A study by Kotze et al. (5) has indicated that CpG dinucleotides may be mutational hotspots among Asian Indians settled in South Africa. However, our study indicates mutational heterogeneity among Indian subgroups, which is not surprising considering the different ethnic groups present in the heterogeneous Indian population.

In addition to the two previously unpublished insertion mutations that we detected, the usefulness of this technique to detect minor changes was tested using mutation-positive control samples for exon 3 (R57R), exon 4 (E207K and D200Y), exon 9 (E387K), and exon 14 (P664L) of the LDLR gene (kindly provided by Dr. M.J. Kotze (Department of Human Genetics, Faculty of Medicine, University of Stellenbosch, Tygerberg, South Africa), Dr. A Soutar (MRC Lipoprotein Team, Clinical Science Centre, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom), and Dr. A Minnich (Clinical Research Institute of Montreal, Montreal, Quebec, Canada)). It remains to be seen whether all possible base pair changes could be detected by the method described, but of the five known point mutations that we have tested (relatively large PCR fragments), four (fragments up to 496-bp) were easily detectable using this technique, whereas one (550-bp fragment in exon 9) was not.

The use of low cross-linked polyacrylamide gels supplemented with urea (9, 17) previously has been demonstrated to be highly sensitive in detecting single-base substitutions as HDXs. Furthermore, we also observed that the mixing of control and mutant DNA samples and an extra denaturing/renaturing step increases the chances of HDX formation between the nonhomologous DNA strands during cross-annealing, making them more apparent and easily visualized with reduced electrophoresis time. The mixing of control and mutant DNA can also facilitate formation of HDXs in homozygous mutant alleles where HDXs do not arise during amplification cycles.

In conclusion, this simplified nonradioactive PCR-HDX analysis gives reliable results with simple procedures within a short time. The method requires only small amounts of PCR-amplified DNA, is easy to handle because radioactivity is not involved, and readily resolves differences between mutant and control DNA. Accordingly, we expect that it could be the preferred initial method of screening and could complement other techniques for detecting minor gene mutations.

Does Iron Concentration in a Liver Needle Biopsy Accurately Reflect Hepatic Iron Burden in β-Thalassemia? Guido Crisponi,1 Rossano Ambu,1 Franco Cristiani,1 Gabriella Mancuso,1 Valeria Marina Nurchi,1 Rosalba Pinna,1 and Gavino Fao2 (1 Dipartimento di Chimica Inorganica ed Analitica, Università di Cagliari, Complesso Universitario di Monserrato, 09042 Monserrato-Cagliari, Italy; 2 Dipartimento di Citomorfologia, Divisione di Anatomia Patologica, Università di Cagliari, Via Ospedale 60, 09124 Cagliari, Italy; * author for correspondence: fax 39-07067547, e-mail crisponi@unicait.it)

β-Thalassemia major is an autosomal recessive disease characterized by absent or decreased synthesis of the β-globin gene (1). Thalassemic children, estimated at 100,000 worldwide, are affected by chronic anemia and need regular blood transfusion (2). Because of the limited capacity of iron excretion in humans, the iron in transfused red cells accumulates in the body. The liver, heart, and pancreas are the target organs of iron-induced injury; therefore, the major pathological manifestations observed in β-thalassemia major are chronic liver disease, evolving to cirrhosis, and dilative cardiomyopathy, both characterized by severe iron deposition (3, 4). The dangerous effects of iron excess can be managed by administration of chelators capable of removing iron from transferrin, fer-
ritin, and other iron stores (5–7). Determination of the hepatic iron concentration (HIC) is one of the most valid procedures in assessing real body iron burden (8–10), which is important for adjusting each patient’s chelation therapy over the years. HIC usually is measured on one part of a needle biopsy core, and the measured value is considered representative of the iron concentration in the whole liver (11). In 1995, a study by our group (3) first showed that iron is unevenly distributed in the livers of β-thalassemic patients, and therefore, the iron content determined in a small liver fragment should be interpreted with caution because it cannot be considered a true representation of the mean HIC. These data were confirmed by us (12–14) and by other authors (15), who reported differences in HIC measurements on liver biopsy specimens.

Recently, a striking difference in HIC was found in biopsy samples from cirrhotic livers (range, 60–285 μg/g dry weight) (16). It therefore seemed of interest to measure iron concentrations in a large number of needle biopsies with the following objectives: (a) to establish whether the determination of iron concentration in a needle biopsy is representative of the mean iron content of the whole liver; (b) to determine whether HIC measured in only a portion of the needle biopsy is indeed representative of the mean liver iron content; and (c) to determine the minimum weight of liver parenchyma needed to obtain a HIC value that would be useful to evaluate the body iron burden. To this end we performed 54 needle biopsies from an autopsy liver of a thalassemic patient and evaluated the effect of each factor by use of analysis of variance (17).

The clinical data of the 29-year-old male affected by homozygous β-0-thalassemia major were reported previously (3).

At autopsy, the right lobe of the liver was divided into 18 areas with diameters of ~1 cm (sampling sites), and three needle biopsies were performed in each area. The three biopsies were subdivided into two, three, and four parts (subsamples), respectively: the first subsample, marked with index 1, corresponded to the subcapsular part, whereas the indices for the other parts increased with depth. Fig. 1A shows the sampling map for these 162 [18 × (2 + 3 + 4)] subsamples.

The digestion procedure to obtain a solution suitable for inductively coupled plasma atomic emission spectroscopy (ICP-AES), the working calibration, and the ICP-AES conditions have been described previously (18).

All samples were weighed on a balance accurate to the sixth digit. It should be highlighted that weighing is fundamental to the precision of the entire procedure, e.g., a four-digit balance could lead to a 10% error when samples of ~1 mg are weighed. The sequence in analyzing the 162 samples was completely randomized to avoid any possible systematic error related to an ordered sequence. The same type of operation on all samples (e.g., weighing, diluting to the mark, spectral recording) was carried out by the same operator to avoid operator-generated errors. All procedures were tested by analyzing NIST bovine liver samples. During spectrophotometric analyses, one calibration out of five solutions was measured to check the reliability of the measurements.

HNO₃ and the ICP calibrator for iron (10 g/L) were from Aldrich; Triton X was from Merck. NIST Standard Reference Material 1577b, Bovine Liver, was used to validate the measurements.

All data are represented as three 18 × p matrices, where 18 is the number of sampling sites, and p = 2, 3, or 4, the number of subsamples into which each needle biopsy was subdivided. The matrix elements xᵢⱼ represent iron concentrations at sampling sites i (1–18) and depths j (1 to p, where 1 is the index of the superficial sample and p is the index of the deepest sample). For each matrix, the total mean value over the 18 × p data points is defined as:

\[
\bar{x} = \frac{\sum_{j=1}^{p} \sum_{i=1}^{18} x_{ij}}{18 \times p}
\]

For each matrix, p mean values (\(\bar{x}_j\)) could be defined among samples at the same depth in different sampling...
sites, and 18 mean values ($\bar{x}_i$) could be defined over the $p$ subsamples in each needle biopsy:

$$\bar{x}_i = \frac{\sum_{j=1}^{p} x_{ij}}{p}$$

The total sum of squares, $SS_T = \sum_{i=1}^{p} \sum_{j=1}^{18} (x_{ij} - \bar{x})^2$, with $18 \cdot p - 1$ degrees of freedom, can be divided into three contributions:

$$SS_S = p \cdot \sum_{j=1}^{18} (\bar{x}_i - \bar{x})^2$$

and measures the part of the total sum attributable to the differences among sampling sites;

$$SS_D = 18 \cdot \sum_{i=1}^{p} (\bar{x}_i - \bar{x})^2$$

and measures the variation attributable to the depth of sampling;

$$SS_R = \sum_{i=1}^{18} (x_{ij} - \bar{x}_i - \bar{x} + \bar{x})^2$$

has $(p - 1) \times 17$ degrees of freedom, and is the part that cannot be explained by a given factor of variation and can be considered an estimate of experimental error.

Analysis of variance can be performed, using the $F$-test (17), by comparing the variances attributable to the various factors with the variance attributable to the experimental error.

The results presented in Table 1 show some characteristics that need some comment:

- The global mean value (20 954) on all 162 data almost coincided with the total mean values over the 2 × 18 (20 878), the 3 × 18 (21 012), and the 4 × 18 (21 018) data for the needle biopsies divided into two, three, and four parts, respectively.

- The mean values of data at the same depth showed a steady decrease in iron concentrations from the subcapsular region to the inner part of liver parenchyma (Fig. 1B).

- The global mean value coincided with the mean calculated on 51 samples of the same liver (~1 g dry weight) presented in a previous work (3).

- The relative standard deviation associated with experimental error (~10%) was approximately twice the value we found when testing the precision and accuracy of the procedure on much heavier samples (18). We measured the concentrations of various elements other than iron on sets of seven samples of NIST bovine liver, each set characterized by a definite weight. For samples weighing 60–250 mg, the standard deviations did not vary significantly, whereas they increased dramatically for samples weighing 4–60 mg. This behavior, observed for all of the elements, cannot be ascribed to a loss of instrumental precision (ICP instrumental percentage...
We thank the Assessorato alla Sanità della Regione Autonoma della Sardegna for financial support.

References