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Actions and consequences: characterization of a deletion in the *CFTR* gene that encompasses a splice site

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Abstract

We report an interesting complex *CFTR* gene mutation in a patient with cystic fibrosis. It is an insertion combined with a deletion that spans an exonic splice site, causes a frameshift and could affect splicing. This rare mutation poses a challenge to provide correct nomenclature and to interpret its clinical significance.

Keywords

Cystic fibrosis; indel; splice mutation; frameshift.

History

A *CFTR* gene sequencing screen was performed on an adult non-Caucasian female with a cystic fibrosis (CF)-related phenotype, as part of her diagnostic evaluation. At the Stanford Molecular Pathology Laboratory, the *CFTR* gene sequencing assay covers 983 bases of the 5′ untranslated region, exons 1 to 24 (24 exons by conventional legacy nomenclature, which includes 27 factual exons), at least 20 bases into the 5′ and 3′ ends of all introns, the *CFTR* poly T and TG tracts in intron 8, intron 19 surrounding the c.3717 + 10 kb C > T [3849 + 10 kb C > T] mutation, and intron 11 surrounding the c.1679 + 1.6 kb A > G [1811 + 1.6 kb A > G] mutation. Analysis for known and novel mutations in these areas of the gene was performed first by unidirectional sequencing followed by confirmation of all observed potential sequence changes in the opposite direction. Sequencing quality assessment and mutation detection were performed as a semi-automated process using Mutation Surveyor™, followed by direct examination of all sequence tracings. Three sequence changes were detected in the patient's *CFTR* gene: (1) A deletion of 7 contiguous nucleotides (AAGTATG) including 2 nucleotides at the 3′ end of legacy exon 5 and 5 nucleotides at the 5′ end of the successive intron. This deletion expands over a splice site (Fig. 1) leading to an unquantified but deleterious effect on the protein product. There is only one prior report of this mutation in the literature[1]. (2) A deletion of 3 nucleotides in legacy exon 10 that causes the loss
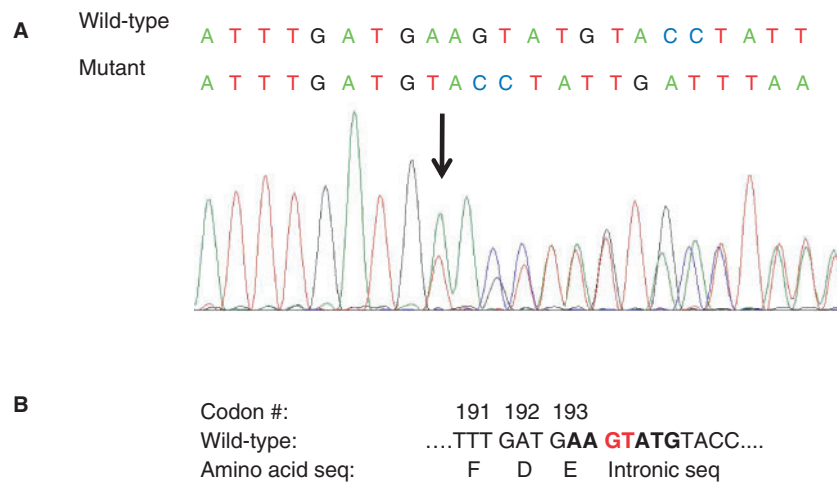

Fig. 1. (A) Forward electropherogram demonstrating the sequence overlap after the heterozygous c.578_579 + 5delAA GTATG [710_711 + 5del7] mutation (arrow). The wild-type and mutant sequences are indicated above the electropherogram. (B) The wild-type DNA and amino acid sequences are compared with the mutant sequences. The deleted nucleotides (AAGTATG) are shown in bold type in the wild-type sequence. In addition, the deleted donor splice site (GT) that marks the exon–intron boundary is shown in red.

of a single amino acid (phenylalanine) at position 508 of the cftr protein[2–5]. (3) A missense change caused by a T-to-C transition at c.3080 that results in an isoleucine-for-threonine substitution at amino acid residue 1027 in exon 19 [legacy exon 17a]. This is a mutation of unclear significance frequently reported with p.Phe508del [delF508] on the same chromosome[6,7].

Diagnostic report

Two pathogenic mutations (c.578_579 + 5delAAGTATG and p.Phe508del) and one sequence change of uncertain significance (c.3080T > C, p.Ile1027Thr) were detected.

Mutation 1: c.578_579 + 5delAAGTATG [710_711 + 5del7]

The c.578_579 + 5delAAGTATG mutation results from a deletion of 2 nucleotides at the 3′ end position of exon 5 and the first 5 nucleotides of intron 5 including the donor splice site. The mutation results in a frameshift (p.Glu193fs) and is expected to adversely affect splicing, thereby having a deleterious effect on the protein product. This pathogenic mutation has been reported in a patient with CF who also carried c.3908delA [4040delA] in exon 24 (legacy exon 21)[1].

Mutation 2: p.Phe508del (delF508)

This mutation is a known deleterious mutation in the *CFTR* gene. The p.Phe508del mutation is the most common *CFTR* gene mutation. It accounts for approximately 70% of CF chromosomes in most Caucasian populations and is also relatively commonly observed in other ethnic groups[2–5,8].

Sequence change: c.3080T > C, p.Ile1027Thr [I1027T]

The significance of the c.3080T > C, p.Ile1027Thr substitution in the *CFTR* gene is unknown and it has been reported variably as a polymorphism or a deleterious mutation. Notably, it is frequently reported in *cis* with p.Phe508del[6,7,9,10].

Discussion

As DNA sequencing has become an integral part of the clinical armamentarium for the diagnosis of certain heritable diseases, molecular pathologists and geneticists are increasingly faced with

the challenge of providing medical interpretation for novel or rare mutations. For common mutations, genetic databases and publications often provide sufficient information to determine whether a mutation accounts for the patient's condition and warrants genetic counseling. However, when it comes to novel or rare mutations, information to help discern whether they are pathogenic or innocuous is frequently lacking. In these circumstances, the type of mutation can provide clues to infer its clinical significance. For missense mutations, in the absence of functional studies, the actual effects on the protein are not readily apparent. Nonetheless, one can take into account changes in polarity and size of the affected amino acid, conservation across species during evolution, the research tool of mathematical prediction algorithms that estimate the functional consequences, segregation of the disease with the mutation in affected families, peer-reviewed literature, and the frequency of the change in the general population. Other mutation types such as a change from an amino acid to a stop codon, a mutation that results in a shift of the reading frame, and changes that affect the process of splicing, result in premature termination of translation with possible nonsense-mediated mRNA decay and are highly likely to be deleterious.

We describe the occurrence of a rare frameshift mutation involving a splice site (c.578_579 + 5delAAGTATG [710_711 + 5del7]) together with a known deleterious deletion (p.Phe508del) and a sequence variant of uncertain significance (c.3080T > C, p.Ile1027Thr) that often occurs in linkage disequilibrium with p.Phe508del[6]. Although the clinical significance of an c.3080T > C, p.Ile1027Thr substitution is unclear, when detected together with p.Phe508del, its contribution to disease may be negligible because p.Phe508del has already impaired the allele. Therefore, in our case, determining whether the additional finding of a 7-bp deletion is potentially pathogenic becomes crucial to providing an adequate interpretation and clinical correlation of the findings. A detailed analysis of that deletion reveals that it causes a shift of the reading frame and expands over the GT donor splice site of intron 5. The GT sequence at the 5′ end of virtually all introns constitutes a recognition site for the spliceosome. When this site is absent, the spliceosome is unable to recognize the exon–intron boundary. As a result, multiple alterations can occur including exon skipping, activation of a cryptic splice site, or intron retention. The specific consequence of mutations affecting mRNA splicing is generally not predictable with certainty; however, the end consequence is the generation of a defective protein[11,12]. The c.3080T > C, p.Ile1027Thr substitution could also be present on the same allele as the 7-bp deletion, but as with p.Phe508del, the pathogenic effect of the 7-bp deletion is unlikely to be further influenced by the c.3080T > C, p.Ile1027Thr substitution.

CF is an autosomal recessive condition that requires 2 mutations on opposite chromosomes for its clinical manifestation. Thus, once the potential deleterious effect of a mutation has been established, determining whether the mutation is on the opposite (*trans*) or the same (*cis*) chromosome as the p.Phe508del mutation becomes essential to predict a clinical effect: only if the mutation is in *trans* to p.Phe508del would it result in CF. When the 2 mutations are present in the same sequenced DNA fragment, this question can be readily elucidated by carefully analyzing the mixed DNA sequence. However, when the mutations are in different sequenced segments, typically different exons, parental studies would need to be performed to answer this question with certainty. The fact that in this case the patient already has been diagnosed with CF indicates that both *CFTR* alleles are compromised and that the cftr protein product from neither allele is functional. A literature and database search revealed that the c.578_579 + 5delAAGTATG [710_711 + 5del7] mutation had been reported in one patient with CF[1], further supporting the association of this mutation with a deleterious effect.

With the rapid expansion of sequencing technologies in the clinical setting, the conundrum of interpreting the clinical significance of rare or novel mutations will continue to become increasingly pressing.

Teaching point

The c.578_579 + 5delAAGTATG [710_711 + 5del7] deletion that spans an exonic splice site causes a frameshift and could affect splicing at the same time. The mutation type and its location are unusual, resulting in relatively complex naming of the mutation. The functional effects on the protein and the clinical impact of this mutation are expected to be deleterious.

References

1. http://www.genet.sickkids.on.ca.
2. Rommens JM, Iannuzzi MC, Kerem B, *et al*. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989; 245: 1059–1065. doi:10.1126/science.2772657.
3. Riordan JR, Rommens JM, Kerem B, *et al*. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245: 1066–1073. doi:10.1126/science.2475911.
4. Kerem B, Rommens JM, Buchanan JA, *et al*. Identification of the cystic fibrosis gene: genetic analysis. Science 1989; 245: 1073–1080. doi:10.1126/science.2570460.
5. Worldwide survey of the delta F508 mutation – report from the cystic fibrosis genetic analysis consortium. Am J Hum Genet 1990; 47: 354–359.
6. Fichou Y, Genin E, Le Marechal C, Audrezet MP, Scotet V, Ferec C. Estimating the age of CFTR mutations predominantly found in Brittany (western France). J Cyst Fibros 2008; 7: 168–173. doi:10.1016/j.jcf.2007.07.009.
7. Penmatsa H, Frederick CA, Nekkalapu S, *et al*. Clinical and molecular characterization of S1118F-CFTR. Pediatr Pulmonol 2009; 44: 1003–1009. doi:10.1002/ppul.21092.
8. Welsh MJ, Tsui L-C, Boat TF, Beaudet AL. Cystic fibrosis. In: Scriver C, Beaudet AL, Sly WE, Valle D, editors. Metabolic and molecular basis of inherited disease. 7th ed. McGraw-Hill3799–3876.
9. Ziedalski TM, Kao PN, Henig NR, *et al*. Prospective analysis of cystic fibrosis transmembrane regulator mutations in adults with bronchiectasis or pulmonary nontuberculous mycobacterial infection. Chest 2006; 130: 995–1002. doi:10.1378/chest.130.4.995.
10. Schrijver I, Ramalingam S, Sankaran R, *et al*. Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diagn 2005; 7: 289–299. doi:10.1016/S1525-1578(10)60557-0.
11. Ward AJ, Cooper TA. The pathobiology of splicing. J Pathol 2010; 220: 152–163.
12. Nakai K, Sakamoto H. Construction of a novel database containing aberrant splicing mutations of mammalian genes. Gene 1994; 141: 171–177. doi:10.1016/0378-1119(94)90567-3.