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Short Communication

A false positive newborn screening result due to a complex allele carrying two frequent CF-causing variants

Anne Bergougnoux [a,*], Amandine Boureau-Wirth [c], Cécile Rouzier [c], Jean-Pierre Altieri [a], Fanny Verneau [a], Lise Larrieu [a], Michel Koenig [a,b], Mireille Claustres [a,b], Caroline Raynal [a,b]

[a] *Laboratoire de Génétique Moléculaire, CHRU Montpellier, 34093 Montpellier Cedex 5, France*
[b] *Laboratoire de Génétique de Maladies Rares, Institut Universitaire de Recherche Clinique, EA 7402, Université de Montpellier, 34000 Montpellier Cedex 5, France*
[c] *Service de Génétique Médicale, Hôpital L'Archet 2, 06200 Nice, France*

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Abstract

The detection of two frequent *CFTR* disease-causing variations in the context of a newborn screening program (NBS) usually leads to the diagnosis of cystic fibrosis (CF) and a relevant genetic counseling in the family. In the present study, CF-causing variants p.Phe508del (F508del) and c.3140-26 A > G (3272-26 A > G) were identified on a neonate with positive ImmunoReactive Trypsinogen test by the Elucigene™ CF30 kit. The CF diagnosis initially suggested, despite three inconclusive Sweat Chloride Tests (SCT), was finally ruled out after the familial segregation study combined with a negative SCT. Haplotype studies, based on the comparison of 80 p.Phe508del haplotypes, suggested a probable *de novo* occurrence of c.3140-26 A > G on the p.Phe508del ancestral allele in this family.

This false positive case emphasizes the importance of SCT in the NBS strategy. Moreover, it raises the need for familial segregation studies in CF and in overall molecular diagnosis strategy of autosomal recessive diseases.

Keywords: Cystic fibrosis; *CFTR* gene; Newborn screening; Complex allele; False positive

1. Introduction

The French national newborn screening (NBS) program for Cystic Fibrosis (CF; MIM #219700) has been set up in 2002 to reduce delays in diagnosis, facilitating preventive care for early respiratory and nutritional involvement [1], using an IRT/DNA algorithm [2]. Infants with elevated IRT are screened for a panel of common CF-causing variants [3]. A sweat chloride test (SCT) is performed for all newborns screened positive for at least one CF-causing variation to estimate a CF or non-CF status and the relevance to search for a second CF-causing variation through an in-depth *CFTR* gene analysis. Finally, a familial segregation study is recommended to assess the segregation of the mutated alleles and to confirm the clinical diagnosis of CF. The identification of two *CFTR* molecular alterations has also significant implications for genetic counseling in families. If both parents are carriers of a CF-causing variation, their risk of having a CF child in future pregnancies is 25%, which fully justifies prenatal or pre-implantation diagnosis.

Here, we report the first case of a healthy carrier of two frequent CF-causing variants on the same allele and the most likely mechanism for its occurrence. This particular case emphasizes the importance of SCT and the following familial segregation study to improve the medical management of infants with positive NBS and their families.

2. Results

This study focused on a family composed by a female neonate (IRT at day 3: 82, 73, 71 ng/ml), her parents and her maternal grandparents (complete pedigree in Fig. 1a). All the methods and

* Corresponding author at: Laboratory of Molecular Genetics, CHU Montpellier—IURC, 641 Avenue Doyen Gaston Giraud, 34093 Montpellier cedex 5, France. Tel.: +33 4 11 75 98 64.
E-mail address: anne.bergougnoux@inserm.fr (A. Bergougnoux).

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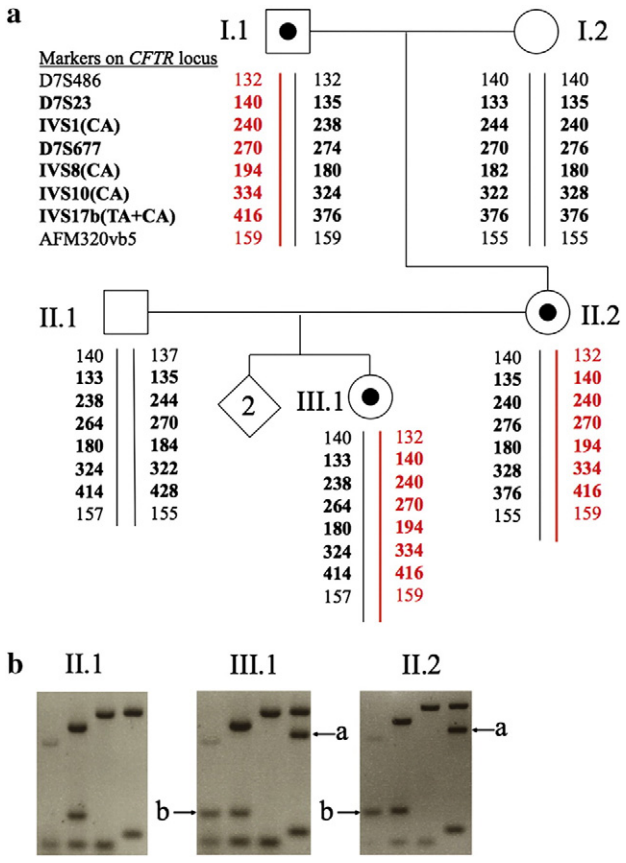

Fig. 1. Genetic analysis of the family. a. CFTR-linked markers analysed on five individuals of the family. Intragenic (bold) or extragenic polymorphic markers, noted in the 5'-3' range, confirmed the haplotype phasing, the absence of sample mix, and the biological paternity. The haplotype linked to the mutant allele is highlighted in red. b. Detection of c.3120-26A>G (band a) and p.Phe508del (band b) using the ElucigeneTM CF30 kit Cystic Fibrosis Screening - ARMS technology in III.1 and II.2

markers used in this study are reported as supplementary materials. Variants c.1521_1523del or p.Phe508del (F508del) and c.3140-26 A > G (3272-26 A > G), representing respectively 67.2% and 0.3% of CF alleles in France [4], were identified on a dried-blood sample of the female newborn (Fig. 1b). As recommended, the local CF center performed a first SCT of the neonate that could not be interpreted due to an insufficient sweat weight. Two other SCT performed three weeks apart also failed. Finally, three months after the NBS positive result, parents were offered genetic counseling for familial segregation studies. Surprisingly, p.Phe508del and c.3140-26 A > G were both found in the maternal sample, on two independent blood samples from the girl and her parents. No other disease-causing variation was found after the in depth *CFTR* analysis. Analysis of microsatellite markers at the *CFTR* locus allowed us to exclude a maternal uniparental disomy or germline recombination, as the girl inherited the complete maternal haplotype. The haplotype analysis on *CFTR* and *MYO7A* loci confirmed the parenthood and ruled out an accidental mix-up of samples (Supplementary Fig. 1 online). The CF center informed us of the negative value of the 4th SCT (29 mmol/l) and the normal elastase measurement (>500 µg/g stool) in the newborn. The mother had also a negative SCT (24 mmol/l). Finally, we showed that the complex allele was inherited from the maternal grandfather (I.1 in Fig. 1a). Altogether, these results confirmed the in-*cis* segregation of the two variations as a novel complex allele c.[1521_1523del;3140-26 A > G] in this family; consequently CF diagnosis was ruled out for this infant.

The complex allele c.[1521_1523del;3140-26 A > G] could have been set up by recombination or *de novo* occurrence. To understand the mechanism of its formation, the *cis*-associated haplotype was compared to haplotypes of eighty p.Phe508del chromosomes and two c.3140-26 A > G chromosomes. Nine out of thirteen *CFTR* markers identified in the proband, her mother and grandfather were similar to the most frequent p.Phe508del-associated ones (Table 1). Moreover, only two *CFTR* markers were common to one of the c.3140-26 A > G haplotypes and were located upstream the p.Phe508del mutation (haplotype 1, Table 1). We found no similarity with the other c.3140-26 A > G chromosome (haplotype 2, Table 1). Although we cannot definitely exclude a recombination, taken together, our results suggest that the c.3140-26 A > G variant arose *de novo* on an ancestral p.Phe508del-mutated chromosome.

3. Discussion

CFTR complex alleles are frequently found in patients, usually associating two or more "mild" variants (e.g. involved in *CFTR*-Related Disorders), and more rarely one CF-causing variant with "non-neutral" or "mild" variations [6]. This work reports the first complex allele on the *CFTR* gene that associates two frequent CF-causing variations detected by NBS strategy. Since such a complex allele was an unexpected genetic event, it was not surprising that the CF center suggested the diagnosis of CF to the parents based on the two hits in the NBS-CF kit, despite three inconclusive SCT. This child should have rather been considered as "CFSPID" until the SCT result is available [7]. Even with the negative SCT, the achievement of the segregation study, demonstrating the association of the two variations on the same allele, was actually the only way to definitely rule out the CF diagnosis of this child [8]. This case highlights the need of precautions in the announcement of CF diagnosis, considering the anxiety induced and the resulting constraints in daily life. It also indicates that SCT and secondary familial segregation need to be performed as quickly as possible to either confirm or exclude a CF diagnosis.

The characterization of the complex allele c.[1521_1523del;3140-26 A > G] has only been possible after an extensive analysis of the proband's family that associated NBS, SCT in the neonate and her mother, and study of the familial segregation of the variants through two generations. To our knowledge, only two other cases of complex alleles with two CF-causing variants were previously described, in CF patients [9,10]. Moreover, the extensive analysis of the *CFTR* gene performed on many hundreds of patients in our laboratory using Sanger sequencing or, massive parallel sequencing of the complete locus never highlighted such an association in *cis* [11,12].

Even if a recombination event could not be completely excluded and despite the low *de novo* occurrence on the *CFTR*