

Short Communication

Comparison of the TaqMan and LightCycler systems in pharmacogenetic testing: evaluation of *CYP2C9**2/*3 polymorphisms

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Abstract

Background: Pharmacogenetic testing for drug-metabolizing enzymes is not yet widely used in clinical practice.

Methods: In an attempt to facilitate the application of this procedure, we have compared two real-time PCR-based methods, the TaqMan[®] and the LightCycler[™] for the pharmacogenetic evaluation of *CYP2C9**2/*3 polymorphisms.

Results and Conclusion: Both procedures are suitable for pharmacogenetic studies. The TaqMan procedure was less expensive in terms of cost per sample, but the TaqMan apparatus is more expensive than the LightCycler apparatus.

Keywords: cytochrome P450 (CYP450); pharmacogenetic testing; polymorphisms.

Pharmacogenetic testing for drug-metabolizing enzymes is not widely used in clinical practice, probably because of a poor evidence base and a slow translation of research results to the clinical setting (1). A major drug-metabolizing enzyme is cytochrome P450 2C9 (CYP2C9), which metabolizes over 100 currently used drugs (2, 3). Over 50 single nucleotide polymorphisms (SNPs) have been identified in the regulatory and coding regions of this gene (2). The coding variants *CYP2C9**2 (Arg¹⁴⁴Cys) and *CYP2C9**3 (Ile³⁵⁹Leu) reduce drug metabolism, which suggests that drug dosage should be appropriately tailored in patients carrying these variants (4). *CYP2C9* genotypes have been investigated mainly in relation to

warfarin, an anticoagulant widely used to prevent thromboembolic events in cardiovascular disease (5).

A simple, sensitive, rapid and accurate genotype test would promote the use of pharmacogenetic testing in laboratory practice. The most widely used genotyping systems are: denaturing high-performance liquid chromatography (dHPLC); polymerase chain reaction (PCR)-restriction fragment length polymorphisms; matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; pyrosequencing; microarrays; and real-time PCR, each of which has advantages and limitations (6).

We have compared the costs, analysis time, success rate and versatility of two real-time PCR-based methods, the TaqMan[®] (Applied Biosystems, Foster City, CA, USA) and LightCycler[™] (Roche Diagnostics, Mannheim, Germany) procedures, in the identification of *CYP2C9* polymorphisms. Using both instruments, we genotyped 114 subjects (65 patients undergoing therapy with warfarin for over 10 years and 49 controls). All participants were recruited from our University Hospital and gave informed consent to the study. The study was approved by the Ethics Committee of the School of Medicine of Naples University "Federico II" and was conducted in accordance with the principles of the Helsinki II Declaration.

Briefly, a fasted blood sample was collected from enrolled subjects in the morning after an overnight fast. DNA was extracted with the Amersham Nucleon BACC 2 Kit (Amersham Biosciences, Europe, Little Chalfont, Buckinghamshire, England), and stored at +4°C until required. Anamnestic and clinical data were collected from all patients.

The *CYP2C9* *1 wild-type (wt), *2 and *3 polymorphic alleles were genotyped with the TaqMan procedure (7, 8) and with the LightCycler Real Time PCR (9, 10), two procedures widely used to identify SNPs. The pre-developed TaqMan assay reagent kit contains one pair of PCR primers, one pair of fluorescent TaqMan probes, and control templates. The PCR is set up in a 96-well plate with a 25-μL mix reaction and requires 50 ng of genomic DNA per assay. The PCR and the subsequent allelic discrimination were carried out on an ABI PRISM 7900 HT system (Applied Biosystems). SDS 2.1 software (Applied Biosystems) was used to analyze the fluorescent PCR products and to genotype the samples. The LightCycler-CYP2C9 Mutation Detection Kit provides primers and hybridization probes for sequence-specific detection, pre-mixed reagents, a solution containing Fast Start Taq polymerase, and a control template. The PCR is set up in glass capillaries using 20 μL of mix reaction and 50 ng of genomic

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Table 1 Costs of analyzing *CYP2C9**2/*3 polymorphisms using the LightCycler and TaqMan procedures.

	Cost/test, €		
	TaqMan		LightCycler
Consumables (tips, well, cover or capillaries)	0.366 ¹ (96-test manual kit)	1.263 ² (384-test automated kit)	1.05 (32-test kit)
Reagent	6.25	1.25	13.06
Total cost	6.6	2.51	14.11

¹Cost for 96 tests in manual; ²cost for 384 tests in automation using the Biomek Workstation.

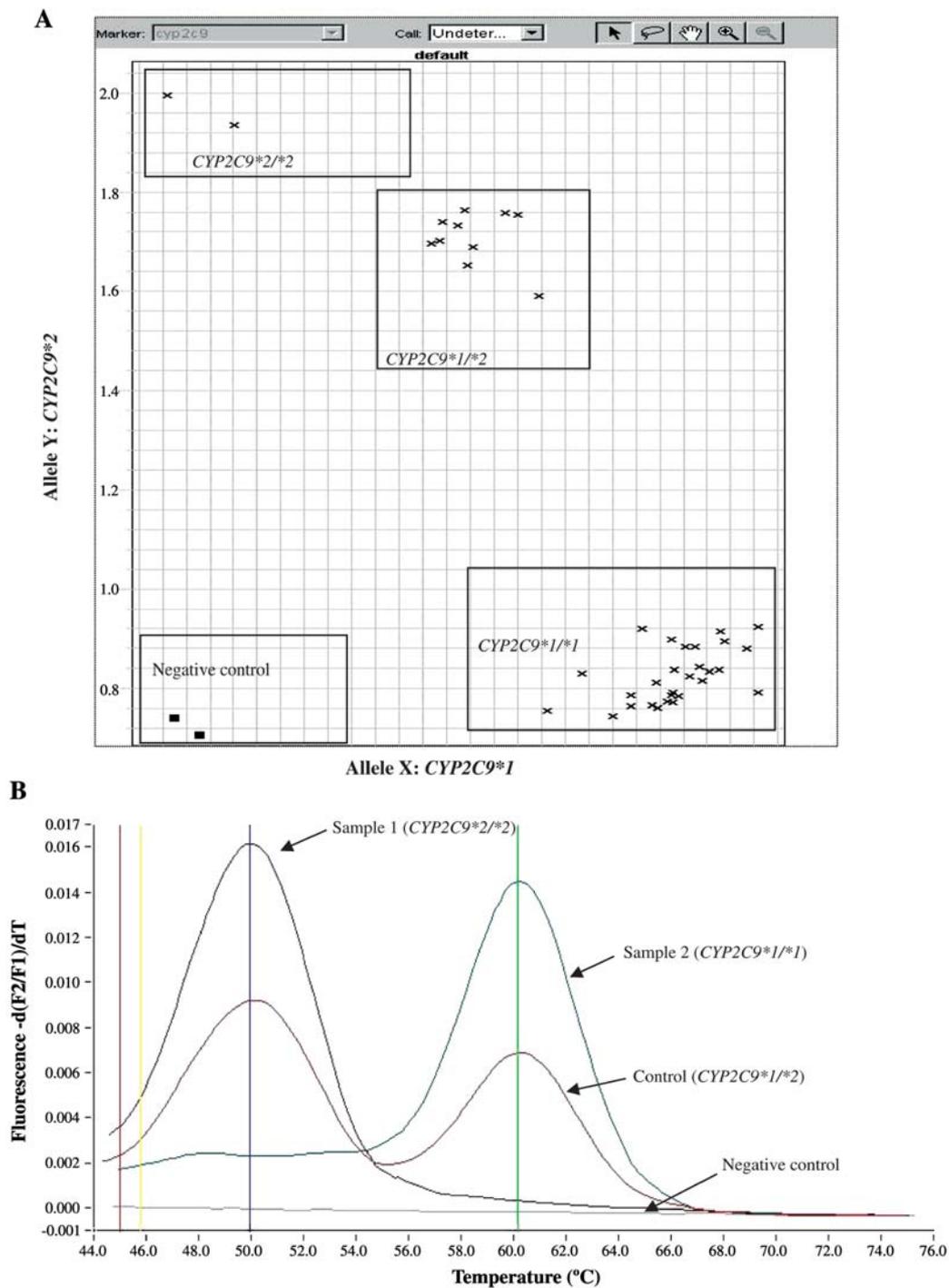


Figure 1 Identification of *CYP2C9* gene polymorphisms with the TaqMan (A) (PCR conditions: 50°C for 2 min, 95°C for 10 min, followed by 35 cycles at 92°C for 15 s and 60°C for 1 min) and LightCycler (B) (PCR conditions: 95°C for 10 min followed by 45 cycles at 95°C for 10 s, 55°C for 10 s, 72°C for 10 s and 95°C for 1 min, 40°C for 1 min) systems.

DNA, and is performed on the LightCycler system (Roche Diagnostics); the three *CYP2C9* alleles are identified based on fluorescence measurements. The costs and times required to isolate DNA and to set-up the PCR mix were similar for both methods and are therefore not included in the test comparison. In addition, the times required to determine *CYP2C9**2/*3 genotypes after DNA isolation were comparable: 106 min with the TaqMan procedure (up to 48 samples) and 60 min with the LightCycler procedure (up to 32 samples). The cost per sample, including reagents and consumables, was €6.61 for the TaqMan set-up and €14.11 for the LightCycler system (see Table 1). Moreover, the TaqMan procedure can be automated using the 384-well apparatus and an automated workstation, thereby further decreasing the cost/test. With the Biomek 2000 Workstation (Beckman Instruments, Fullerton, CA, USA) the test/cost was €2.51. Both methods recommend a quality control procedure, consisting of one negative and two positive (mutated and wt) homozygous controls (TaqMan), or of one negative and one positive (heterozygous mutated) sample (LightCycler).

The advantages of the LightCycler were: (a) less risk of contamination because a single glass capillary is used; and (b) the cost per test is the same, irrespective of the number of samples (from 1 to 32), which is useful when dealing with a low number of samples. However, the glass capillaries are easily broken and the cost per test is high. The advantages of the TaqMan were: (a) a low cost per test, particularly for high-throughput screening (from 96 to 384); and (b) possible automation that further decreases the costs. The TaqMan procedure has the disadvantage that it is not suitable for a low number of assays and the open-well plate entails a risk of contamination.

We tested the same sample 15 times to evaluate reproducibility of the *CYP2C9* assay, and the results invariably overlapped with the two instruments. The success rate, i.e., the number of runs successfully completed with each instrument, was 97% (36/37 runs) with the TaqMan and 91% (29/32 runs) with the LightCycler method. Failures were due to non-amplification of the positive control (TaqMan) and/or to the quenching of 1/2 fluorochromes (LightCycler).

There was 100% concordance in the genotyping results obtained with the two procedures. The *CYP2C9**1, *CYP2C9**2 and *CYP2C9**3 alleles were present in 80.2%, 13.1% and 6.6%, respectively, of the genotypes, which agrees with data reported for other Italian samples (11, 12). Both kits produced unambiguous real-time sequence outputs (Figure 1A, B) and were equally easy to use. The initial equipment costs varied, depending on the model purchased, i.e., between €38,500 and €110,000 for the TaqMan system, and between €31,750 and €63,000 for the LightCycler system.

In conclusion, the TaqMan and the LightCycler systems appear to be suitable for the pharmacogenetic genotyping of *CYP2C9* gene polymorphisms. The TaqMan procedure was less expensive in terms of cost per sample, but the TaqMan apparatus is more expensive than the LightCycler apparatus.

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