hybridization. We here describe a simpler alternative for the identification of haplotype 2 mutant alleles. DNA sequence data on the PAH gene indicate that the restriction enzyme MnlI cuts twice in exon 12. However, the more upstream of the two recognition sites includes the C → T transition in exon 12 that causes the amino acid substitution at position 408. Thus, this MnlI recognition site is lost in mutated DNA.

To test the usefulness of this approach, we have analysed PKU families with respect to this mutation by DNA amplification of exon 12 of the PAH gene, using PCR and direct analysis by MnlI without hybridization. Genomic DNA was isolated from blood samples from Norwegian PKU family members and control persons, and 1 µg of DNA was used for each PCR reaction. To amplify a specific 245-bp DNA region containing exon 12 and flanking regions, we used oligonucleotides A (5'-ATGCCACTGAGAACCTCTCTT-3') and B (5'-AGTCTTCATTACTGAGAAA-3') as primers (3). The PCR reaction was carried out for 25 cycles, each consisting of 1 min at 95 °C for denaturation, 2 min at 55 °C for annealing, and 3 min at 72 °C for polymerization (GeneAmp; Perkin-Elmer Cetus, Norwalk, CT). The amplification product was then precipitated in an equimolar mixture of isopropanol and 1 mol/L ammonium acetate, resuspended, and digested with 4 units of the restriction enzyme MnlI (NEB, Beverly, MA) for 2 h. Samples were analyzed by gel electrophoresis in 3% NuSieve GTG agarose (FMC BioProducts, Rockland, ME).

As predicted from the PAH cDNA sequence, two MnlI restriction sites were consistently found in the PCR products of DNA from randomly selected individuals. We then applied the method to five Norwegian PKU patients and their immediate family members. RFLP analyses from a population-based study of PKU in Norway (Apold et al., manuscript in preparation) had already shown that these PKU patients possessed at least one haplotype 2 allele. Using the PCR/MnlI approach, we found that all patient were heterozygous for the haplotype 2 mutation (see, e.g., Figure 1), in agreement with the previously performed haplotype analyses.

We conclude that this PCR-based approach can accurately identify the haplotype 2 PKU mutation in a few hours without the use of radioactively labeled probes.

References


**Falsey Normal Values of Intact Parathyroid in Serum of Patients with Mild Primary Hyperparathyroidism? S. Minisola, M.T. Pacitti, E. Romagnoli, L. Scarneccia, V. Carnevale, A. Scarpiello, and G. Mazzuoli (Servizio Aggregato "Malattie del Ricambio Minerale," Università degli Studi di Roma "La Sapienza," Viale del Policlinico 155, 00161 Rome, Italy)

We performed the following study to evaluate whether hypothesized (1) pulsatile secretion of parathyroid (PTH, parathyroid hormone) might account for the occasional finding of normal values for PTH in serum from patients with mild primary hyperparathyroidism.

We studied four patients with primary hyperparathyroidism (three postmenopausal women and one man), who had previously showed persistently increased concentrations of ionized calcium while their concentrations of intact PTH in serum were fluctuating around the upper limit of normal established for our laboratory. In one of the women, clinical diagnosis was subsequently confirmed by the surgical removal of a parathyroid adenoma. (The indication for surgery was the appearance of a radio-opaque kidney stone.) We compared results obtained for these four patients who had mild hyperparathyroidism with those for a 65-year-old woman with a highly increased concentration of intact PTH.

Five whole-blood samples were collected without an indwelling venous catheter from each subject at 30-min intervals, from 0800 to 1000 hours; the patients had fasted at least 12 h. To minimize activity, the subjects were restricted to the study site.

Blood was collected anaerobically into red-stoppered Vacutainer Tubes and immediately analyzed for ionized calcium with a Nova 8 ion-selective electrode (Nova Biochemical, Waltham, MA). Both the intact and midmolecule (MM) PTH concentrations were quantified in the same sample. Intact PTH concentrations in serum were measured with a two-site immunoradiometric assay (IRMIA) as a commercial kit (Incstar Corp., Stillwater, MN). The detec-
ion limit of the method (defined as the apparent concentration at 2 SD from the counts at minimum binding) is about 1.2 ng/L. Intra- and interassay coefficients of variation were respectively <3.0% and 5.5%. Our reference range for PTH, as determined in 30 normal subjects, is 10.6–54.0 ng/L (mean ± SD = 26.7 ± 12.7 ng/L), which compares well with that stated by the manufacturer. IRMA values for PTH in serum were <10.6 ng/L in seven patients with surgical or idiopathic hyperparathyroidism and <12.2 ng/L in six patients with hypercalcemia of cancer. For the MM assay, which determines both the bioactive intact hormone molecule and its biologically inactive metabolites containing the 44–68 amino acid sequence, we used a radioimmunoassay (2).

Figure 1 illustrates the changes in ionized calcium, MM PTH, and intact PTH during the 2-h sampling period. The concentrations of intact PTH in patients with mild hyperparathyroidism oscillated throughout the observation period, and were considered to be within or above the normal range, depending on the time of blood sampling. For results by this IRMA, the intra-individual variation in the four patients during the 2-h examination period (13.9%, 10.5%, 13.9%, and 9.5%) was much greater than the assay variability. The fifth patient, with extremely high PTH values, showed similar fluctuations in intact PTH measurements.

In the contrary, the MM PTH measurements showed a different pattern: except for one patient (□, Figure 1), intra-individual variations were always less than the intra-assay variability of the method. Ionized calcium values remained increased throughout the observation period.

In our opinion these fluctuations in intact PTH measurement are compatible with a pulsatile secretion, which is also typical of other peptide hormones. On the other hand, the occurrence of micropulses and larger secretory bursts of macropulses has been already demonstrated, at least in normal subjects (3).

In the presence of increased concentrations of ionized calcium in serum, unsuppressed concentrations of PTH clearly suggest that parathyroid gland overactivity is responsible for the hypercalcemia. However, we suggest that, as with other hormones secreted in a pulsatile fashion such as pituitary gonadotropins), more than one blood sampling may be necessary to demonstrate above-normal concentrations of intact PTH in patients with mild hyperparathyroidism.

References
1. Hesch RD, Brabant G, Rittinghaus EF, Atkinson MJ, Harms H. Pulsatile secretion of parathyroid hormone and its action on a type

![Figure 1](image-url) Changes in serum concentrations of intact and midmolecule PTH and of ionized calcium at 30-min intervals in four patients with mild hyperparathyroidism and in one patient (A) with definite hyperparathyroidism. The shaded areas indicate the normal ranges in our laboratory.