BRIEF REPORT | Genetics inMedicine

Report on the p.Ser489X (p.Ser489*) *CFTR* mutation, a variant with severe associated phenotype and high prevalence in a Quebec French-Canadian cystic fibrosis patient population

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Purpose: This study reports on the phenotype of cystic fibrosis patients identified to be carriers of the p.Ser489X (p.Ser489*; c.1466C>A) cystic fibrosis transmembrane conductance regulator (CFTR) mutation, a variant rarely described in the cystic fibrosis literature, as well as on its allelic frequency in a French-Canadian cystic fibrosis patient cohort.

Methods: Reported phenotypes and allelic frequency of this variant were collected based on the data from a large French-Canadian cystic fibrosis patient cohort.

Results: Cystic fibrosis patients found to carry the p.Ser489X variant generally presented with classic gastrointestinal manifestations of this condition in infancy. The allelic frequency of this variant was calculated to be 0.7% for this population.

Conclusion: The p.Ser489X *CFTR* variant is a severe disease-causing *CFTR* allele that is relatively frequent in the French-Canadian cystic fibrosis patient population, warranting its inclusion into *CFTR* molecular testing panel for this population.

Genet Med 2012:14(10):883–886

Key Words: c.1466C>A; *CFTR*; cystic fibrosis; meconium ileus; p.Ser489X

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive childhood disorder in the Caucasian population, occurring in about 1 in 2,500 live births. CF has a high incidence in the French-Canadian population, particularly in the geographically isolated region of Saguenay–Lac-Saint-Jean (SLSJ), Quebec, where the carrier rate has been determined to be 1 in 15 (refs. 1,2). In this population, three Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations account for ~94% of disease alleles, namely p.Phe508del (p.F508del; [delta]F508, c.1521_1523delCTT), 621+1G>T (c.489+1G>T), and p.Ala455Glu (p.A455E; c.1364C>A).[3,4]

Molecular *CFTR* testing is usually performed to confirm a clinical diagnosis of CF, to determine carrier status in individuals at risk, or to corroborate results of newborn screening. To date, nearly 2,000 mutations have been identified within the *CFTR* gene (Cystic Fibrosis Mutation Database: http://www.genet.sickkids.on.ca/Home.html). However, in most laboratories, *CFTR* mutation testing is performed using commercially available platforms assaying a fixed panel of mutations because only a handful of mutations reach significant frequency in most populations. Following the recommendations of the American College of Medical Genetics and Genomics, a minimal *CFTR* molecular testing panel of 23 pan-ethnic disease-causing variants that are present in at least 0.1% of CF chromosomes[5,6] has been defined. However, laboratories must be aware of additional mutations that reach significant frequencies in their target population.

With this publication, we report a severe disease-causing *CFTR* variant that has only scarcely been described in the scientific literature and presents at a relatively high frequency in the French-Canadian CF patient population of Quebec.

MATERIALS AND METHODS

As the designated provincial molecular testing center for CF, our laboratory carries out the majority of molecular *CFTR* testing for the province of Quebec (Canada). Our *CFTR* molecular testing panel currently covers 72 variants that are detected either through PCR amplification and restriction digest assays or through a commercial 71-*CFTR*-variant panel (xTAG Cystic Fibrosis Assay; Luminex, Austin, TX). Before 2005, only 35 mutations were detected with our *CFTR* molecular testing panel.

The p.Ser489X variant is currently detected in our laboratory by PCR amplification of *CFTR* exon 11 followed by restriction digest. The 193-base-pair (bp) PCR product is digested with *Mse* I, as the c.1466C>A change introduces an additional restriction site for this enzyme. Presence of the variant is determined by the appearance of two additional DNA fragments of

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Submitted 12 March 2012; accepted 18 April 2012; advance online publication 24 May 2012. doi:10.1038/gim.2012.57

41 and 19 bp. Alternatively, the p.Ser489X variant can also be detected by sequencing *CFTR* exon 11.

Our laboratory database was interrogated for all patients identified to carry at least one copy of the p.Ser489X *CFTR* variant. Because all these patients were reported to be of French-Canadian ethnicity, our database was then interrogated for all the patient or prenatal samples that were ethnically identified as "French Canadian" and referred for diagnostic testing over the period ranging from 1986 to 2010. Samples from the patients identified as being of mixed ancestry (French Canadian and "other") were not included. The p.Ser489X variant was not detected in any of these excluded patients. *CFTR* variants identified through molecular testing were then recorded and allele frequency was calculated for these patient chromosomes on the basis of the reported genotype of the patients. For the purpose of allelic frequency calculations and to the extent that the available pedigree/family history allowed it, closely related probands were excluded.

Phenotypes of the patients carrying the p.Ser489X *CFTR* variant were reported on the basis of clinical information provided by referring physicians.

RESULTS

Using the selection criteria described above, 13 CF patients referred for molecular testing were found to be carriers of the p.Ser489X *CFTR* variant. All these patients were reported to be of French-Canadian ethnicity.

All but one of these CF patients ascertained in our French-Canadian cohort were compound heterozygotes for the p.Ser489X variant and another severe disease-causing *CFTR* allele, namely p.Phe508del, 621+1G>T (c.489+1G>T), 711+1G>T (c.579+1G>T), p.Ile1023_Val1024del (3199del6; c.3067_3072delATAGTG), or p.Gly85Glu (p.G85E; c.254G>A) (**Table 1**). Most patients presented clinically before 2 years of age, although specific symptoms were not reported for half of our cohort. All of the individuals found to be carriers of the p.Ser489X variant for whom clinical information was available presented with severe neonatal- or infantile-onset CF, usually in the context of meconium ileus, intestinal obstruction, growth restriction, and failure to thrive. A single p.Ser489X homozygote was identified who presented as a neonate with bowel malrotation, meconium ileus, and elevated trypsinogen, clinical manifestations that further support the association of the p.Ser489X *CFTR* variant with the severe classical CF phenotype.

Allele frequency of the p.Ser489X variant was assessed by comparing its frequency with that of other disease-causing variants identified in our French-Canadian CF patients. Nine hundred and ninety-four samples were identified in our laboratory database as originating from French-Canadian CF probands, and 193 were designated as originating specifically from SLSJ. Eighty-eight of those represented close relatives, usually sibs. Thus, a total of 1,712 CF chromosomes (172 from SLSJ probands) were considered for calculation of allelic frequencies. Allelic frequencies of *CFTR*-related disease-causing alleles identified in this French-Canadian patient population that were calculated as being above 0.1% are depicted in **Table 2**. As can be seen from this table, the variant p.Ser489X represents 0.7% of disease-causing alleles.

DISCUSSION

The p.Ser489X (p.Ser489*, c.1466C>A; reference sequence NM_000492.3) *CFTR* variant is a disease-causing allele that has

Table 1. Genotypes and phenotypes of French-Canadian cystic fibrosis patients who were carriers of the p.Ser489X *CFTR* variant

Patient no.	*CFTR* genotype	Age at diagnosis	Reported symptoms	Note
1	p.[Ser489X];[Ser489X]	Newborn	Volvulus, meconium ileus, positive trypsinogen	
2	p.[Phe508del];[Ser489X]	3 Months	Meconium ileus and positive sweat test	
3 (Sib of 2)	p.[Phe508del];[Ser489X]	1 Week	Meconium ileus	
4	p.[Phe508del];[Ser489X]	2 Weeks	Meconium ileus	
5	p.[Phe508del];[Ser489X]	5 Months	Positive sweat test, intestinal obstruction, and steatorrhea	
6	p.[Phe508del];[Ser489X]	15 Months	Positive sweat test, no additional symptoms reported	
7	p.[Phe508del];[Ser489X]	Unknown	Unknown	Tested at 7 years of age
8	p.[Phe508del];[Ser489X]	Unknown	Unknown	Tested at 9 years of age
9	p.[Phe508del];[Ser489X]	Unknown	Unknown	Diagnosis made in infancy; molecular testing performed as an adult (26 years old)
10	[621+1G>T];p.[Ser489X]	2 Months	Growth restriction, failure to thrive	
11	[711+1G>T];p.[Ser489X]	3 Months	Positive sweat test, no additional symptoms reported	
12	p.[Ile1023_Val1024del];[Ser489X]	Unknown	Positive sweat test, no additional symptoms reported	Tested at 13 years of age
13	p.[Gly85Glu];[Ser489X]	Unknown	Unknown	Diagnosis made in infancy, molecular testing performed as an adult (18 years old)

Table 2. Frequencies of *CFTR* disease-causing alleles in the French-Canadian cystic fibrosis patient population

	All variants		p.Phe508del		621+1G>T		711+1G>T		p.Ala455Glu		p.Ser489X		p.Arg334Trp		3199del6		p.Gly542X		p.Gly85Glu		p.Ile507del	
***CFTR* variant**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**	**FC**	**SLSJ**
Number of CF chromosomes	1712	172	1321	116	143	29	61	1	51	13	12	0	9	0	8	2	6	1	5	1	4	1
% Of total CF chromosomes	100.00	100.00	77.16	67.44	8.35	16.86	3.56	0.58	2.98	7.56	0.70	0.00	0.53	0.00	0.47	1.16	0.35	0.58	0.29	0.58	0.23	0.58

CF, cystic fibrosis; FC, French-Canadian; SLSJ, Saguenay–Lac-St-Jean.

been mostly characterized in a CF mouse model,[7,8] and has only scarcely been reported in the human CF literature.[4,9–11] This variant results in premature chain termination and is predicted to behave as a functional class I allele, causing reduced *CFTR* protein synthesis. This is expected to correlate with a classic pancreatic insufficiency CF phenotype. Indeed, in our patient cohort, the symptom most often reported was meconium ileus, a clinical finding associated with pancreatic insufficiency. However, phenotypes reported for our cohort may reflect ascertainment bias, because half of those patients referred for *CFTR* testing presented as neonates with severe gastrointestinal manifestations of CF. Pulmonary phenotype was not reported in our patient cohort, therefore we cannot comment on the severity of pulmonary symptoms associated with this variant.

Two groups reported on the phenotype of a CF p.Ser489X homozygous null mouse model, engineered through disruption of the mouse *CFTR* analogue coding sequence by the introduction of an in-frame stop codon at position 489 of exon 11, a region of 100% identity between human (accession number NP_000483.3) and mouse (accession number NP_066388.1) *CFTR* protein sequences.[7,8] These mice are reported to present with failure to thrive, intestinal obstruction leading to perforation and peritonitis, and alteration of mucous and serous glands. Over 90% of these animals die from intestinal obstruction by 30 days of age, often within the first week of life.

A brief recall of the history of French-Canadians is necessary for the comprehension of the population genetics of this particular ethnicity. The province of Quebec currently has ~7.9 million residents, 80% of whom are French speaking (http://www.stat.gouv.qc.ca/). The majority of these "French Canadians" descend from 8,500 French settlers, who arrived in "Nouvelle France" between 1608 and 1759 under the rule of the French government. Following the British Conquest of 1760 and the British deportation campaign, between 2,000 and 4,000 Acadians, descendants of French pioneers from Acadia (represented these days by the Canadian provinces Nova Scotia, New Brunswick, and Prince Edward Island), also settled in Quebec, and others were deported to the United States, giving rise to the Cajuns of the South. At the end of the 18th century, a group of American Loyalists came to Quebec after the American War of Independence, whereas during the late 19th century, immigration occurred mostly from the British Isles (Scotland, Ireland, and England).[12] Recent immigration waves have come from more diverse international areas.

Equally important in terms of population genetics is the emigration to the United States between 1840 and 1930 of approximately 900,000 French Canadians who were in search of economic opportunities.[13] At the time, these French Canadians mostly settled on the east coast. In the 2000 US Census, it was reported that more than 2.3 million Americans are of French-Canadian descent, French Canadians represented ~10% of New Hampshire's population, and French Canadian was the most common ancestry in Maine.[14] It is therefore not surprising that common French-Canadian CF mutations have been reported in Americans of French-Canadian descent.[15]

In the 17th century, the rapid expansion of the French-Canadian population led to a series of regional founder and bottleneck effects, in the context of relative isolation elicited by both geographical and cultural barriers.[12] Expansion of this initially small population led to genetic drift, with overrepresentation of some rare genetic variants. Recent work has confirmed the importance of the early French immigrants to the contemporary French-Canadian gene pool, with the highest contribution of these founders to all regional samples despite the heterogeneity of the pool of founders. The distinct genetic identity of regional Quebec gene pools can thus be explained by the early French settlement and the subsequent demographic history of this founder population.[16]

It is currently difficult to assess the exact carrier frequency of the p.Ser489X allele in the Quebec French-Canadian population because of ascertainment bias; carrier testing is offered to the relatives of the confirmed *CFTR* mutation carriers. Yet the fact that p.Ser489X represents 0.7% of disease-causing alleles in our French-Canadian patient population supports its inclusion in our current *CFTR* molecular testing panel. However, because the frequency of this disease-causing *CFTR* allele has not yet been reported in other populations, inclusion of this variant in other pan-ethnic screening panels is currently not warranted.

None of the CF probands of our cohort identified as originating from the SLSJ region were found to be carriers of the p.Ser489X variant, whereas Madore et al.[4] had reported this variant in one of their SLSJ CF patients, with an estimated allele frequency of 0.59% for this population.The allele frequencies of two of the three most common SLSJ *CFTR* mutations, namely p.Phe508del and p.Ala455Glu, are similar in our cohort to those reported by Madore et al.[4] for this population. However, the 621+1G>T allele frequency in our population is significantly lower than that reported by this group. Ascertainment bias may be an explanation for this discrepancy, because CF patient samples from the SLSJ region may be sent to other Quebec molecular testing facilities.

Patients found to be carriers of the p.Ser489X variant were primarily identified as originating from the Laurentians, Lanaudieres, and Outaouais regions, with a single family reportedly originating from the Centre-du-Québec region. This may represent a geographical aggregate similar to that of the SLSJ, because these three regions (Laurentians, Lanaudieres, and Outaouais) are adjacent territories, although numbers are too small at this time to confirm the hypothesis.

In conclusion, the p.Ser489X *CFTR* variant is a severe disease-causing *CFTR* allele that is relatively frequent in the French-Canadian CF patient population. Carrier frequency for this variant has yet to be established in Quebec, as well as in other populations. Data from the Canadian Consortium for CF Genetic Studies and *CFTR*2 consortium may help in establishing the worldwide frequency of this severe *CFTR* variant.

DISCLOSURE

The authors declare no conflict of interest. No funding was received for completion of this work.

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