the Ektachem 250 at the Johnson & Johnson laboratory and the other was analyzed with the SMA II in our laboratory. The results are summarized in Table 1.

The correlation coefficient (r) was very near 1 for the five analytes (Na^+, K^+, Ca^{2+}, urea, creatinine), which is consistent with the visually linear relation between results by slide chemistry and flow chemistry. The intercepts were very low except for the urea comparison; however, in relation to the expected values for urea, which are in the range of 200 mmol/L, the intercept is of no clinical significance. Moreover, by paired t-tests there was no significant difference between the two methods.

Differences between the two experiments may reflect differences in the calibrations used in the two laboratories. The differences between the tested methods were less than the published urine analytical goals of 400–700 mmol/24 h for urea and 8–16 mmol/24 h for creatinine *[7]*.

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R325X Mutation in Exon 15 of the Hydroxymethylbilane Synthase Gene Identified in Two Danish Families with Acute Intermittent Porphyria

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Mutations in the hydroxymethylbilane synthase (HMB synthase; porphobilinogen deaminase; EC 4.3.1.8) gene are associated with the disease acute intermittent porphyria (AIP). Although >60 mutations have been characterized (for a review, see *1*), no genetic defect within the HMB synthase gene has previously been described in the Danish population. While establishing a denaturing gradient gel electrophoresis (DGGE)-based mutation screening technique, we observed an abnormal (four bands) DGGE pattern in exon 15 of DNA samples from two apparently unrelated Danish families, each with a history of AIP. From family 1, we investigated a father and his 4-year-old son, neither of whom had ever shown clinical signs of AIP (Fig. 1A). The HMB synthase activity in erythrocytes from the father was below normal (17 nkatal/L; reference values: noncarriers 21–45 nkatal/L, AIP heterozygotes <27 nkatal/L); erythrocytes from the son were not investigated. From family 2, a female proband with clinical signs of AIP but only moderately low erythrocyte

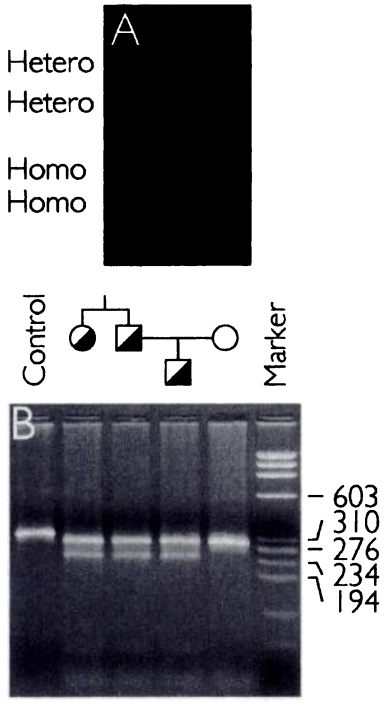

Fig. 1. Results from analysis of exon 15 of the HMB synthase gene in DNA from a Danish family with a history of AIP.

(*A*) Denaturing gradient gel electrophoresis of PCR-amplified DNA from a father with decreased erythrocyte HMB synthase activity, his 4-year-old son, and the healthy mother of the child (lanes receiving the PCR amplificates are as indicated in the pedigree). The four-band pattern typical for heterozygosity for the C→T transversion at 973 is seen in the lanes receiving DNA from the father and his son. *Upper bands* (*hetero*) represent heteroduplexes formed by hybridization between single-stranded DNA representing different alleles, and *lower bands* (*homo*) represent the corresponding homoduplexes. PCR primers used as described by Gu et al. *[2]*; PCR mixes, cycling protocol, and DGGE as described by Nissen et al. *[3]*, except that a 30–70% gradient was used. Gel was run for 5 h at 150 V. (*B*) Exon 15 in DNA from the proband, his diseased sister, his son, and his wife was amplified by PCR as above, digested with *Nla*III, run on a 3% NuSieve® agarose gel (FMC BioProducts, Rockland, ME), and finally stained with ethidium bromide. *Lanes* correspond to the pedigree above. The *control lane* represents undigested PCR product from a healthy volunteer; the *marker lane* received ϕX174/*Hae*III as a molecular mass standard. The 282-bp fragments represent the normal allele and 224-bp fragments represent the mutant allele. A constant 34-bp fragment and an additional 58-bp fragment from the mutant allele are not seen because of their low mass in the gel.

HMB synthase activity (25 nkatal/L) was investigated (data not shown).

To characterize the genetic change underlying the abnormal DGGE pattern, we sequenced DNA samples from each individual. This revealed a C→T transition at position 973 (numbering starting at first translated codon in the sequence of the nonerythropoietic form of the human HMB synthase cDNA *[4]*, which changes the highly conserved arginine codon 325 *[5]* to a stop codon (R325X). The translation product from such an affected allele would be expected to be lacking 37 C-terminal amino acids. The half-time of the transcript from this allele, however, may be too short to allow translation *[6]*. Therefore, the R325X mutation easily would explain the occurrence of AIP within these families.

Two mutations in exon 15 have been characterized by others, 1062insC *[7]* and 1073delA *[8]*, each causing a frame shift.

The 973C→T mutation generates a cleavage site for the restriction endonuclease *Nla*III in the vicinity of a nonpolymor-

phic *Nla*III site in exon 15. This can be utilized in a PCR-based restriction fragment length polymorphism (RFLP) analysis to verify the mutation targeted by the DGGE analysis, or by itself can serve as a simple and fast analysis for this specific mutation. Results from RFLP analysis of family 1 (including the father's sister, who had experienced several AIP attacks) are shown in Fig. 1B.

In conclusion, a new mutation, 973C→T, in the HMB synthase gene was targeted in DNA from Danish AIP probands by means of the DGGE technique and sequencing. The mutation, which might explain the occurrence of AIP within these families, is easily screened for by use of *Nla*III in a RFLP analysis.

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Myoglobin/Carbonic Anhydrase III Ratio: Highly Specific and Sensitive Early Indicator for Myocardial Damage in Acute Myocardial Infarction,

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Measurements of cardiac markers in serum play an important role in the diagnosis of acute myocardial infarction (AMI), but most [e.g., creatine kinase (CK), CK-MB isoenzyme, and lactate dehydrogenase] increase only at late times after AMI. Myoglobin increases rapidly in blood after skeletal or cardiac muscle damage, and serum myoglobin has been used for evaluation of skeletal muscle damage, for early detection and monitoring of myocardial infarction, and for detecting coronary reperfusion or reinfarction *[1]*. Cumulative myoglobin concentrations in serum have been proposed for infarct sizing *[2]*. Carbonic anhydrase III (CA III), another soluble protein, has been shown to exist in high amounts only in skeletal muscle and not in the myocardium *[3, 4]*. Our previous results suggest that the simultaneous measurement of serum myoglobin and CA III can be used to differentiate between myocardial and skeletal muscle damage *[5]*. However, because of the possibility that myoglobin/CA III ratio does exclude concomitant massive skeletal muscle and myocardial injury, we undertook the present study. Our aims were to evaluate serum myoglobin/CA III ratio as a biochemical marker in the early diagnosis of AMI, and to compare this ratio with other serum markers commonly used in the diagnosis of myocardial infarction.

We studied 267 patients (118 women and 149 men) consecutively admitted to the University Hospital of Oulu during a 3-month period with a history of symptoms characteristic for AMI. AMI was diagnosed by the patients' physicians in 37 patients—17 women (median age 74 years, range 57 to 88) and 20 men (median age 62 years, range 48 to 79)—according to WHO criteria without knowledge of the serum myoglobin or CA III results. On admission, S-T segment elevation and (or) Q-waves were present in 17 cases, ischemia in 13 cases, and a left bundle branch block in 3. The electrocardiogram (ECG) was unchanged from an earlier one in three cases and was normal in one case. Only those patients were included for whom the time of onset of chest pain was established with certainty and the time between this onset of chest pain and admission was <24 h. The interval between onset of symptoms and hospital admission was 8 ± 6 h (mean ± SD). The remaining 230 patients were diagnosed as having "unstable" angina pectoris (20), stable angina pectoris (102), or noncardiac chest pain (108).

Blood samples were taken at the time of admission to hospital. Serum CK and CK-MB activities were determined in the routine laboratory. For the immunological determination of CK-MB mass concentration, myoglobin, and CA III, serum samples were frozen at −20 °C until analysis. The study protocol was approved by the ethical committee of the University Hospital of Oulu.

CK activity was measured *[6]* at 37 °C with a Monarch 2000 analyzer (Instrumentation Laboratory, Lexington, MA) and commercial reagents (CK NAC-activated; Boehringer Mannheim, Mannheim, Germany). The upper reference limit for CK was 170 U/L for women and 270 U/L for men. The proportion of CK-MB isoenzyme was determined with a commercial agarose gel electrophoresis kit (REP CK Isoenzyme kit; Helena Labs., Gateshead, UK), the upper reference limit being 15 U/L. CK-MB activities were used only for the final diagnosis of AMI. We measured the mass concentration of CK-MB with the microparticle enzyme immunoassay (MEIA) *[7]* from Abbott (STAT CK-MB; Abbott Labs., Abbott Park, IL), for which the detection limit is 0.7 μg/L and the intra- and interassay imprecisions are 5.1% and 5.0%, respectively. The reference range for the mass concentration of CK-MB was validated by using the results for the serum samples from the patients diagnosed as not having AMI. In this group, 95% of the results were <5.0 μg/L.

The myoglobin/CA III assay, involving polyclonal antibodies and the fluorescent lanthanides europium (Eu) and samarium (Sm), is a solid-phase immunoassay based on competition between Eu- and Sm-labeled antigen and sample antigen for polyclonal rabbit antibodies *[8]*. The upper reference limit for