Increased Frequency of Fanconi Anemia Group C Genetic Variants in Children With Sporadic Acute Myeloid Leukemia

To the Editor:

Acute myeloid leukemia (AML) constitutes about 15% of childhood (0 to 15 years) acute leukemia in the United Kingdom, with an age-standardized incidence rate of approximately 6 cases/million/yr. Although the causes of childhood AML are uncertain, dietary and chemical exposure to substances that inhibit DNA topoisomerase II have been implicated. However, it is not clear whether genetic susceptibility to such environmental factors may also be involved. Children with the autosomal recessive disorder Fanconi anemia (FA) have one of the highest predictable risks of developing bone marrow failure leading to AML. This may be related to the hypersensitivity of their hematopoietic stem cells and myeloid progenitors to the clastogenic effects of bifunctional DNA cross-linking agents. What is not clear is whether children who are heterozygous asymptomatic carriers of FA mutations are also at risk of developing AML from exposure to environmental carcinogens. The notion of an increased cancer risk to such heterozygotes was originally proposed by Swift, but this was not confirmed in a follow-up study. Based on the high estimated frequency of FA gene mutation carriers in the population (~1/300), a role for such mutations in the susceptibility of heterozygotes to sporadic AML after exposure to environmental carcinogens seems a plausible hypothesis that has never been tested. In this preliminary study, we report findings from the screening of children with AML and normal infant controls for mutations and variations in the FAC group C gene.

The availability of detailed sequence information for the FAC gene permits exon-by-exon screening of genomic DNA as a means of determining the relationship between constitutional FAC sequence variation and the risk of childhood leukemia. Although this has been established in FA probands, there is no published information on FAC sequence variation in children with sporadic AML. We have therefore screened genomic DNA from 24 children with sporadic AML and 104 infant controls for variations in 6 of the 14 FAC exons (1, 4, 5, 6, 13, and 14), including the exons known to carry mutations most commonly found in FA. The patient series consisted of the following French-American-British (FAB) subtypes: 3 M1, 3 M2, 1 M3, 5 M4, 6 M5, 5 M7, and 1 MDS/RAEB-T. Two of the patients had Downs syndrome (trisomy 21 [T21]). The controls consisted of a random anonymous series of cord blood samples from 104 normal full-term newborn infants, of whom 82 were European, 18 were Asian, 1 was Afro-Caribbean, and 3 were Oriental. Polymerase chain reaction (PCR) primer sequences used to amplify FAC exons were those designed by Gibson et al. Forward and reverse primers were 5’ end-labeled with the fluorescent dye Cy5 during synthesis, and PCR products were analyzed by electrophoresis in MDE gels using a Pharmacia ALFexpress sequencer (Amersham Pharmacia Biotech, Little Chalfont, UK). Screening by combined single-strand conformation polymorphism and heteroduplex analysis was performed using 3 to 6 different electrophoresis conditions (including different concentrations of MDE gels), and each FAC variant was analyzed twice by reamplification of the test DNA and electrophoresis under the same conditions.

Eight FAC variants were identified in 7 of the 24 children with AML, compared with 9 variants in 9 of the 104 controls (patients vs control variant frequency, 29.1% vs 8.65%; odds ratio [OR], 4.3; 95% confidence interval [CI], 1.5 to 12.4; two-sided Fisher’s, $P = .025$). Of the 48 AML patient and 208 control chromosomes tested for FAC variants, the patient and control frequencies were 16.6% and 4.32%, respectively (OR, 4.42; 95% CI, 1.69 to 11.52; two-sided Fisher’s, $P = .011$). FAC variants were only detected in AML M4 and M5 patients. Neither of the T21 patients had FAC variations. No FAC variants were detected in 4 Asian AML patients, but 1 variant was present in 1 Asian control. One AML M4 patient, but no controls, had FAC variants in 2 exons (exons 5 and 6); otherwise, all variants detected in the exons so far screened were heterozygous. Acute-phase AML cells have not yet been tested to determine whether additional, somatic FAC variants also occur.

DNA sequencing performed on the FAC variants found in 2 of the AML (both AML M4) and 2 controls showed that none conformed to the mutations associated with FA. Both AMLs had identical C-to-T transitions in exon 1 that altered serine to phenylalanine at position 26 (S26F). One of the 2 controls carried a previously unpublished variant that altered a proline to arginine at position 211 in exon 6 (887 C > G; P211R). The other control had a glycine to glutamine conversion in exon 4 (9671 G > A; G139E). Both the S26F and G139E variants are previously reported FAC polymorphisms.10,11

Among the 6 FAC exons studied to date, we have found a significantly increased frequency of FAC sequence variants in children with AML compared with controls. The finding of an identical polymorphism (S26F) in 2 AML patients is interesting. We can only speculate on the nature of the association. Perhaps small variations in the FAC protein might be sufficient to render hematopoietic stem cells more sensitive to certain environmental DNA cross-linking agents, leading to an increased risk of AML. Corroborative evidence will require further FAC variant analysis in a larger series of AML patients and controls, together with an analysis of epidemiological data of environmental carcinogen exposure. The UK Childhood Cancer Study, a 5-year epidemiological case-control study designed to investigate the role of environmental factors in childhood cancer and leukemia, provides a unique opportunity to test the hypothesis that variation in FA genes may contribute to AML susceptibility.

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