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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 RsaI 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 RsaI 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 (5mC 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 Rsal. 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 J enny et al.2
Differentiating Juvenile Myelomonocytic Leukemia From Infectious Disease

To The Editor:

Two recent articles in BLOOD\(^1\)\(^2\) review the findings and outcome of juvenile myelomonocytic leukemia (JMML). Both omit an important aspect of JMML: its differentiation from infectious disease. Several disseminated microbial infections of infancy can result in persistent fever, failure to thrive, hepatosplenomegaly, skin lesions, anemia, thrombocytopenia, and myelomonocytosis, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes virus-6 (HHV-6), histoplasma, mycobacteria, and toxoplasma. Thorough investigation for infection is needed in infants with these findings to avoid erroneous diagnosis and mistaken interventions.

Herrod et al\(^3\) reported two infants with persistent EBV infection and findings consistent with JMML, including increased numbers of F and i cells and abnormal granulocyte-macrophage colony formation in vitro. Both recovered without treatment and remained well. This raises the possibility that some of the long-term survivors reported by Niemeyer et al and Arico et al had similar infections rather than leukemia. Neonatal CMV and HHV-6 infections can also mimic JMML.\(^4\)\(^5\) Might erroneous diagnosis account for the better prognosis reported for patients with JMML who are less than 6 months old?\(^6\)

The excellent reviews of Niemeyer et al and Arico et al suggest that JMML represents a group of diseases rather than a single entity. Careful investigation for microbial associations, including more recently identified herpesviruses, might contribute to understanding pathogenesis as well as to diagnosis and management.

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Response

We are grateful to Dr Pinkel for his carefully considered remarks. We fully agree that some infectious disease can mimic JMML, thus jeopardizing the interpretation of some cases. In particular, Dr Pinkel raises the question of whether some of the long-term survivors we described, in fact, have JMML. Although such suspicion is obviously warranted, our experience indicates that the vast majority of cases that fit the diagnostic picture of JMML represent leukemia and not an infectious process. Even when the patients show rapid recovery with or without “minimal treatment,” reactivation of the disease may occur. In some cases these recurrences have an accelerated phase, mirroring that in patients with rapidly fatal JMML. We cannot rule out infectious diseases as the origin of some of the more unusual cases of JMML that have been reported in the medical literature, but we do not believe these exceptions account for more than 10% of the total number. Perhaps current advances in the diagnosis will help to resolve this intriguing issue.

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Response

We have recently published the results of a retrospective analysis of 110 children with chronic myelomonocytic leukemia (CMML).\(^7\) There has since been an international consensus to rename the disease juvenile myelomonocytic leukemia (JMML). The new term JMML will include all leukemias of childhood previously classed CMML,\(^1\)\(^2\) juvenile chronic myelogenous leukemia (JCML),\(^3\)\(^4\) or infantile monosomy 7.
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