (1) or 414 and 415 (4) positions of the coding sequence; a TAC insertion between 412 and 413 (2) and an ACT insertion between 413 and 414 (3) positions. Any of these rearrangements will lead to duplication of the CTA codon without changing the reading frame, leading to duplication of leucine at position 138. According to the current nomenclature, the L138ins mutation should be designated as c.411_412insCTA (p.Leu138dup).

This mutation is located in the second membrane penetrating motif of the first transmembrane domain (MSD1) involved in the formation of the pore of the CFTR chloride channel. The likely consequence of this mutation is the impairment of the conductive properties of the chloride channel. The L138ins mutation was first described by

Table 1. Mean values of clinical and functional indices in CF patients with different genotypes

	L138dup/F508del	3849+10kbC>T/F508del	F508del/F508del
Sweat chloride (mmol/l)	86.99 ± 17.02	78.29 ± 24.29	103.49 ± 22.89
Age at diagnosis (yrs)	6.71 ± 9.73	14.34 ± 8.89	1.99 ± 0.16
PI (% of patients)	19.1	33.3	94.1
BMI (kg/m^2)	21.87 ± 2.58	18.84 ± 2.73	18.64 ± 2.46
FEV1(%)	77.56 ± 29.43	54.52 ± 21.88	72.34 ± 27.21
Chronic *P. aeruginosa*	12.5%	62.5%	37.1%
Age of patients with chronic *P. aeruginosa* (yrs)	26.44+10.33	27.67 ± 10.18	16.24 ± 8.13
Liver damage	9.1%	6.4%	33.3%

Dörk et al. in 1996 in a 34-year-old patient with a congenital bilateral absence of the vas deferens (CBAVD). The patient was pancreatic sufficient, without lung lesions, a sweat chloride level of 53 mmol/l, with the 5T variant in the second allele [7]. The CFTR1 database [8] describes two insertions of three nucleotides in the region under consideration: in one case the mutation, using the legacy nomenclature referred to as L138ins, is an ACT insertion between nucleotides 412 and 413, which leads to the insertion of histidine between two leucines located at 137 and 138 (c.412_413insACT; p.Leu137_Leu138insHis). In another case, the 546insCTA (c.414_415insCTA) mutation was described, however, the wrong amino acid sequence at the protein level was reported as p.Leu139X (i.e., a premature stop codon at position 139); the correct designation of this mutation should be p.Leu138dup (i.e., leucine duplication at position 138).

Since 31st August 2018, the mutation understudy was included in the CFTR2 database, referred to as c.413_415dupTAC (p.Leu138dup, L138ins, rs397508679) [9]. It is also included in the EXAC database of 1000 genomes [10] and the ClinVar [11]. The first reference identified was the correct description of the mutation in the work of McGinniss et al. (2011) [12].

In our previous paper regarding the genotype-phenotype correlation in Russian CF patients with c.411_412insCTA (p.Leu138dup) mutation, we showed that the p.Leu138dup mutation could be considered as a disease-causing mutation, leading to a variable but relatively mild course of CF (**Table 1**) [13].

To date, in the Laboratory of Genetic Epidemiology, mutation L138ins (c.411_412insCTA, p.Leu138dup), *in trans* with other CF-causing mutations, has been detected in 37 Russian CF patients from 33 families. The average patient age is 13.13 ± 11.42 years (1.00–43.00), with a ratio by sex: 0.43 m: 0.57 f (16:21). Eleven different genotypes have been identified and are described in **Table 2**.

Table 2. Genotypes of the examined patients

Genotype	Number of patients
L138ins/F508del (c.[411_412insCTA];[1521_1523delCTT], p.[Leu138dup];[Phe508del])	15
L138ins/CFTRdele2,3 (c.[411_412insCTA];[54-5940_273+10250del21kb], p.[Leu138dup];[Ser18Arg*fsX16])	4
L138ins/2184insA (c.[411_412insCTA];[2052_2053insA], p.[Leu138dup];Gln685ThrfsX4])	3
L138ins/2143delT (c.[411_412insCTA];[2012delT], p.[Leu138dup];[Leu671X])	1
L138ins/N1303K (c.[411_412insCTA];[3909C>G], p.[Leu138dup];[Asn1303Lys])	1
L138ins/R1162X (c.[411_412insCTA];[3484C>T], p.[Leu138dup];[Arg1162X])	4
L138ins/712-1G>T (c.[411_412insCTA];[580-1G>T]	1
L138ins/E92K (c.[411_412insCTA];[274G>A], p.[Leu138dup];[Glu92Lys])	1
L138ins/ 2183AA->G (c.[411_412insCTA];[2051_2052delAAinsG], p.[Leu138dup];[Lys684SerfsX38])	1
L138ins/ L138ins (c.[411_412insCTA];[411_412insCTA], p.[Leu138dup];[Leu138dup])	1
L138ins/W1282R (c.[411_412insCTA];[3844T>C], p.[Leu138dup];[Trp1282Arg])	1
L138ins/not identified (c.[411_412insCTA];[?], p.[Leu138dup];[?])	4

The most common genotype, L138ins/F508del (c.[411_412insCTA];[1521_1523delCTT], p.[Leu138dup];[Phe508del]), has been found in 15 CF patients (40.5%); the second allele remains unidentified in four patients. The L138ins (c.411_412insCTA, p.Leu138dup) mutation is always linked to one haplotype of the intrinsic marker IVS1CA-IVS6aGATT-IVS8CA-IVS17bCA — 22–7-16–13, suggesting that the L138ins (c.411_412insCTA, p.Leu138dup) variant occurred as the result of a single mutation event.

Most CF patients with the L138ins (c.411_412insCTA, p.Leu138dup) mutation reside in Moscow (17) or the Moscow region (7) and all patients belonged to a Slavic ethnic group (Russians, Ukrainians). The L138ins (c.411_412insCTA, p.Leu138dup) mutation has been detected in six of the nine Federal Regions (predominantly Russian) of the Russian Federation, with a relative proportion ranging from 0.35% to 3.11%. Relative frequencies of the L138ins (c.411_412insCTA, p.Leu138dup) mutation (according to RCF-PR 2017) are given in **Table 3** [2].

Table 3. Distribution of the L138ins (c.411_412insCTA, p.Leu138dup) mutation in various regions of the Russian Federation

Federal regions	The number of patients tested in the laboratory of genetic epidemiology	The proportion (%) of the total number of mutant alleles (based on the RCF-PR 2016)
Central	8	1.30
Moscow	16	1.52
North-Western		< 1.00
St. Petersburg		< 1.00
Southern		< 1.00
Volga Region	2	1.38
Ural		3.00
Siberian	2	< 1.00
Far Eastern		–
North Caucasus		–

Conclusions

The L138ins (c.411_412insCTA, p.Leu138dup) mutation identified in Russian CF patients (a Slavic ethnic group) is a duplication of three nucleotides (CTA) in exon 4 of the *CFTR* gene and is categorised as a small in-frame insertion/deletion.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Kapranov NI, Kashirskaya NY (eds.). Cystic fibrosis (Mucoviscidosis). Moscow: Publishing House "MEDPRACTIKA-M"; 2014. p. 672.
2. Cystic Fibrosis Patients Registry in Russian Federation. 2016. Krasovsky SA, Chernyak AV, Voronkova AY, Amelina EL, Kashirskaya NY, Kondratyeva EI, Gembitskaya TE (eds.). Moscow: "Medpractika-M" Publishing House; 2018. p. 64.
3. Petrova NV, Kondratyeva EI, Krasovsky SA, Polyakov AV, Ivachshenko TE, Pavlov AE, Zinchenko RA, Ginter EK, Kutsev SI, Odinokova ON, Nazarenko LP, Kapranov NI, Sherman VD, Amelina EL, Asherova IK, Gembitskaya TE, Ilyenkova NA, Karimova IP, Merzlova NB, Namazova-Baranova LS, Neretina AF, Nikonova VS, Orlov AV, Protasova TA, Semykin SY, Sergienko DF, Simonova OI, Shabalova LA, Kashirskaya N.Y. National Consensus Project «Cystic fibrosis: definition, diagnostic criteria, treatment» Section «Genetics of Cystic Fibrosis. Molecular genetic diagnosis of cystic fibrosis». Medical Genetics. 2016;15(11):68–84.
4. Petrova NV. Analysis of four polymorphisms in CFTR gene in families of cystic fibrosis patients. Medical Genetics. 2006;5(12):27–32.
5. Petrova NV, Timkovskaya EE, Zinchenko RA, Ginter EK. Analysis of the frequency of some mutations in the CFTR gene in different populations of Russia. Medical Genetics. 2006;5(12):32–39.
6. Rukavichkin DV. Clinico-genotypic polymorphism of cystic fibrosis among the population of the Krasnodar Territory: Diss. Cand. Med. Sciences: 03.00.15. Krasnodar; 2007. p. 27.
7. Dörk T, Dworniczak B, Aulehla-Scholz C, Wieczorek D, Böhm I, Mayerova A, Seydewitz HH, Nieschlag E, Meschede D, Horst J, Pander HJ, Sperling H, Ratjen F, Passarge E, Schmidtke J, Stuhrmann M. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Hum Genet. 1997 Sep;100(3–4):365–77.
8. Cystic Fibrosis Mutation Database. URL: http://www.genet.sickkids.on.ca. Accessed 20 Aug 2019.
9. CFTR2. Clinical and Functional Translation of CFTR. URL: https://www.cftr2.org. Accessed 20 Aug 2019.
10. ExAC Browser (Beta) | Exome Aggregation Consortium, URL: http://exac.broadinstitute.org. Accessed 20 Aug 2019.
11. National Center for Biotechnology Information. ClinVar; [VCV000053905.2], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000053905.2 (accessed March 2, 2020).
12. Matthew J. McGinniss, Arlene M. Buller, Franklin Quan, Mei Peng, Weimin Sun. Cystic Fibrosis Gene Mutations. United States Patent, US008076078B2, Dec. 13, 2011.
13. Petrova NV, Kashirskaya NY, Vasilyeva TA, Voronkova AY, Kondratieva EI, Sherman VD, Novoselova OG, Krasovskiy SA, Chernyak AV, Amelina EL, Ginter EK, Kutsev SI, Zinchenko RA. Phenotypic features in patients with cystic fibrosis with L138ins (p.Leu138dup) mutation. Pediatria. 2017;96(6):64–72 .

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