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LETTER

Testing the parents to confirm genotypes of CF patients is highly recommended: report of two cases

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Molecular genetic analysis of cystic fibrosis (CF) is routinely performed in hundreds of laboratories worldwide. The Orphanet database (www.orpha.net) lists 302 CF diagnostic laboratories (44 for Germany). Most laboratories use commercial kits that are expected to be robust and deliver reliable results. For most European populations, CFTR mutation detection rates of 70–90% can be achieved by testing the most frequent mutations (which are mainly included in commercial kits), thereby identifying homozygosity or compound heterozygosity for CFTR mutations in approximately 50–80% of CF patients. Very often, the referring clinicians (mostly paediatricians or geneticists) request testing of only the CF patients and not of their parents. At the Institute of Human Genetics in Hannover, we have tested 2168 patients with the clinical diagnosis of CF, but we have received samples only from the parents (mostly after having tested the index patients) in 198 (9.1%) of these cases. However, as again stated in the best practice guidelines for molecular genetic analysis of CF- and CFTR-related disorders in the *European Journal of Human Genetics*, 'identification of mutation(s) on both parental alleles is required to confirm the diagnosis. Homozygous and compound heterozygous status should thus be confirmed by studying the parents'.[1] Here, we present two recent cases from our laboratories that strengthen the need to correctly assign the parental alleles, particularly in the context of prenatal diagnosis of CF or carrier testing.

Case 1

CFTR testing using the Elucigene CF29 kit was performed in 2003 in a newborn Turkish girl with meconium ileus. Owing to an apparent F508del homozygous test result, the tentative diagnosis of CF was confirmed. The patient suffered her first infection of the respiratory tract at the age of 2 months. Since then, several episodes of bronchitis occurred (treated by antibiotic therapy and inhaled corticosteroids). At the age of 4 years, she is on constant therapy with pancreatic enzyme supplements and hypertonic saline inhalations. In 2008, the mother of this CF patient became pregnant and requested genetic counselling and prenatal diagnosis. DNA samples of the mother and the fetus were tested for F508del by PCR (primers are located in CFTR exon 10) and polyacrylamide gel electrophoresis (PAGE),[2] resulting in a homozygous wild-type test result for the fetus. The mother, however, was heterozygous for the two base-pair deletion 1677delTA (commonly used traditional nomenclature; corresponding to mutation designation c.1545_1546delTA, HGVS nomenclature, www.hgvs.org/). Subsequent testing of the index case by PCR and PAGE as well as by sequencing confirmed compound heterozygosity for F508del and 1677delTA. Retesting of the patient's DNA with this version of the Elucigene CF29v.2 kit (Tepnel Diagnostics, Abingdon, UK) yielded an F508del homozygous test result, whereas F508del heterozygosity was observed with the INN0-LIPA CFTR 36 kit (Innogenetics, Zwijnaarde, Belgium) and the Abbott CF genotyping assay (Abbott, Wiesbaden, Germany). The results of the different assays are compiled in Table 1 and Supplementary Figure S1. *Note*: testing of both parents with the Elucigene kit would be expected to have resulted in an F508del heterozygous result in the father and would have resulted in a wild-type homozygous result in the mother. The discrepancy to the apparently homozygous F508del result of the patient would have been recognized, and further testing would have been initiated. (Figure 1)

Case 2

In January 2007, CF was diagnosed clinically in an almost 2-year-old girl with recurrent infections of the respiratory tract, failure to thrive, pancreatic insufficiency and a pathological sweat test result (sweat chloride: 126 mmol/l). Molecular genetic testing with PCR and PAGE as well as with the INNO-LIPA 19 kit indicated homozygosity for F508del. The parents were tested using the same test methods. Whereas the father was confirmed to be heterozygous for F508del, the mother appeared to be homozygous for wild-type CFTR. Subsequent analysis with Multiplex Ligation-dependant Probe Amplification (MLPA, mix P091, MRC-Holland, Amsterdam, The Netherlands) resulted in the detection of heterozygosity for a large deletion including CFTR exons 3–24, in both the mother's and the patient's DNA (see Supplementary Figure S2).

Thus, in both cases, the initial test results (homozygosity for F508del) had to be revised after testing the parents.

Table 1 Case 1: summary of the test results from the different diagnostic kits/assays

	F508del	*wt*	*1677delTA*	*Genotype*
Elucigene CF29 v2003	+	−	Not included	F508del/F508del
Elucigene CF29 v.2	+	−	Not included	F508del/F508del
Inno−Lipa CF38	+	+	Not included	F508del/wt
Abbott OLA v3	+	+	Not included	F508del/wt
PCR & PAGE	+	−	+	F508del/1677delTA[a]
Sequencing	+	−	+	F508del/1677delTA

Case 1 is a female Turkish CF patient, compound heterozygous for F508del and 1677delTA, as confirmed by testing the mother. + = mutation or wild-type (wt) present. − = mutation or wt not present.
[a]Genotype deduced from typical band pattern of homo- and heteroduplices. None of the commercial kits test for 1677delTA ('not included'). Supplementary Figure S1 shows all test results.

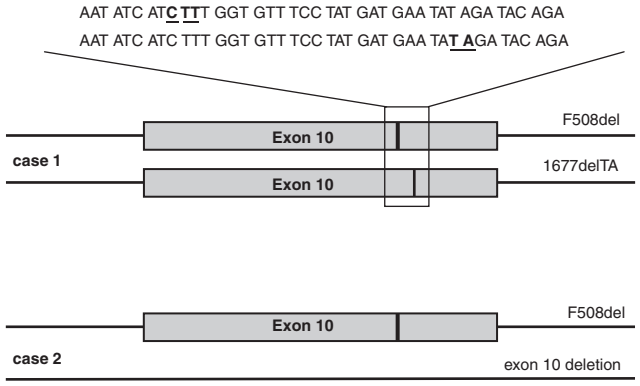

Figure 1 Schematical depiction of the genotypes found in the presented patients. Patient 1 is compound heterozygous for the mutations F508del and 1677delTA (traditional nomenclature; c.1521_1523delCTT and c.1545_1546delTA according to the HGVS nomenclature), whereas patient 2 carries F508del on one allele and the second allele (including exon 10) is deleted. Vertical bars represent the mutations. The relevant part of the sequence is written above (traditional nomenclature: nt 1645–1686; HGVS nomenclature: nt 1513–1554) and represents the boxed region of exon 10. Deleted nucleotides are underlined and written in bold.

Homozygosity for F508del is the most frequent genotype of CF patients. Therefore, the initial test results were completely unremarkable until the later problems were found. Several scenarios are imaginable, in which testing for F508del only could lead to erroneous conclusions in case of prenatal diagnosis or carrier testing. In family 1, an F508del/1677delTA compound heterozygous (affected) fetus could have been misdiagnosed as an F508del heterozygous (healthy) fetus. Carrier testing of relatives of the mothers from both cases could have resulted in the erroneous exclusion of carriership.

Case 1 also illustrates that genotyping with commercial kits does not always guarantee correct results. In this case, we failed to amplify the non-F508del allele by using the allele-specific amplification technology (Elucigene kits), although the reverse hybridization line probe assay (INNO-LIPA kit) and the oligo-ligation assay (OLA, Abbott kit) correctly identified the presence of one non-F508del allele. As F508del (22%) and 1677delTA (4.5%) are the most and second most frequent mutations in the Turkish population, respectively,[3] compound heterozygosity for these two CFTR mutations is assumed to occur relatively frequently among Turkish CF patients. As long as commercial kits do not test for 1677delTA, we recommend testing Turkish patients for this mutation with an additional test method such as PCR and PAGE or sequencing. Companies usually optimize their panels for American and western European populations, and the sensitivity of the test is therefore lower in certain regions, notably central, southern and eastern Europe. Suppliers of commercial kits should be aware of possible genotyping problems and should take care to solve these problems. From the annual external quality assessment scheme of the European CF-network (www.cfnetwork.be), it is known that other genotypes than those described above caused genotyping errors with commercial kits, that is, compound heterozygosity for W1282X and S1251N was mistyped as homozygosity for W1282X (OLA), and R553X/G551D compound samples appeared as homozygous for R553X and heterozygous for G551D (reverse hybridization line probe assay). By changing the assay and/or disclaimers on the instruction manuals, both companies (Abbott and Innogenetics) reacted to solve these problems.

We conclude that although commercial CFTR kits may usually be robust and reliable, they cannot guarantee correct results for all possible combinations of mutations. As highly recommended in European[1,4] and national[5] guidelines, CFTR genotypes of CF patients should always be confirmed by testing the parents. The report of the testing laboratory to the referring clinician should clearly state that the patient's genotype is provisional, dependent on confirmation by testing the parents. The assignment of correct parental alleles is needed if prenatal diagnosis of CF is required or if testing relatives.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)