in the evening. Two patients (with AIHA and AA) used commercially available grapefruit juice and the others (with ITP and AIHA + CLL) a juice obtained by a commercially available self-squeezed grapefruit.

The CsA blood concentration was monitored every 2 weeks by Sandimmum-kit radioimmunoassay as well as blood pressure, glucose, serum creatinine, serum glutamic oxaloacetic, and pyruvic transaminase and lactate dehydrogenase levels. In all patients treated with CsA plus grapefruit juice, after 2 weeks of treatment we noted a progressive increase of CsA blood concentration and an increase of hemoglobin (Hb) and platelet levels; we also noted an increase in the white blood cell levels in the AA patient.

This allowed a reduction of CsA doses, especially in ITP patient (Fig 1). For all patients, the clinical outcome progressively improved (Fig 1). Neither nephrotoxicity nor other side effects were observed. The ITP patient, with drug requiring, CsA-dependent hypertension was also able to withdraw the antihypertensive drugs. In the control patients treated with CsA with water, the hematological remission was maintained over time with full CsA doses and without any possibility of CsA dose reduction.

Our data suggest that coadministration of CsA with grapefruit juice in drug-dependent hematological patients could increase CsA blood concentration and that the effect is sustained over time. This interaction, if unmonitored, is potentially dangerous, increasing toxicity of CsA. On the other hand it allows the reduction of daily required dose of drug, improving the clinical outcome on the whole with stable results and without side effects, at least in patients with hematological disorders. Moreover, the reduction of drug dosage can cut its side effects, as in one of our patients, as well as the cost of treatment.

We think that it would seem reasonable to warn the hematologists, the patients under long-term CsA treatment, and the pharmacists of this food-drug interaction.

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## An Erythroid-Specific Exon Is Present in the Human Hexokinase Gene

*To the Editor:*

Human hexokinase type I (EC 2.7.1.1) is the predominant glucose-phosphorylating enzyme in red blood cells. By a number of methods[1] it has been proved, and is now widely accepted, that this enzyme is largely heterogeneous and present in multiple molecular forms. Hexokinase subtypes have similar kinetic properties but a different age-dependent decay and a different intracellular distribution in reticulocytes.

It is presently unknown if the multiple hexokinase subtypes reflect posttranslational modifications or different gene products. We previously showed that, at least in human placenta, the heterogeneity of hexokinase type I is caused by the presence of truncate forms arising postsynthetically.[2]

In the February issue of BLOOD, Murakami and Piomelli[3] reported evidence for a red cell–specific hexokinase cDNA containing a unique sequence of 60 nucleotides at the beginning of the coding region. We have now identified this red cell–specific hexokinase sequence in the human genome and found that it is located 3.1 kb upstream from the somatic exon 1 (GenBank accession number AF016350). Determination of the splice-junction by direct sequencing confirmed the hypothesis that a true hexokinase isozyme may exist in humans, likely as a product of an alternative splicing event. However, human erythrocytes show a multiplicity of forms that cannot be explained only on the bases of two alternative hexokinase isoforms.[4,5] Thus, the origin of hexokinase multiplicity remains at least in part to be determined.

Finally, we would like to note that expression of recombinant human hexokinase type I lacking the porin-binding domain results in an enzyme with normal kinetic and regulatory properties (Bianchi et al, submitted for publication). Thus, in cases of hexokinase mutations with altered enzymatic properties, the mutation must be searched for downstream of exon 1. This exon could instead confer stability to the enzyme by favoring binding to intracellular organelles and be responsible for enzyme defects with accelerated in vivo hexokinase decay.[6]

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## A New Factor V Gene Polymorphism (His 1254 Arg) Present in Subjects of African Origin Mimics the R2 Polymorphism (His 1299 Arg)

*To the Editor:*

We have recently detected a genetic component in the factor V (FV) gene that contributes to activated protein C (APC) resistance both in the presence and in the absence of FV Leiden.[1] It is a highly conserved FV gene haplotype with a wide geographical distribution, which encodes several aminoacid changes. This haplotype is marked by the R2 polymorphism,[2] an A to G transition at position 4070 in exon 13 (B domain) that predicts the His1299Arg substitution, and is detectable by *Rsa* I restriction. The R2 haplotype was found to be conserved and similarly frequent (0.075) in four different populations (Somali, Southern Indians, Italians, and Greek Cypriots).

Genotyping of subjects of Somali origin at the 4070 polymorphic site revealed a new restriction pattern (Fig 1), suggesting the presence of a different polymorphic *Rsa* I restriction site in the same amplicon. Direct sequencing (Fig 1) of the polymerase chain reaction (PCR) fragment in these subjects revealed an A to G transition at position 3935 (R3 polymorphism), which predicts the incorporation of an Arg in the place of His1254 (CAT/CGT).[3] Six out of 40 Somali in our sample carried the new polymorphism in the heterozygous condition (frequency of the G allele 0.075). The 3935G allele could also be detected in Cypriots (1 out of 146 subjects was heterozygous, allele frequency 0.0034), whereas none of 40 Indians and 500 Italians was a carrier.

The R2 polymorphism is located in a highly repeated area of exon 13, characterized by 31 tandem repeats of the same 27-bp sequence, and affects the 20th nucleotide of the 16th repeat. We find it interesting that the new marker reproduces exactly the R2 polymorphism in the homologous position of the 11th repeat of exon 13, suggesting that the same molecular mechanism ($^{5m}$C to T determination at a CpG dinucleotide) was probably responsible for both transitions. Both polymorphisms appear to be ancient, as indicated by their presence in the Somali population, which reflects the ancestral genetic state of mankind,[4] but R3 shows a more limited geographical distribution. The finding of a new sequence variation in exon 13 of the FV gene confirms the extremely polymorphic nature of this exonic area, which seems to be subjected to loose sequence constraints.

Since the R2 marker is likely to be extensively investigated in various populations because of its involvement in APC resistance, we would like to point out that the new polymorphism can be easily distinguished from it by its restriction pattern (Fig 1).

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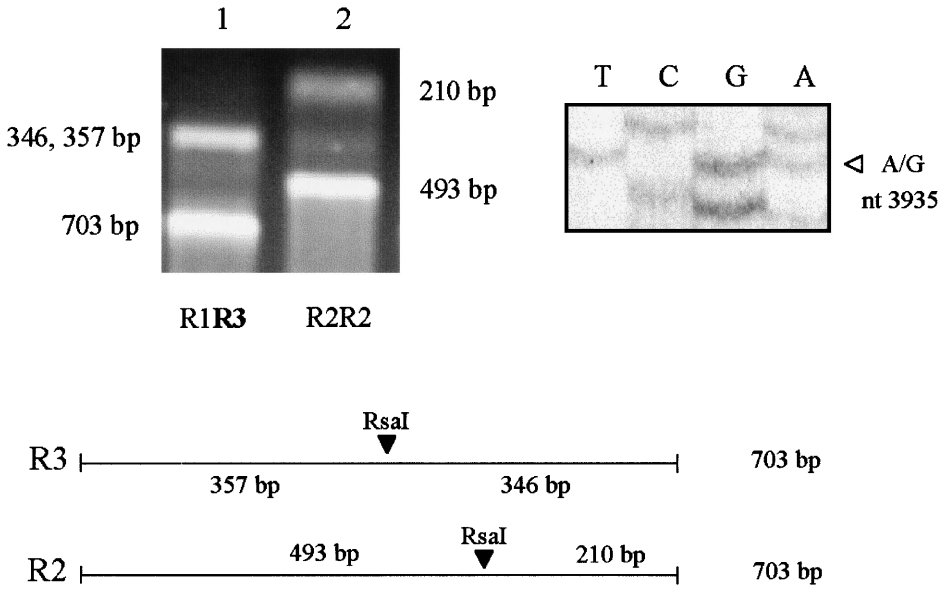


**Fig 1. Detection and characterization of a new polymorphic marker in exon 13. Upper left, separation by electrophoresis of the PCR product digested with *Rsa* I. The gel (2% agarose) was run at 70 V for 1 hour. Lane 1, subject heterozygous for the R3 allele and for the frequent R1 allele. Lane 2, subject homozygous for the R2 allele. The restriction map for R2 and R3 alleles is reported below. Upper right, detection of the R3 polymorphism by sequencing. The A/G transition is indicated by the open arrow. Nucleotide numbering according to Jenny et al.[3]**