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A rapid and simple LC-MS/MS diagnostic test for the exclusion of methylmalonic acidemia

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Introduction

Methylmalonic acidemia is an autosomal, recessive, congenital, metabolic disorder, which causes a disturbance in the catabolism of amino acids such as valine, isoleucine, threonine and methionine, as well as of odd-chain fatty acids and cholesterol (Fig. 1). The disease is ascribed to the so-called organoacidopathies [1] with an incidence of about 1:50,000 [2] and was described for the first time in 1967 [3, 4]. The cause is a genetic mutation, which leads to functional failure of the vitamin B_{12}-dependent enzyme methylmalonyl-CoA mutase. This enzyme is a genuine isomerase which, in the presence of the coenzyme adenosylcobalamin (AdoCbl), catalyses the conversion to succinyl-CoA, thus providing the initial intermediate for the tricarboxylic acid cycle (Krebs cycle) [1, 4]. Furthermore, the disease can also be caused by the lack of supply of AdoCbl from vitamin B_{12} due to a poor synthesis activity. In the cases mentioned, a characteristic accumulation of methylmalonic acid (MMA) occurs, firstly in the blood and subsequently in the urine, the concentration of which can vary greatly, depending on the type of disease [5]. The large increase in MMA leads to intoxication and to life-threatening metabolic imbalances and crises [5–10]. No causal therapy has been described to date, however the MMA concentrations arising in the disease caused by the lack of AdoCbl can be considerably reduced in some cases by the administration of cobalamin in pharmacological doses [11]. A deficiency of the apoenzyme methylmalonyl-CoA mutase can be treated with cobalamin, subsequently reducing the MMA levels in blood and urine. However, this is applicable only for cases with a partial deficiency. For the diagnosis of methylmalonic acidemia, the detection of MMA in urine can be used [1, 12].

Patients/Methods

In this study, a gas chromatographic reference method [13, 14] was compared with a new LC-MS/MS method. Routine metabolic laboratory urine samples from 41 patients, for exclusion diagnostics of methylmalonic acidemia or for monitoring the disease, were analysed by both methods and the results were compared (Ethics Committee Approval Heidelberg 071/2005). The ages of the patient cohort varied between 10 days and 53.3 years and both genders were represented in almost equal numbers.
The sample preparation for the LC-MS/MS method consisted of only the addition of 25 µL of a deuterated internal standard to 200 µL of patient urine, and dilution with a buffer. Of this, 20 µL were injected into the Agilent 6460 LC-MS/MS system and the analysis was carried out in negative ionisation mode (ESI negative) [15].
In order to perform the gas chromatography, the MMA initially derivatised by silylation (trimethylsilyl group) was enriched from the previously acidified urine by liquid-liquid extraction using ethyl acetate. For the analysis, a gas chromatograph (Agilent, GC G1530N) was used, coupled to a mass detector (MSD 5975A). The measurement was performed in SIM mode with deuterated MMA as internal standard. For chromatographic separation of the components to be determined, a DB-5MS capillary column was used (Agilent J&W) [14].

Results

Evaluation of the comparison measurements (GC-MS reference method versus the new LC-MS/MS method) using linear regression analysis showed good agreement, with $r^2 = 0.994$ (Fig. 2). The values measured for MMA in urine varied in the range of 1.4 to 10,000 mmol MMA/mol creatinine, and very high individual values for MMA of up to 14,500 mmol/mol creatinine were measured in some cases. This indicates a considerable formation of MMA as the result of a disorder in the vitamin B_{12}-dependent conversion of L-methylmalonyl-CoA to succinyl-CoA.

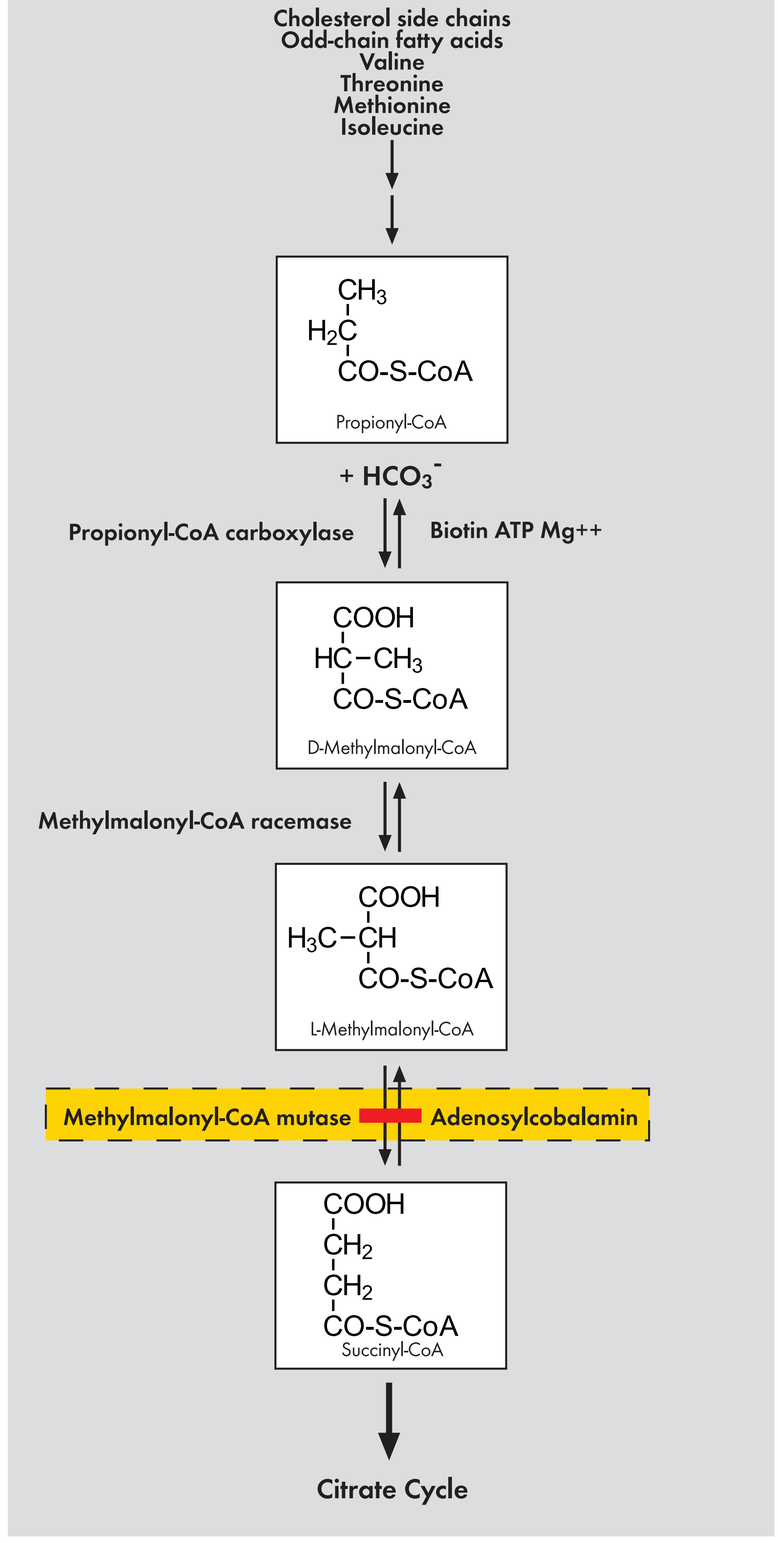

Figure 1:
Overview on the degradation of the amino acids isoleucine, methionine, threonine and valine, of odd-chain fatty acids and of cholesterol side chains. The metabolic step affected by methylmalonic acidemia is highlighted in yellow.

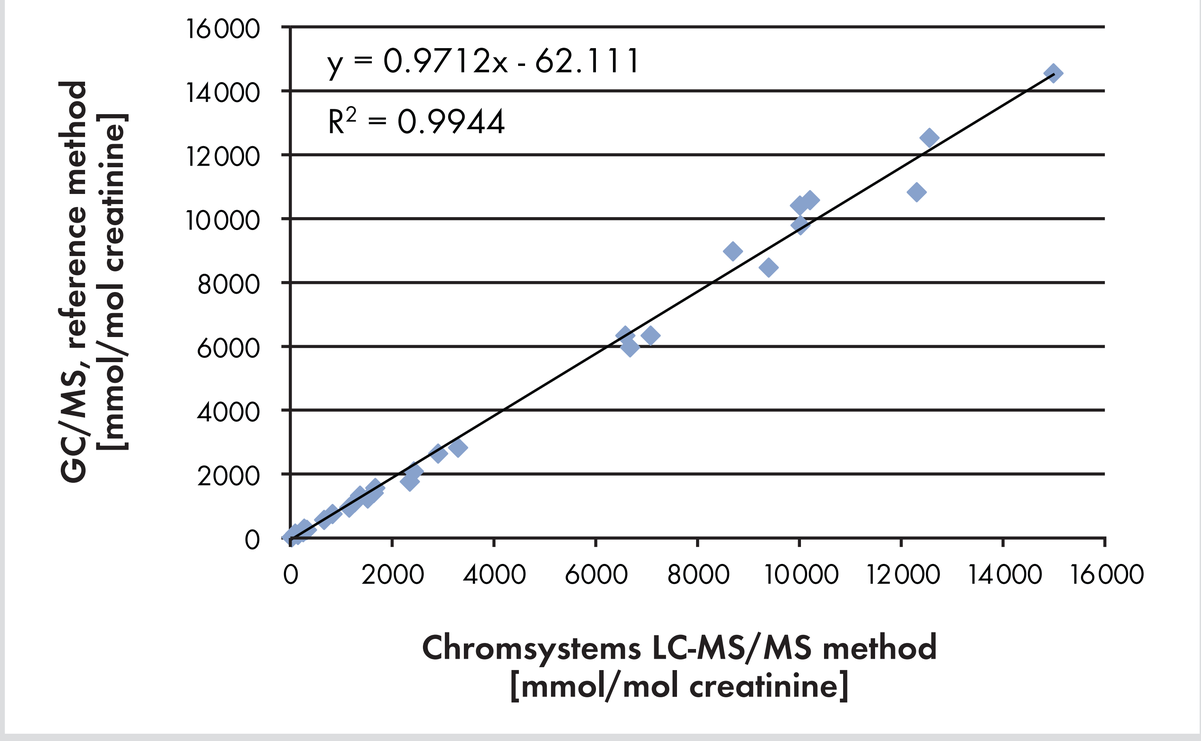

Figure 2:
Comparative correlation analysis of the quantitative determination of methylmalonic acid in patient urine samples. The GC-MS reference method was compared with the Chromsystems LC-MS/MS method.

Conclusion

The LC-MS/MS method presented here for the analysis of MMA in urine is suitable for identifying methylmalonic acidemia and, with regard to quality, is comparable with the GC-MS reference method [13, 14]. Here, the sample preparation consists only of the addition of an internal standard to the urine sample and a dilution step. A similar conclusion has previously been drawn from a larger comparative study in which, in contrast with this study, only urine samples from healthy individuals were used [15].

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