CASE STUDY

Identification of a new cystic fibrosis transmembrane regulator mutation in a severely affected patient

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Identification of a new cystic fibrosis transmembrane regulator mutation in a severely affected patient. E. Spitzer, D. Staab, R. Hanke, U. Wahn, R. Grosse. ©ERS Journals Ltd 2002.
ABSTRACT: By using a combination of multiplex polymerase chain reaction and allele-specific labelled probes, the oligo-ligation assay is designed to detect known cystic fibrosis transmembrane regulator mutations. This study shows that this assay may also be useful to detect new mutations.

The second child of a family of Bosnic origin showed all the symptoms of intestinal and pulmonary manifestations of cystic fibrosis. No signal could be obtained for the allele-specific probe 1898+1G>A. This could be explained by a nearby localized sequence change that prevented polymerase chain reaction primers or oligonucleotide probes from binding to the target sequence. Indeed, sequence analysis revealed a new 1894G>T exchange (Glu587Stop). Both parents and the healthy brother carried this mutation. Thus, the index patient was homozygous for 1894G>T, which was inherited from both parents.
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Cystic fibrosis (CF) is an inherited disorder that is characterized by respiratory disease and pancreatic dysfunction [1]. CF results from mutations in the gene encoding the CF transmembrane conductance regulator (CFTR). The CF phenotype can be caused by >900 different mutations in the CFTR gene [2]. With the exception of the more common 3-base pair deletion in exon 10 (ΔF508), the vast majority of mutations are rare or restricted to certain populations. In order to make an efficient screening for the more common known mutations possible, the oligo-ligation assay (OLA) has recently been introduced [3, 4]. By using a combination of multiplex polymerase chain reaction (PCR) and allele-specific fluorescently-labelled probes ligated to CFTR mutation sites, deoxyribonucleic acid (DNA) fragments harbouring each of the 31 more common mutations can be produced. Identification of a CFTR genotype is achieved by size separation and gene scanning on an ABI 377 device (Applied Biosystems, Foster City, CA, USA). The OLA assay is designed to detect known mutations for which specific probes must be available. Nevertheless, this paper shows that this assay has made the detection of a new nonsense mutation possible in a family of Bosnic origin.

Case report

A family of Bosnic origin with two children was studied. After normal delivery, the second child showed symptoms of dyspnoea, dilated bowel and intestinal obstruction at the age of 3 h. After meconium aspiration, connatal pneumonia was diagnosed. The child was treated with antibiotics and the intestinal obstruction was resolved with enemas. At the age of 4 months, he was re-admitted to the hospital because of an atelectatic pneumonia. At that time, the diagnosis of CF was verified by a pathological sweat test and he was referred to a CF centre. The second and third sweat test were found to be pathological (sodium: 74 and 83 mmol·L⁻¹) and pancreatic insufficiency was diagnosed. The child showed all symptoms of intestinal and pulmonary manifestation of CF. The elder brother was healthy and there was no history of CF or early deaths in childhood in the family, but the grandparents of both families were born in the same village.

DNA samples were obtained from all members of the family from dried blood stains prepared as previously described [5]. They were routinely examined by means of the CF assay kit from Applied Biosystems.

Discussion

Studies carried out in southern European populations, including the former Yugoslavia, identified ΔF508, G542X, G551D, 621+1G>T, W1282X and N1303K as the most common CF mutations [6, 7]. To the authors' knowledge, no data has been published about the Bosnic population.

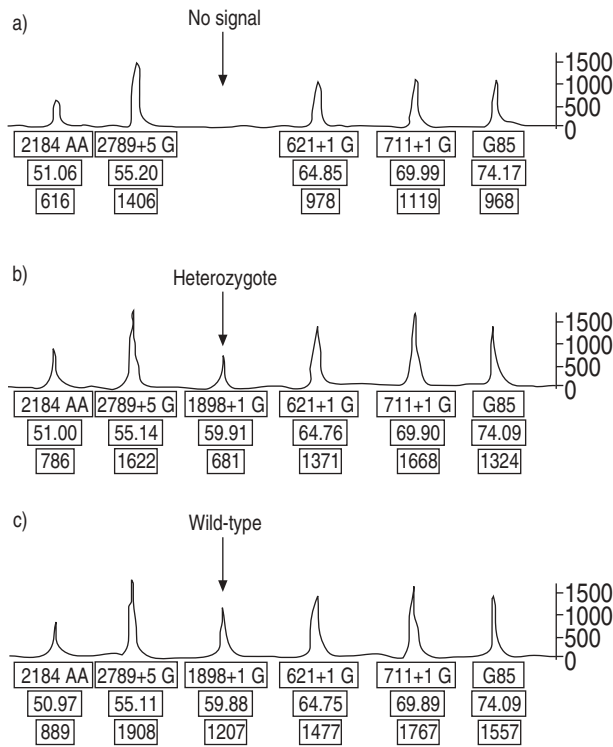In order to screen for CFTR mutations, the DNA

Fig. 1. – Oligo-ligation-assay. The plots of the electropherograms for six different mutation sites in the cystic fibrosis transmembrane regulator (CFTR) gene are shown. The numbers in the boxes indicate the mutation site, the size of the fragment and the height of the peak. a) The plot obtained from the index patient′s sample. No signal could be detected for 1898+1G in intron 12 of the CFTR gene (arrow). b) The plot obtained from the blood sample of the index patient′s father. The signal obtained for 1898+1G was reduced in comparison with the five other mutation sites of the same panel and the wild-type signal for 1898+1G shown in c). The same results were obtained with blood samples from the mother and the healthy brother of the patient (data not shown). c) The plot obtained using a wild-type control sample.

Fig. 2. – Sequencing analysis. The results of sequencing polymerase chain reaction fragments including exon and intron 12 of the cystic fibrosis transmembrane regulator gene. a) The sequence from the index patient′s sample shows a homozygous G>T exchange at position 1894. b) The sequence from the sample of the index patient′s father. The heterozygous exchange G>T at nucleotide position 1894 was found and confirmed for the patient′s mother and the healthy brother (data not shown). c) The sequence from a wild-type control sample.

sample obtained from the index patient was analysed by means of the OLA assay. For 30 of the allele-specific probes the assay revealed no differences to the wild-type sequence (fig. 1). However, no signal could be obtained for either the wild-type or the mutated site of the allele-specific probe 1898+1G>A in intron 12 [8]. This may be explained by a sequence change that prevented PCR primers from binding to the target sequence, thus inhibiting amplification of the DNA fragment harbouring the 1898+1G>A genotype. Alternatively, a sequence alteration in the binding domain for the oligonucleotide ligation probes could have prevented fragment analysis. Nevertheless, the data suggest a sequence deviation in the vicinity of the site 1898+1G. To test this assumption, exon 12, including the exon-intron boundaries, was sequenced. Sequence analysis showed a G>T exchange at the base position 1894, which generated a termination signal at amino acid position 587 (Glu>Stop; fig. 2).

The lack of an OLA signal at position 1898G>A in exon 12 could therefore be interpreted as a heterozygous G>T exchange associated with a simultaneous deletion in the region of exon/intron 12 on the other allele, or, alternatively, might have been due to a
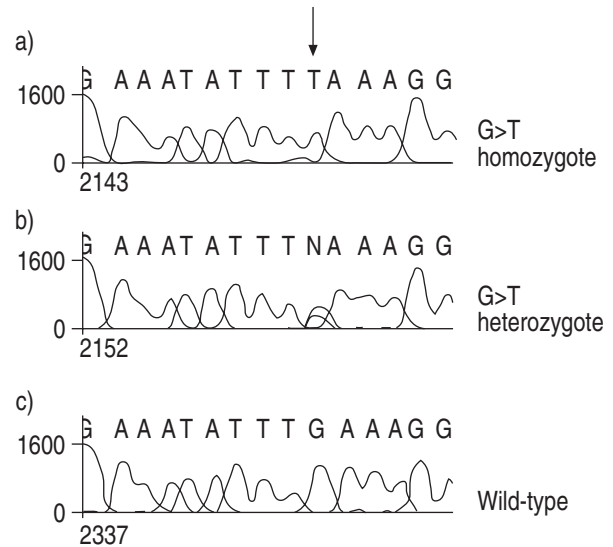
homozygous G>T exchange at position 1894. Both parents and the healthy brother of the index patient were therefore examined for the presence of 1894G>T. All three individuals were found to be heterozygous carriers of the mutation 1894G>T (figs. 1 and 2). Thus, the index patient inherited the mutation from both parents and was classified as homozygous for the mutation 1894G>T (Glu587Stop).

The data presented show the sensitivity and efficiency of the oligo-ligation assay for detecting single-base substitutions. If under routine conditions the allelic ligation probes fail to produce specific fragments and fluorescence signal using the oligo-ligation assay, it is therefore straightforward to test for sequence variants by sequencing the respective deoxyribonucleic acid fragment or exon. Finally, it would be interesting to further explore the frequency of the mutation 1894G>T among the Bosnic population. This can be efficiently achieved through the use of the oligo-ligation assay.

References

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