To the Editor:

In her recent letter to BLOOD, Dr Lynas reports the development of a cheaper and more rapid polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) technique for detection of mutations in HFE, the candidate gene for hemochromatosis. The PCR-RFLP method discussed by Dr Lynas uses Rsa I and Bcl I to cleave products amplified using primers designed by Feder et al. However, many authors, including Jouanolle et al. in November 1996, Roberts et al. in February 1997, Martinez et al. in the March 1997 issue of this journal, and Carella et al. and Merryweather-Clarke et al. in April 1997, have already published PCR-RFLP methods incorporating the use of Rsa I for detection of the C282Y mutation, and Roberts et al. and Martinez et al. have already published the application of Bcl I for detection of the H63D mutation. Dr Lynas considers this technique to be superior to other RFLP-PCR methods because these two enzymes are cheaper than SnaBI (used by Jazwinska et al. for C282Y RFLP-PCR) and Mbo I (used by Jouanolle et al., Carella et al., Merryweather-Clarke et al., and Jazwinska et al. for H63D RFLP-PCR). In reply, we point out that our reasons for using the more frequent 4-bp cutter Mbo I instead of the 6-bp cutter Bcl I are particularly pertinent for the diagnostic applications referred to by Dr Lynas and afforded greater accuracy in a worldwide study of the HFE H63D/C282Y genotype of 5956 chromosomes. We redesigned one of the primers used to amplify the DNA fragment encoding the H63D mutation (H63DF primer, 5’ACA TGG TTA AGG CCT GTT GC; H63DR primer, 5’CTT GCT GTG GTG ATT TTC GC) to produce a 294-bp fragment instead of the 208-bp fragment amplified by Feder et al. The extra 86 bp incorporated in this fragment contain an additional Mbo I recognition site, which provides an internal control for restriction digestion. Following agarose gel electrophoresis, it is therefore possible to distinguish between a partially or nondigested product of 294 bp, which may be of either genotype, and a digested product containing the H63D mutation, which yields fragments of 57 bp and 237 bp on Mbo I digestion. (The wild-type PCR product yields Mbo I digestion products of 138 bp, 99 bp, and 57 bp.) This internal control against misdiagnosis due to partial or nondigestion is not possible in the method discussed by Dr Lynas, as the fragment amplified from an H63D allele remains uncleaved and is thus indistinguishable from a nondigested fragment from either allele.

Dr Lynas places considerable emphasis on cost. We suggest that instead of using the QIAamp kit for extracting DNA from blood (Qiagen, Crawley, UK; £1362 for 1,000 extractions from 200 mL of blood), the more economical Nucleon Kit (Amersham Little Chalfont, UK; £95 for 2,500 extractions from 200 mL of blood) may be more economical. The Nucleon Kit (Amersham Little Chalfont, UK; £95 for 2,500 extractions from 200 mL of blood) may be more economical. We have found the Nucleon Kit perfectly adequate for producing DNA for PCR, a procedure taking only 30 minutes. Another rapid method of DNA extraction enables one to process 20 samples in 1 h at a total consumables cost of 5 pence per sample. We have also found that the H63D PCR works using whole blood as a template if it is first subjected to 3 cycles of 95°C for 5 minutes followed by 30°C for 5 seconds, as suggested by Rees et al. Another cheap and rapid method of C282Y typing is mutagenically separated (MS)-PCR. This allele-specific technique is ideally suited to routine analysis of blood samples in a diagnostic laboratory, as whole blood may be used as the template in a single PCR reaction per sample and restriction enzymes are not required. Other methods more suited to automation include heteroduplex analysis and PCR-SSP.

The availability of many different methods for the diagnosis of HFE mutations C282Y and H63D means that each will have its own particular advantage in different situations and that diagnostic labs can confirm diagnoses by the simultaneous use of independent methods. However, while we congratulate Dr Lynas on the adaptation of PCR-RFLP for use in her laboratory and applaud her focus toward the diagnosis of hemochromatosis patients, we contest her claim to have developed a new method.

Finally, there has been a plethora of nonpeer-reviewed material on the subject of HFE, and we welcome the interest this stimulates in the field of hemochromatosis. However, we encourage editors and authors alike to take more care over both the nomenclature used (the gene is correctly called HFE, not HLA-H) and the emphasis given to nonpeer-reviewed literature over peer-reviewed publications. Perhaps this would help to avoid the confusion and duplication of methods observed in this case.

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Fc-εRII/CD23 Receptor on Circulating Human Eosinophils

To the Editor:

The expression of receptors for IgE on human eosinophils remains controversial, as reviewed in a recent comprehensive editorial.1 Although human eosinophils from subjects infected by Schistosoma mansoni were recently reported to express different types of receptors for IgE (high-affinity Fc-ε receptor [Fc-εRI]),2 low-affinity Fc-ε receptor [Fc-εRII],3 and Mac-2a/βP,4 murine eosinophils purified from hepatic granulomas of mice affected by S. mansoni parasitosis were shown not to express IgE receptors either by flow cytometry or by reverse transcriptase polymerase chain reaction analyses.5 In this respect, further studies were encouraged by Kita and Gleich1 to solve the existing controversies concerning the expression of Fc-εRII in human hypereosinophilic diseases.

We investigated the expression of CD23 antigen on peripheral blood eosinophils from 14 patients affected by vernal keratoconjunctivitis (VKC) with mild-to-moderate eosinophilia and from 10 matched normal controls. All patients showed skin reactivity to grass (n = 5), Dermatophagoides pteronyssimus (n = 5), Parotaria officinalis (n = 2), or multiple allergens (n = 2). In brief, 150 µL of unfractionated peripheral blood were incubated for 30 minutes at 4°C with the following fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAbs): CD23 (EBVCS-5 clone, derived from BALB/c mice immunized with in vitro–transformed EBV cell line; IgG1); CD16 (NKP15 clone; IgG1); Becton Dickinson, Mountain View, CA), and CD9 (MM2/57 clone; IgG1).6 After sequential fixation and permeabilization of unfractionated peripheral blood allows the identification and electronic gating of eosinophils and the

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Hemochromatosis-Related Mutation Detection

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