

A Guanine to Thymidine substitution, located 1643 nucleotides into Intron 11 of the CFTR gene (1811+1643G>T), could represent a novel splicing mutation, found exclusively in CF patients of Hispanic descent.

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ABSTRACT

The CFTR sequence variant 1811+1.6kbA>G, or more specifically 1811+1634A>G, has been reported as a mutation that causes severe cystic fibrosis with pancreatic insufficiency (1). This mutation creates a new donor splice site in intron 11 that produces a new exon of 49bp between exons 11 and 12, so-called exon 11b (2). In this study, we report a novel sequence variant, 1811+1643G>T, located only 9bp downstream from the 1811+1.6kbA>G mutation. We detected this novel mutation in 11 unrelated CF patients from a pool of diagnostic samples analyzed between January 2007 and March 2009 by gene sequence analysis. All 11 patients are of Hispanic descent. One is homozygous for the mutation and the other 10 carry a second known CF mutation (three 3876delA, two Delta F508, and one each D1152H, G1244E, G542X, P205S, 3199del6). There were 587 unrelated Hispanic families in this pool, which makes the frequency of this allele approximately 1% (12/1174) in the Hispanic population. Clinical data from the 11 patients indicate that 1811+1643G>T is a deleterious mutation with varying age of onset depending on the severity of the second mutation. For example, one patient whose second mutation is D1152H, was diagnosed only with infertility (azoospermia) and pneumonia at the age of 43. Similarly to 1811+1.6kbA>G, the G to T substitution at 1811+1643 likely creates a new donor splice site, 4bp further downstream, which would produce a new exon of 53bp.

INTRODUCTION

Cystic Fibrosis (CF) is one of the most common autosomal recessive diseases across many ethnic groups, characterized by chronic pulmonary obstructive disease, exocrine pancreatic insufficiency, elevated sweat chloride levels and male infertility. CF is caused by mutations in the cystic fibrosis conductance regular gene (CFTR, OMIM 602421) (3, 4). Over 1300 mutations have been described for the CF gene, which encodes a 1480 amino acid protein whose main function is to regulate chloride transport. Even though the most common mutation of ΔF508 accounts for ~60% of disease alleles (4), especially in Caucasians, the distributions and mutation frequency is extremely diverse. The majority of mutations are found within the coding sequences and the gene's exon-intron boundaries and only a few deep intronic mutations have been described. One of the intronic mutations located more than 10 kilobases (kb) into intron 19 of the CFTR gene, thus named 3849+10kbC>T, has been found frequently in Ashkenazi Jewish population (5). This mutation has been classified as a mild CF mutation with normal or slightly elevated sweat chloride. Subsequently, a deep intronic mutation 1811+1.6kbA>G, detected in CFTR intron 11, was reported in Hispanic population to cause severe cystic fibrosis with pancreatic insufficiency (1). This mutation was shown to create a new donor splice site in intron 11 that produces a new exon of 49 bp between exons 11 and 12, so called exon 11b, and causes a frameshift to the genes normal coding sequences.

In the present study, we report a novel sequence variant, 1811+1643G>T, located only 9 bp downstream from the described 1811+1.6kbA>G mutation. We detected this mutation in 11 unrelated Hispanic CF patients, who also carried a second CF mutation. From the patients' clinical symptoms, this mutation likely causes a severe CF phenotype with high sweat chloride levels.

TABLE 1:
11 patients found to carry c.1811+1643G>T novel variant in this study

Patient	Clinical Symptoms	Ethnicity	Sweat Chloride	Age (yrs) **	Genotype *
1	Azoospermia, infertility, pneumonia, CBAVD	Hispanic		43	D1152H, c.1811+1643G>T
2	Pseudomonas, pancreatic insufficient, bronchiectasis	Hispanic	89, 94	25	DeltaF508, c.1811+1643G>T
3		Hispanic		1	3876delA, c.1811+1643G>T
4		Hispanic		7	DeltaF508, c.1811+1643G>T
5		Hispanic	>60	6	c.1811+1643G>T, c.1811+1643G>T
6		Hispanic		<1	3876delA, c.1811+1643G>T
7		Hispanic		<1	G1244E, c.1811+1643G>T
8		Hispanic	>60	7	G542X, c.1811+1643G>T
9		Hispanic		6	P205S, c.1811+1643G>T
10		Hispanic		16	3876delA, c.1811+1643G>T
11		Hispanic	101, 106	5	3199del6, c.1811+1643G>T

TABLE 2:
6 patients found to carry c.1811+1634A>G known mutation in this study

Patient	Clinical Symptoms	Ethnicity	Sweat Chloride	Age (yrs) **	Genotype *
1	elevated IRT	Caucasian	abnormal	<1	2622+1G>A, c.1811+1634A>G
2		Hispanic		33	W1089X, c.1811+1634A>G
3	IBS	Caucasian		4	c.1811+1634A>G
4		Caucasian	110	<1	DeltaF508, c.1811+1634A>G
5	meconium ileus, wt gain, some coughing	AA/Hispanic	96	<1	DeltaF508, c.1811+1634A>G
6		AA		20	S1255X, I1203V, c.1811+1634A>G

* Genotyping includes CFTR exon deletion / duplication analysis
 ** Patients' ages when the blood samples were collected

FIGURE 1A:

Potential strategy used for the characterization of exon 11b for either sample with c.1811+1643G>T or 1634 A>G sequence variants. cDNA is reversed transcribed using a sequence specific primer of any CFTR exon downstream from exon 12 or random primers. First PCR is then performed with 10s and 12as primers and semi-nested PCR is with 11s and 12as primers. Bs - a putative branch point; Pt - a polypyrimidine tract; map is not to scale.



FIGURE 1B:

Sequence exon 11b (capitals). The letter **a** denotes the putative branch point and the polypyrimidine tract is underlined. c.1811+1634A>G and +1643G>T substitutions are denoted by the letter **g** on top of the reference sequence **a** and **t** on top of the reference sequence **g**, respectively. Both the A>G (at +1634) and G>T (at +1643) substitutions create a perfect donor splice site (**gtaagt**) (Kuivenhoven JA et al, 1996). The +1643 G>T substitution, however, moves the donor splice site 4 base pairs downstream from the +1634 A>G substitution creating a novel 53 bp exon 11b, instead of 49 bp.

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aataagttactataaagggtgttttagacttttaaggcttgcattggttttaaaaaa
atthtaaatggcttaaaaaatttcttaattgtgtgctgaatacaattttttattacagA
AGTACCAACAATTACATGTATAAACAGAGAATCCTATGTACTTG
AGATgataagttaagggttactatcaatcacacctgaaatttaagtgtatgaagaaa
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CONCLUSION

- In this study, an A>G base pair substitution, located 1643 bp into intron 11 of the CFTR gene, thus named 1811+1643A>G, is first described.
- This mutation likely creates a new donor splice site that produces a new exon of 53 bp between CFTR exons 11 and 12.
- It was detected in 11 unrelated Hispanic CF patients, in a pool of CF diagnostic samples containing 587 unrelated Hispanic families.
- Majority of the 11 patients were diagnosed with CF at the early ages and with high sweat chloride levels. Only 1 patient is diagnosed with infertility due to CBAVD at the age of 43 (his second mutation, however, is a known mild CF mutation, D1152H).
- We detected only 6 other patients who carried the described 1811+1.6kbA>G mutation. This equals only ~0.5% frequency, much less than the published frequency. In addition, this mutation seems not to be Hispanic specific although it is only 9 bp away from the 1811+1643A>G sequence variant.

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