linear with respect to a lysate volume of 0–300 μL (r² = 0.997). The influence of the substrate concentration on TPMT activity is shown in Fig. 1. B and C. Kₘₛ were 227 and 4.9 μmol/L for 6-MP and SAM, respectively, and the maximum velocities (Vₘₐₓ) were 28.1 and 24.9 nmol/h per mL of packed cells. These values are similar to those reported previously using a radiochemical assay (10, 15). The TPMT activity determined in a population of Caucasian subjects was 10.4–41.7 nmol/h per mL of packed cells with a mean value of 28.9 nmol/h per mL of packed cells. These preliminary data are in close agreement with the results reported previously in an adult Caucasian European population (9, 13). One subject (2.4%) had TPMT in the intermediate range, 40 (97.6%) subjects had high TPMT activity, and no patient had low or undetectable TPMT activity. Although the number of subjects was small, our preliminary results were similar to the gaussian distribution reported recently in a European population (9, 12). In the method presented, according to Szumlanski et al. (15), the chelation step was omitted to reduce the time of analysis and to simplify the assay. Likewise, allopurinol was not added to the incubation mixture because of the absence of the enzyme xanthine oxidase in the erythrocytes. The simple and rapid sample treatment procedure described allows one to simultaneously stop the enzymatic reaction and obtain a clean extract that can be analyzed directly by HPLC. This procedure avoids the use of acid solutions that may induce potential degradation of thiopurine nucleotides even at ambient temperature (data not shown), and it avoids the time-consuming extraction step.

In conclusion, we believe the thiopurine methyltransferase assay described is rapid and reliable because of the lack of laborious liquid-liquid or solid-phase extraction. We also believe that this method could be implemented easily in the clinical laboratory for the phenotypic analysis of TPMT in patients scheduled for thiopurine therapy and could help optimize and individualize thiopurine treatment.

References

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Detection of Nucleotide c985 A→G Mutation of Medium-Chain Acyl-CoA Dehydrogenase Gene by Real-Time PCR, Luis M. Real,1,2 Antonio J. Gayoso,1 Mercedes Olivera,1 Antonio Caríz,1 Agustín Ruiz,1 and Fidel Gayoso1 (1 Servicio de Bioquímica, Hospital Universitario Virgen de El Rocío, Avda/Mansuelo S/i, 41013 Seville, Spain; 2 Biomedal, CE Pabello de Italia, C/Isaac Newton s/n, 41092 Seville, Spain; * author for correspondence: fax 34-954081279, e-mail liban@inicia.es)

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common hereditary defect of fatty acid oxidation in humans. This deficiency is an autosomal recessive disorder clinically characterized by episodic hypoglycemia, encephalopathy, apnea, and sudden death among children (1). A single A-to-G nucleotide transition at position 985 (nt c985 A→G) of the MCAD gene represents >81% of alleles causing MCAD deficiency (2). The frequency of this allele variant exhibits considerable geographical variation with a high prevalence in Northern Europeans (3).

PCR-based technologies are now widely used for the identification of the nt c985 A→G mutation for the MCAD deficiency (4, 5); however, they involve multiple steps and are time-consuming. We used real-time PCR amplification coupled to fluorescence resonance energy transfer and melting curve analysis (6) to detect nt c985 A→G mutation of the MCAD gene using the single-step LightCycler technology.

In this study, we used genomic DNA isolated from EDTA blood from individuals who had been typed previously by PCR-restriction fragment length polymorphism analysis as was described by Matsubara et al. (7). DNA was isolated using the High Pure PCR Template Preparation reagent set (Roche Diagnostics) according to the manufacturer’s instructions. PCR was performed in a
reaction processing and rapid analysis; it therefore affords both morphism analysis. Obtained previously by restriction fragment length polymorphism analysis at the 3’ end with fluorescent probe 5’-AGTACTGTTATCCATTAT-3’ was labeled at the 3’ end with fluorescent dye; the sensor probe 5’-AGCTAGTTCAACTTTCATTGGCATA-3’ was labeled with LightCycler Red 640 at its 5’ end and modified at its 3’ end by phosphorylation to block extension. As reaction buffer in the PCR, the LightCycler DNA-Master hybridization Probes 10× buffer (Roche Diagnostics) with a final 10 mM MgCl2 concentration of 3.5 mM was used. Cycling conditions were as follows: 95 °C for 1 min and 40 cycles of 95 °C for 0 s, 59 °C for 20 s, and 72 °C for 20 s (ramping rate, 20 °C/s). Fluorescence was monitored at the end of each 20-s annealing phase. After amplification, melting curves were generated by denaturation at 95 °C for 0 s, holding the samples at 50 °C for 20 s, and then heating the sample to 75 °C at 0.2 °C/s, simultaneously monitoring the decline in fluorescence. Melting curves were converted to melting peaks by calculating the negative derivative of the fluorescence with respect to temperature (–dF/dT) against temperature (T).

Typical results for genotyping using this method are shown in Fig. 1. The melting peak of the wild-type sample (curve 1) was at 66.9 °C, whereas the mutant homozygous sample (curve 2) produced a melting peak at 64.5 °C. The heterozygous sample produced two melting peaks at 66.9 and 64.5 °C (curve 3).

The whole process, including DNA extraction, was completed within 70 min. With this method we analyzed 18 individuals from four different MCAD-deficient families and 25 healthy controls (50 chromosomes). The results were consistent with those obtained previously by restriction fragment length polymorphism analysis.

In conclusion, this new method combines simple sample processing and rapid analysis; it therefore affords both high-throughput genotyping and rapid results.

![Fig. 1. Melting peaks for MCAD genotyping.](image)

Curves 1, 2, and 3 represent wild-type, homozygous mutant, and heterozygous samples, respectively. Each analysis included a heterozygous DNA control and a water control, which was negative (data not shown).

References


New Generation Cardiac Troponin I Assay for the Access Immunoassay System, Per Venge,1* Bertil Lindahl,2 and Lars Wallentin2 (Department of Medical Sciences, 1 Clinical Chemistry and 2 Internal Medicine, University of Uppsala, SE-751 85 Uppsala, Sweden; fax 46-186113703, e-mail Per.Venge@clm.uas.lul.se)

The measurement of troponins in blood has rapidly become an alternative to conventional methods of detecting myocardial damage (1–8), particularly in unstable angina, and several studies have indicated the prognostic importance of increased troponins in various clinical settings (9–14). These studies, however, have also pointed out the need for more sensitive methods because patients with even small increases of troponin seem to be at increased risk of cardiac events. Currently, cardiac troponin I (cTnI) can be quantified by assays from several manufacturers (15–19), whereas only one company currently commercializes a cardiac troponin T assay (2, 20, 21). The aim of this work was to evaluate the analytical performance of a new generation of the Access cTnI assay. We also provide data on values in apparently healthy subjects.

Venous blood was drawn from 70 patients admitted to our Coronary Care Unit because of suspicion of an acute coronary syndrome. Only patients found to have increased myocardial markers such as creatine kinase-MB and troponin I were included. The study was approved by the ethics committee of the Medical Faculty of Uppsala University. Serum samples were also obtained from 122 apparently healthy subjects (70 women and 52 men; median age, 41 years; range, 26–73 years) as part of a health-screening program.

The new ACCESS cTnI assay (Beckman Coulter, Inc.,...