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Spectrum of mutations in CFTR in Finland: 18 years follow-up study and identification of two novel mutations

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Abstract

Background: The incidence of cystic fibrosis (CF) is low in the isolated Finnish population and the Finnish CF mutation spectrum has differed from many European countries.
Methods: We have analyzed the mutation spectrum and the geographical distribution of CF mutations in Finland covering the last 18 years (1987–2004).
Results: A total of 14 mutations were identified; two of them new, 774insT and S589T (G>C at 1898). The overall coverage of mutations was 97% (99/102 chromosomes). The most frequent mutations were F508del and 394delTT, found in 36% (37/102) and 35% (36/102) of the CF chromosomes respectively. Of the rare mutations, a mutation of presumable Slavic origin, CFTRdele2.3 (21 kb), was enriched in a rural isolate with a frequency of 5,9% (6/102), and a mutation that possibly indicates Swedish influence, 3659delC, was scattered throughout the country with a similar frequency of 5,9% (6/102). G542X, R1162X, R117H, 3732delA, 1898+3A>C, S1196X, S945L, W57R, 774insT and S589T were each identified in a number of chromosomes from one to three.
Conclusions: Our observations of the Finnish CF mutation spectrum fit well with the characteristics of Finland as a population of multiple local founder effects.

Keywords: Cystic fibrosis; CFTR; Mutation; Finland

1. Introduction

The incidence of cystic fibrosis (CF) in Finland, 1:25 000, is almost tenfold lower than in most European populations. CF is caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR; MIM# 602421), [1] a chloride channel expressed at the membrane of epithelial cells. More than 1300 different mutations in the CFTR protein have been identified worldwide [2]. The most common mutation, F508del (c.1520_1522delTCT, p.Phe508del), ranges from less than 50% to almost 90% in European CF chromosomes. In the countries near the Baltic Sea (Finland, Sweden, Norway, Denmark, Estonia, Russia) the 'Nordic mutation' 394delTT (c.262_263delTT, p.Leu88fs) is also found at relatively high frequencies [3]. The mutation has been suggested to have originated in this area, based on haplotype associations [4].

The spectrum of the CFTR mutations in Finland has differed from many European countries [5–7]. The major CFTR-mutation F508del was found only in 45% and 394delTT for 30% of the CFTR chromosomes in Finland.

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Only two other mutations, G542X (c.1624G>T, p.Gly542X) and 3732delA (c.3600delA, p.Asp1201fs), were each identified in one CF chromosome out of 40. That time 20% (8/40) of Finnish CF mutations remained unidentified. The exceptional distribution of the mutations can be explained by founder effect, genetic drift and national isolation of the Finnish population. The unusually low frequency of F508del explains the rarity of CF in Finland and also makes other mutations appear more prevalent in relative terms. Thus the relative portion of 394delTT in CF chromosomes is larger in Finland than in other Nordic countries, but the absolute prevalence in the above countries is similar. The geographical distribution of the major mutations showed that F508del is most prominent in areas of old and dense settlement and 394delTT is enriched in rural isolates in areas of relatively young settlement.

The objective of our study was to reassess the CFTR mutation spectrum in Finland by combining the data collected during the last 18 years from Finnish CF patients.

2. Materials and methods

We studied 51 unrelated Finnish patients with clinically confirmed or suspected diagnosis of CF. These include all the CF patients in Finland from the 18 year period from the year 1987 to 2004. 31 patients were new, whereas 20 patients had been analyzed earlier [7]. From the 20 patients, 8 carried an unidentified mutation. They were reanalyzed in this study, together with the new patients.

The initial mutation screening was done by PCR and reverse-hybridization technique; using Inno-Lipa CFTR17+Tn Update and CFTR19 test strips (Innogenetics, Gent, Belgium). The InnoLipa assay recognizes 36 mutations: E60X (c.178G>T, p.Glu60X), G85E (c.254G>A, p.Gly85Glu), 394delTT, R117H (c.350G>A, p.Arg117His), I148T (c.443T>C, p.Ile148Thr), 621+1G>T (c.489+1G>T), 711+1G>T (c.579+1G>T), 711+5G>A (c.579+5G>A), 1078delT (c.948delT, p.Phe316fs), R334W (c.1000C>T, p.Arg334Trp), R347P (c.1040G>C, p.Arg347Pro), A455E (c.1364C>A, p.Ala455Glu), I507del (c.1519_1521delATC, p.Ile507del), F508del, 1717−1G>A (c.1585−1G>A), G542X, G551D (c.1652G>A, p.Gly551Asp), Q552X (c.1654C>T, p.Gln552X), R553X (c.1657C>T, p.Arg553X), R560T (1679G>vC, p.Arg560Thr), 1898+1G>A (c.1766+1G>A), 2143delT (c.2012delT, p.Leu671fs), 2183AA>G (c.2051_2052delAAinsG, p.Lys684fs), 2184delA (c.2052delA, p.Lys684fs), 2789+5G>A (c.2657+5G>A), 3120+1G>A (c.2988+1G>A), 3199del6 (c.3067_3072del, p.Ile1023_Val1024del), 3272−26A>G (c.3140−26A>G), R1162X (c.3484C>T, p.Arg1162X), 3849+10kbC→T, 3659delC (c.3528delC, p.Lys1177fs), S1251N (c.3752G>A, p.Ser1251Asn), 3905insT (c.3773dupT, p.Leu1258fs), W1282X (c.3846G>A, p.Trp1282X), N1303K (c.3909C>G, p.Asn1303Lys), CFTRdele2,3(21kb) and Tn-polymorphism on intron 8.

The samples that carried a mutation that remained unidentified after the InnoLipa assay were analyzed further. The whole coding region and intronic boundaries of the CFTR gene were analyzed using multiplex denaturing gradient gel electrophoresis (DGGE) and single-strand conformation polymorphism analysis (SSCP/Heteroduplex) (Genephor, Amersham Pharmacia Biotech, Buckinghamshire, UK). The fragments with an abnormal migration pattern were characterized by sequencing using the BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA) on an ABI 377 sequencer.

Microsatellites IVS8CA and IVS17bTA were analyzed as previously described [8]. Primers were fluorescent end-labeled and analyzed with the Applied Biosystems 672 Genescanner system.

The geographical distribution of the mutations was studied by linking each mutation to the University hospital districts where the patient had been diagnosed.

The CFTR cDNA sequence (GenBank NM_000492.2) is used as the reference sequence. Traditional nucleotide numbering starts from the beginning of the transcript, but current recommendations suggest numbering the A of the translation initiation codon as +1. We use traditional CFTR mutation nomenclature, but the recommended mutation names are also given in parenthesis upon first mention of the mutations. Both the nucleotide and protein designations are given, when appropriate. The current recommended nomenclature follows Human Genome Variation Society (HGVS) guidelines (http://www.hgvs.org/mutnomen/).

3. Results

3.1. Mutation frequencies

A total of 14 mutations were found in 99/102 (97%) of affected chromosomes in CFTR in the isolated Finnish population (Table 1). Two of them, 774insT (c.642_643insT, p.Ile215fs) in exon 6a and S589T (c.1766G>C, p.Ser589Thr) in exon 12, have not been previously described.

The most prevalent mutations were F508del and 394delTT, that together accounted for 72% (73/102) of the Finnish CF chromosomes. The frequency of F508del was 36% (37/102) in this study. This figure is even lower than previously observed (45%, 18/40; [7]). The frequency of 394delTT was 35% (36/102) compared to 30% (12/40). The main mutations F508del and 394delTT had a geographical distribution that was similar to previous observations. Ten other mutations were also identified (Table 1), eight of them never found in Finland before. One of these seven mutations, CFTRdele2,3 (21 kb), was enriched in a rural isolate. 3659delC, on the other hand, was found in small numbers throughout the country and could be a sign of Swedish influence in the CFTR mutation spectrum in Finland.

Table 1
Spectrum of CFTR mutations in Finland

Mutation	Recommended nomenclature/nucleotide	Recommended nomenclature/protein	Exon/Intron	*N*	%
F508del	c.1520_1522delTCT	p.Phe508del	E 10	37	36
394delTT	c.262_263delTT	p.Leu88fs	E 3	36	35
CFTRdele2,3(21kb)			E2 and E3	6	5.9
3659delC	c.3528delC	p.Lys1177fs	E 19	6	5.9
1898+3A>C	c.1766+3A>C		I 12	3	2.9
R117H	c.350G>A	p.Arg117His	E 4	2	2
S945L	c.2834C>T	p.Ser945Leu	E 15	2	2
W57R	c.169T>C	p.Trp57Arg	E 3	1	1
774insT	c.642_643insT	p.Ile215fs	E 6a	1	1
G542X	c.1624G>T	p.Gly542X	E 11	1	1
S589T	c.1766G>C	p.Ser589Thr	E 12	1	1
R1162X	c.3484C>T	p.Arg1162X	E 19	1	1
S1196X	c.3587C>G	p.Ser1196X	E 19	1	1
3732delA	c.3600delA	p.Asp1201fs	E 19	1	1
Unknown				3	2.9
Total				102	100

Reference sequence is Genbank NM_000492.2.

3.2. Geographical distribution of CFTR mutations

The geographical distribution is summarized in Fig. 1. The two novel mutations originate from the early settlement region from Southern and Western Finland.

F508del was most prevalent in the university hospital districts of Turku, Helsinki and Tampere. On the contrary the mutation 394delTT was more prevalent in the university hospital districts of Kuopio and Oulu. Three out of six CFTRdele2,3(21 kb) mutations were enriched in a rural isolate in an area overlapping with both the Oulu and Tampere university hospital districts [7]. 3659delC was more evenly distributed in Finland, with one or two mutation chromosomes in each university hospital district. The three 1898+3A>C (c.1766+3A>C) mutations were situated in the most southern university hospital districts: Helsinki, Tampere and Turku. Both of the R117H mutations were situated in Northern Finland. The other mutations that were present in only one or two chromosomes were situated randomly.

3.3. Novel mutations 774insT and S589T

A novel mutation, 744insT, was discovered in exon 6a of the CFTR gene. The mutation is an insertion of a thymine after nucleotide 774 (recommended numbering 642), and it results in a frameshift and a premature stop codon. The CFTR gene with 744insT mutation is expected to produce a severely truncated protein and thus barely functional CFTR chloride channel. 744insT was found in one chromosome out of the 102 chromosomes studied. The patient with 774insT carried 394delTT on the other chromosome and had a classical CF-phenotype. The patient originates from the early settlement region, Southwestern Finland.

The second novel mutation, S589T, was found in exon 12. The mutation is a transversion of a guanine to a cytosine at nucleotide 1898 (nucleotide 1766 on recommended numbering). The 1898 G is the last nucleotide at the 3′ end of exon 12. We speculate that the mutation could be disease causing by two mechanisms. First, the substitution of serine by threonine may prevent the correct folding or function of CFTR chloride channel. Secondly, the 1898G>C transversion may also lead to inappropriate splicing since it disrupts the consensus splice donor site at the 3′ end of exon 12. Other mutations have also been reported to be caused by a mutation of a G in the last position of an exon, for example 1341G>A (c.1209G>A) at exon 8 [9]. The patient with S589T had F508del on the other chromosome and the phenotype was pancreatic sufficient without meconium ileus. The ancestors of the mutation chromosome were from Western Finland, close to the sea [7].

4. Discussion

The Finns have their own disease heritage that has been explained by the national isolation of the Finnish population and the regional isolation within the sparsely inhabited country [10]. Some recessive disorders belonging to the Finnish disease heritage are more common in Finland than in other Caucasian populations. CF forms a low incidence counterpart to these diseases. In this study, the frequency of the most prevalent mutation F508del was 36%, compared to the earlier estimate of 45%. This suggests that F508del is even rarer in Finland than previously thought. The frequency of 394delTT was slightly higher than previously observed (35% vs. 30%). The geographical distribution of the two most prevalent mutations F508del and 394delTT were similar to previous observations. F508del was most prevalent in areas of old and dense settlement in the University hospital districts of Helsinki, Turku and Tampere, which resemble most closely the genetic make-up of the early Finns. 394delTT has been suggested to have a

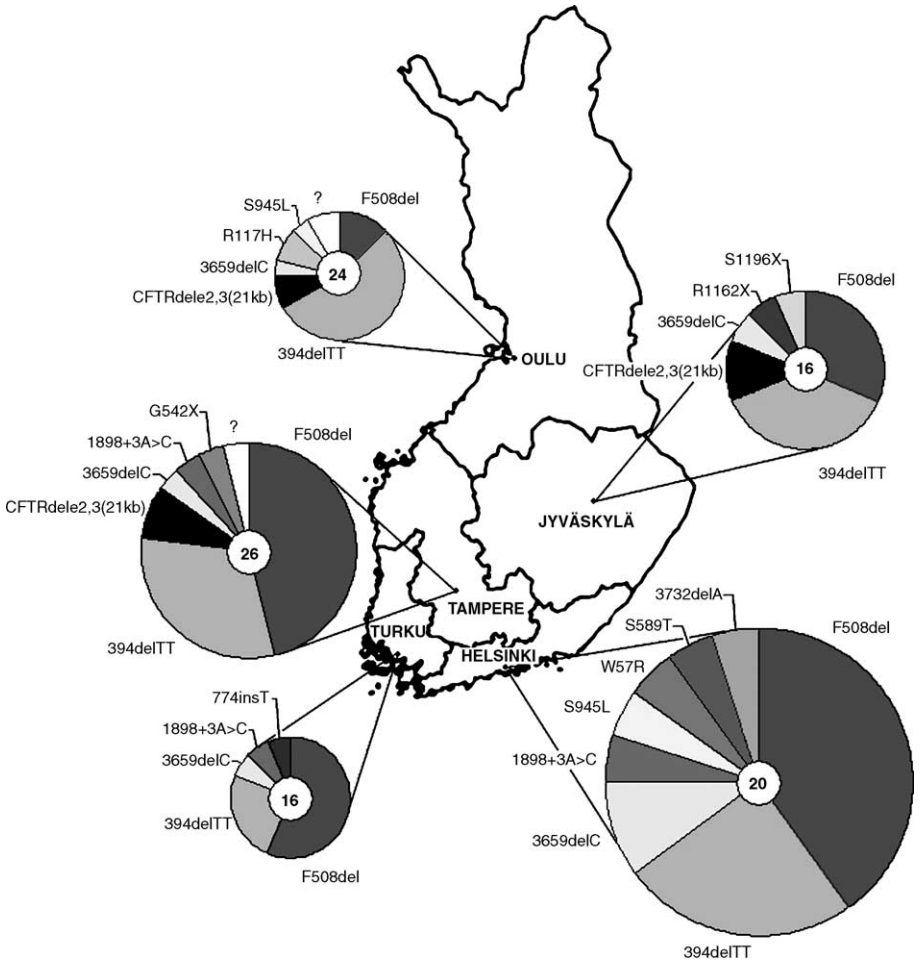

Fig. 1. The geographical distribution of CFTR mutations in Finland by university hospital districts. The radius of the pie chart is proportional to the total population in each district (population information from the year 2002 from the Association of Finnish Local and Regional Authorities: http://www.localfinland.fi). The total number of CF alleles in each district is presented in the center of the pie chart.

common Nordic origin and have been imported to Finland and enriched in rural isolates. The unusually high prevalence of 394delTT in Oulu and Kuopio regions, which are areas of relatively young settlement, further supports this hypothesis.

The third most prevalent mutations in Finland were 3659delC and CFTRdele2,3(21 kbdel). 3659delC was found in 5,9% (6/102) of the CF chromosomes. The mutation is found frequently in Sweden [3]. In a study that comprised 75% of the Swedish CF population, the mutation was found in 7,9% of the CF chromosomes [11]. The even geographical distribution of 3659delC in Finland suggests that it is possible that the mutation is not new in Finland and that it may have come to Finland by the slow but continuous importation of genes from Sweden.

Three out of the six CFTRdele2,3(21 kb) mutations were enriched in a rural isolate on the west coast of Finland. The existence of the cluster had already been hypothesized based on haplotype analysis and genealogical data [7], but the mutation had remained unidentified. Interestingly, CFTRdele2,3(21 kb) is a mutation of Slavic origin, associated with a severe phenotype [12]. We hypothesize that the CFTRdele2,3(21 kb) mutation may have been imported to Finland from Central or Eastern Europe, where the mutation is common [12]. This hypothesis is further supported by the fact that five Finnish CFTRdele2,3(21 kb) chromosomes carried the same infrequent intragenic microsatellite haplotype 16-33 (IVS8CA-IVS17bTA), that has been detected in all chromosomes with CFTRdele2,3(21 kb) so far [12].

Nine mutations were found in Finland with a relative frequency equal or smaller than 2%. Most of these mutations appear to be rare throughout the world. An exception is G542X, which is one of the five mutations that have relative world frequencies higher than 1%. It has been suggested to have a Phoenician origin [13], and it is present in most European countries with the highest mean frequency in the Mediterranean area (6,1%) [3]. Some other rare mutations that were detected in Finland also reach relatively high frequencies in specific areas: 3732delA in Russia; R117H in Norway and in the Celtic countries; R1162X in Northern Italy (although multiethnicity and recurrence has been demonstrated for this mutation) [3,14].

Both of the novel mutations 744insT and S589T were found in the coastal areas of Finland that have traditionally had most connections to the neighboring countries and are genetically most mixed. Thus the new mutations may be imported, although we cannot exclude the possibility that they are de novo mutations.

There were three patients with a mutation that remained unidentified. One of the patients carried F508del on the other chromosome and had strong evidence for the diagnosis of CF: pathological sweat test, malabsorbtion and malnutrition. The other two patients were both from northern Finland and carried R117H on the other chromosome. Both R117H mutations were in combination with 7T variant on the T-tract of intron 8. The phenotypes of both of these patients were of a less classical form. It is possible that the three patients with only one CFTR mutation identified might represent a CFTR related phenotype, or they might have a large deletion or an intronic mutation that remained unidentified.

14 mutations explain 97% of CF mutations in Finland. The heterogeneity of Finnish CFTR mutations is relatively small when compared to for example in Spain, where 75 mutations explain 90% of CFTR mutations [15]. This observation fits well with the characteristics of Finland as a population of multiple local founder effects. The results of our study are of evident interest for clinical testing of Cystic Fibrosis in Finland.

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