identification of MEN 2A carriers and can reduce morbidity and mortality through early intervention (6).

The methods used to detect RET mutations are time-consuming and require optimization of the PCR to avoid nonspecific PCR products that may interfere with the result. We used real-time PCR and melting curves on a LightCycler (Roche Molecular Biochemicals) to analyze the two most frequent RET mutations, C634Y and C634R, and the less frequent C634S (TGC to TCC), C634Y, and C634R, and the less frequent C634S (TGC to TCC).

All subjects had been genotyped previously by sequencing of exon 11 of the RET proto-oncogene. DNA was isolated from peripheral blood leukocytes by standard methods. The primers used for the amplification were RET 11 Forward (5'-CCCTCT-GCGGTCAGCAAGCT-3') and RET 11 Reverse (5'-CCTGACCAGGAAG- GTGGG-3'), which gave a 217-bp PCR product. The wild-type probe stretched from codon 624 to codon 637. The sequence of the sensor 3' fluorescein-labeled probe was 5'-ACGCAGCGAGCATG-3', and the sequence of the anchor probe, labeled 5' with LC-Red 640, was 5'-TCGACAGTGGATCTGTGGTG- G-3'.

PCR reactions were performed in a total volume of 20 µL in the LightCycler glass capillaries. The reaction mixture contained 9.6 µL of distilled water, 2.5 µL of MgCl₂ (25 mM), 1 µL of each primer (10 µM), 1 µL of each probe (4 µM), 2 µL of DNA-Master Hybridization Probes (Roche Molecular Biochemicals), and 2 µL of genomic DNA (100–500 ng). We used the master mix for all samples (to reduce sample-to-sample differences) and a control without DNA. PCR conditions were as follows: initial denaturation at 94 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 0 s, annealing at 68 °C for 5 s, and extension at 72 °C for 10 s. After amplification, the melting analysis was performed by denaturation at 94 °C for 0 s, annealing at 50 °C for 0 s, and increasing the temperature to 90 °C with a ramp rate of 0.5 °C/s. The fluorescence emitted was measured during this process, and the melting curves (F/T) were automatically converted to melting peaks (∆F/∆T).

The wild-type melting curve showed a single peak at 69.5 °C (Fig. 1), whereas each of the mutation carriers showed two different peaks, one for the wild-type allele at 69.5 °C and a lower one for the mutated allele. The T-to-C transition of the C634R mutation produced a melting peak at 61.5 °C, the G-to-A transition of the C634Y mutation produced a melting peak at 60 °C, and the G-to-C transversion of the C634S mutation produced a melting peak at 58 °C. The assay variation of the melting temperatures was assessed. The interassay variation (CV) was <1.5% and the intraassay variation was <0.5% for the wild-type and the mutant alleles studied.

This method has several advantages over other methods used. The possibility of contamination is reduced because no post-PCR handling is necessary. Nonspecific PCR products will not affect the result because they will not be recognized by the probes. Finally, this method is very rapid and reduces labor and reagent costs. We think it is useful in the screening of RET for the most common mutation in MEN 2A and especially to establish the carrier status in members of families with MEN 2A and FMTC already characterized as having the 634 mutation. All family members can be analyzed simultaneously and in a very short time.

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References


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S100B Protein Concentrations in Urine Are Correlated with Gestational Age in Healthy Preterm and Term Newborns

To the Editor:

S100B is an acidic calcium-binding protein concentrated in the nervous system, where it is located mainly in glial cells (1). Measurements of the protein in cerebrospinal fluid and blood are used to detect brain distress, both in infants and in adults (2, 3), and in the perinatal period are correlated with brain maturation (4). Because collecting urine is a simpler procedure than collecting cerebrospinal fluid or blood, especially in infants in whom anemia of premature infants attributable to blood sampling is common, this study investi-
gated whether S100B can be detected in urine of preterm and term infants.

Eighty-three women with normal physiological pregnancies (23 preterm, 60 term) whose deliveries occurred between 26–42 weeks of gestation were enrolled in the study. Gestational age was defined by the presence of ultrasonographic signs according to Campbell and Thoms (5) and by postnatal confirmation. Exclusion criteria were multiple pregnancies, intrauterine growth retardation, and third trimester maternal diseases. The study protocol was approved by the local Ethics Committee after consent was given by parents.

After birth, the first urination was collected, and S100B concentrations were measured in duplicate in all samples using a Lia-mat Sangtec 100 (AB Sangtec Medical, Bromma, Sweden) according to the manufacturer’s recommendations. Cord blood samples were collected at the same time and measured in 42 patients selected without conscious bias. The detection limit of the assay was 0.02 μg/L. The S100B concentrations in the groups are expressed as the mean ± SE. Statistical analysis was performed by Kolmogorov–Smirnov one-way ANOVA and Mann–Whitney U-test when data were not normally distributed. The relationship between the urine concentration of S100B and weeks of gestation was analyzed by linear regression analysis. P <0.05 was considered significant.

Normal clinical conditions and an absence of overt neurological injury were observed on discharge of all infants from the hospital. In the preterm group, gestational age at birth and birth weight were significantly lower than in the term group (P <0.01), whereas no differences were observed regarding delivery mode and Apgar score at the 1st and 5th min (P >0.05, not significant). Tests of renal function were within reference intervals and did not differ between the two groups (P >0.05, not significant). Urine concentrations of S100B were significantly higher in the preterm group, peaking in the earliest weeks of gestation and progressively decreasing near term, being undetectable or at the detection limit of the assay in the term group (3.17 ± 1.02 vs 0.70 ± 0.23 μg/L; P <0.001). The same pattern was found when S100B cord blood concentrations were measured in the same selected patients (data not shown). When urine S100B concentrations in preterm newborns were subgrouped according to gestational age and weight at birth (6), they were significantly higher in the very low birth weight group compared with both the low birth weight and term groups (P <0.001) and significantly higher in the low birth weight group than in the term group (P <0.001). A significant correlation between S100B urine concentrations and gestational age was observed when all newborns were considered (r = −0.79; P <0.001).

The data presented constitute the first observation of S100B in urine and offer a urinary S100B concentration reference curve (available from the authors) at different gestational ages. These findings also fit previous observations on cord blood S100B concentrations (4). Because renal function tests were normal in all examined infants, it did not appear to influence the results. The higher concentrations of S100B in preterm newborns may be related to the neurotrophic role possibly exerted by S100B (1). This possibility seems to be consistent with high concentrations of the trophic factor at earlier gestational ages, when brain maturation processes are more active, and lower concentrations at later stage of brain maturation.

In conclusion, the present S100B urinary pattern could provide the basis for a new and easier means of studying brain pathophysiological conditions, which have hitherto been investigated by measuring the protein in cerebrospinal fluid or blood.

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Is It Necessary to Order Aspartate Aminotransferase with Alanine Aminotransferase in Clinical Practice?

To the Editor:

We read with great interest the articles by Dufour et al., “Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests” (1) and “Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory