

Mutation scanning of the *CFTR* gene by high resolution melting analysis (HRM)



Krenkova P, Stambergova A, Norambuena P, Macek Jr. M

Cystic Fibrosis Centre, Department of Biology and Medical Genetics,
Charles University Prague -2nd School of Medicine and University Hospital Motol, Prague,
Czech Republic



Introduction

To this date more than 1500 mutations have been identified in *CFTR* gene (1) which consists of 27 exons (2), making the detection of non common mutations by sequencing laborious, expensive and time-consuming. To simplify the analysis of such a broad mutation spectrum, a rapid and reliable method is necessary. In this study we evaluated High Resolution Melting (HRM) which presents a rapid, high-throughput, closed-tube method for mutation scanning and genotyping. The sample preparation consisted of a standard PCR reaction with a dsDNA binding fluorescent dye and does not require any post PCR handling. Products are after PCR amplification directly analysed on special designed instruments for melting analysis. The homozygous, heterozygous and wild-type samples are differentiated according the melting profile which is represented by plotting fluorescence over the temperature range.

Materials and Methods

HRM was used for mutation scanning of selected samples derived from CF patients with a known *CFTR* genotype. We tested 19 different disease causing *CFTR* mutant genotypes (table 1) located within 6 exons of the *CFTR* gene (4, 7, 10, 11, 14b and 22). Analysed mutations included all SNP classes and 1- or 3-base-pair deletions and represent at least 76.5% of all CF alleles detected in the Czech Republic.

Evaluated amplicons varied in size from 101bp to 380bp and had a GC content ranging from 33.7% to 45.9%. Melting curves of tested samples were evaluated against melting curves of sequencing-verified wild-type samples. DNA amplification and melting analysis was performed on RotorGene 6000 (Corbett Life Science) using the fluorescent double-strand DNA intercalating dye LCGreen plus (Idaho Technology).

Results and Discussion

Tested samples were evaluated by initial visual inspection of melting curves and by software analysis. All examined samples with mutant heterozygous genotype were unambiguously distinguished from wild type samples by a different shape of the melting curves (Figure 1). Heterozygous profiles of G551D versus R553X and L1335F versus L1335P were not distinguishable (Figures 1F and 1H).

Homozygous genotype for F508del and M470V mutations showed similar melting pattern as the wild type control samples and the T_m shift was not easily distinguished (Figure 1C). The presence of the homozygote mutations was confirmed by mixing (1:1) the PCR product with the wild type PCR product, denaturing and reanalysing the melting. That way, heterozygosity of both mutations was established and the detection of the homozygote mutations by melting analysis was obvious - the melting pattern was similar as the original sample with heterozygous genotype M470V/F508del (Figure 1D).

In total, we analysed 120 wild type melting curves and 140 mutant melting curves with 100% sensitivity and 96% specificity.

Table 1. Mutations analyzed in the study

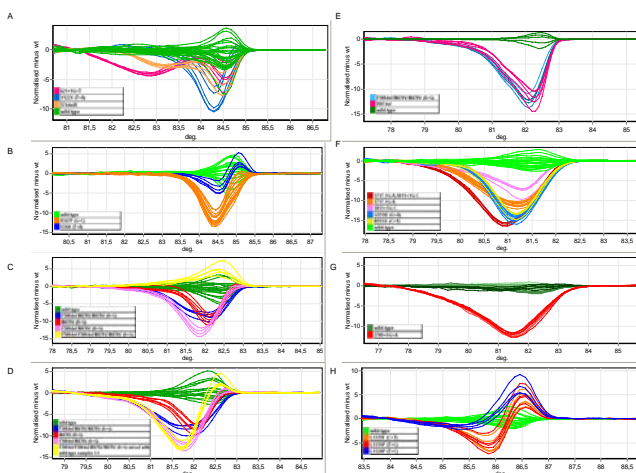
Exon	Genotype	No. of Samples	Nucleotide Change	SNP class
4	621+1G>T	1	G>T	2
	Y122X	1	T>A	4
	5749delA	1	del	-
7	R347P	3	G>C	3
	I336K	1	T>A	4
	F508del/M470V/M470V	1	del/A>G/A>G	1
10	F508del/F508del/M470V/M470V	1	del/del/A>G/A>G	1
	F508del/M470V	1	del/A>G	1
	M470V	1	A>G	1
	I507Gdel	1	del	-
	1717-1G>A	2	G>A	1
11	1811+1G>C	1	G>C	3
	1717-1G>A / 1811+1G>C	1	G>A / G>C	1/3
	G551D	2	G>A	1
	R553X	1	C>T	1
	2789+5G>A	2	G>A	1
14b	L1335F	1	C>T	1
	L1335P	1	T>C	1
	L1324P	1	T>C	1

In our hands HRM allowed easy detection of all SNP classes as well as 1- and 3-base-pair deletions.

This method exhibits a very high specificity and sensitivity suitable for its use as a pre-screening method in diagnostics.

In conclusion, HRM analysis is an economical, sensitive and specific close-tube method that can dramatically reduce the need of sequencing. Consequently it has a high utility for the detection of unknown mutations in CF DNA diagnostics.

Figure 1. Normalized difference graphs



Each genotype was melted and displayed in five replicates. A - exon 4. B - exon 7. C - exon 10. D - exon 10; homozygous sample (yellow colour) was mixed 1:1 with wild type PCR product, danaturated and reanalyzed. E - exon 10, F - exon 11, G - exon 14b, H - exon 22.

References

- (1) Cystic Fibrosis Mutation DataBase - <http://www.genet.sickkids.on.ca/cfr>
- (2) Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science, 1898;245:1066-1073.

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Contact

petra.krenkova@fnmotol.cz