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

Nika V Petrova
Research Centre for Medical Genetics, Moscow
https://orcid.org/0000-0001-5933-6594

Nataliya Y Kashirskaya
Research Centre for Medical Genetics, Moscow
https://orcid.org/0000-0003-0503-6371
Corresponding author: kashirskayanj@mail.ru

Tatyana A Vasilyeva
Research Center for Medical Genetics, Moscow
https://orcid.org/0000-0002-6744-0567

Elenai I Kondratyeva
Research Center for Medical Genetics, Moscow
https://orcid.org/0000-0001-6395-0407

Andrey V Marakhonov
Research Center for Medical Genetics, Moscow
https://orcid.org/0000-0002-0972-5118

Milan Macek Jr
2nd Faculty of Medicine, Charles University, Prague; Motol University Hospital, Prague
https://orcid.org/0000-0002-5173-5280

Evgeny K Ginter
Research Center for Medical Genetics, Moscow
https://orcid.org/0000-0001-6920-6726

Sergey I Kutsev
Research Center for Medical Genetics, Moscow
https://orcid.org/0000-0002-3133-8018

Rena A Zinchenko
Research Center for Medical Genetics, Moscow; National Institute of Public Health named after N.A. Semashko
https://orcid.org/0000-0003-3586-3458

DOI: https://doi.org/10.20883/medical.383

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

Published: 2020-01-30

How to Cite: Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva EI, Marakhonov AV, Macek Jr M, Ginter EK, Kutsev SI, Zinchenko RA. Characteristics of the L138ins (p.Leu138dup) mutation in Russian cystic fibrosis patients. JMS [Internet]. 2020 Mar 31;89(1):e383. doi: 10.20883/medical.383

© 2020 by the author(s). This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC) licence. Published by Poznan University of Medical Sciences

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.

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
The spectrum of CTR 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 Federation [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 CTR mutations in European countries. The purpose of this study is to describe the genotypic features of the L138ins mutation in Russian CF patients.

**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 CTR gene was first observed (Figure 1). Sequencing confirmed the presence of a CTR 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]. Subsequently, 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 CTR mutations routinely tested for in the Russian population [3].

Analysis of the chromatogram (Figure 1) shows that the initial sequence of 5'-CACTGCTCTACCCAGCC is changed to 5'-CACTGCTCtACTACCCAGCC. Formally, four dif-
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 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

|                  | 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/m²)              | 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%           |
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 under study was included in the CFTR2 database, referred to as c.413_415dupTAC (c.413_415dup, 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.

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-IVS17-

### Table 2. Genotypes of the examined patients

| Genotype | Number of patients |
|----------|-------------------|
| L138ins/F508del (c.[411_412insCTA];[1521_1523delCTT], p.[Leu138dup];[Phe508del]) | 15 |
| L138ins/CFTRdel2,3 (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/712-10>T (c.[411_412insCTA];[580-10>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/W1282R (c.[411_412insCTA];[3844T>C], p.[Leu138dup];[Trp1282Arg]) | 1 |
| L138ins/not identified (c.[411_412insCTA];[?], p.[Leu138dup];[?]) | 4 |

### 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  | —                                                                   |                                                                                   |
bCA — 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].

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.

Acknowledgements

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

Funding sources
The work was carried within the state assignment of Ministry of Science and Higher Education of the Russian Federation for Medical Genetics and in part with the financial support of the Russian Fund for Fundamental Research (RFFR) (project No. 20–015–00061) (expeditions, DNA research, writing the manuscript) and Czech Ministry of Health CZ.2.16/3.1.00/240220PPK; IP00064203/6003 for University Hospital Motol (general management of the project, reviewing the manuscript).

References
1. Kapranov N, Kashirskaya N, eds. Cystic fibrosis (Mucoviscidosis). Moscow: Medpractika-M; 2014.
2. Krasovsky S, Chernyak A, Voronkova A, Amelina E, Kashirskaya N, Kondratyeva E, Gembitskaya T, eds. Cystic Fibrosis Patients Registry in Russian Federation. 2016. Moscow: Medpractika-M; 2018.
3. Petrova N, Kondratyeva E, Krasovsky S, Polyakov A, Ivachshenko T, Pavlov A, Zinchenko R, Ginter E, Kutsev S, Odinokova O, Nazarenko L, Kapranov N, Amelina E, Asherova I, Gembitskaya T, Ilyenkova N, Karmova I, Merzlova N, Namazova-Baranova L, Neretina A, Nikonova V, Orlov A, Protasova T, Semykin S, Sergienko D, Simonova O, Shabalova L, Kashirskaya N. 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):29-45.
4. Petrova N. Analysis of four polymorphisms in CFTR gene in families of cystic fibrosis patients. Medical Genetics. 2006;5(12):27-32.
5. Petrova N, Timkovskaya E, Zinchenko R, Ginter E. Analysis of the frequency of some mutations in the CFTR gene in different populations of Russia. Medical Genetics. 2006;5(12):32-9.
6. Rukavichkin DV. Clinico-genotypic polymorphism of cystic fibrosis among the population of the Krasnodar Territory. Krasnodar: Diss. Cand. Med. Sciences: 03.00.15; 2007.
7. Dörk T, Dworkiczak B, Aulehla-Scholz C, Wieczorek D, Böhm I, Mayerova A, Seydewitz HH, Nieschlag E, Meschede D, Horst J, Pander H, Sperling H, Ratjen F, Passarge E, Schmidtke J, Stuhrmann M. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Human Genetics. 1997 Aug 4;100(3-4):365-377. https://doi.org/10.1007/s004390050518
8. Cystic Fibrosis Mutation Database. http://www.genet.sickkids.on.ca. Accessed 2019 August 20.
9. Clinical and Functional Translation of CFTR. https://www.cftr2.org. Accessed 2019 August 20.
10. Exome Aggregation Consortium. http://exac.broadinstitute.org. Accessed 2020 March 2.
11. ClinVar; [VCV000053905.2]. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000053905.2. Accessed 2020 March 2.
12. McGinniss MJ, Buller AM, Quan F, Peng M, Sun W. Cystic Fibrosis Gene Mutations. 2011 Dec 13; US008076078B2 (United States).
13. Petrova N, Kashirskaya N, Vasilyeva T, Voronkova A, Kondratieva E, Sherman V, Novoselova O, Krasovskiy S, Chernyak A, Amelina E, Ginter E, Kutsev S, Zinchenko R. Phenotypic features in patients with cystic fibrosis with L138ins (p.Leu138dup) mutation. Pediatria. Journal named after G.N. Speransky. 2017 Dec 11;96(6):64-72. https://doi.org/10.24110/0031-403x-2017-96-6-64-72