A Lack of a Functional NAD(P)H:Quinone Oxidoreductase Allele Is Selectively Associated with Pediatric Leukemias That Have MLL Fusions


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ABSTRACT

Rearrangements and fusion of the MLL gene with various alternative partner genes occur in ~80% of infant leukemias and are acquired during fetal hemopoiesis in utero. Similar MLL gene recombinants also occur in topoisomerase II-inhibiting drug-induced leukemias. These data have led to the suggestion that some infant leukemia may arise via transplacental fetal exposures during pregnancy to substances that form cleavable complexes with topoisomerase II and induce illegitimate recombination of the MLL gene. A structural feature shared by many topoisomerase II-inhibiting drugs and other chemicals is the quinone moiety. We assayed, by PCR-RFLP, for a polymorphism in an enzyme that detoxifies quinones, NAD(P)H:quinone oxidoreductase (NQO1), in a series (n = 36) of infant leukemias with MLL rearrangements versus unselected cord blood controls (n = 100). MLL-rearranged leukemias were more likely to have genotypes with low NQO1 function (heterozygous CT or homozygous TT at nucleotide 609) than controls (odds ratio, 2.5; P = 0.015). In contrast, no significant allele bias was seen in other groups of pediatric leukemias with TEL-AML1 fusions (n = 50) or hyperdiploidy (n = 29). In the subset of infant leukemias that had MLL-AF4 fusion genes (n = 21), the bias increase in low or null function NQO1 genotypes was more pronounced (odds ratio, 8.12; P = 0.00013). These data support the idea of a novel causal mechanism in infant leukemia involving genotoxic exposure in utero and modulation of impact on a selective target gene by an inherited allele encoding a rate-limiting step in a carcinogenic detoxification pathway.

INTRODUCTION

Pediatric acute leukemia is a diverse cancer in terms of its underlying biology and clinical response (1, 2). Infants with ALL or AML usually have acquired MLL gene fusions as their major consistent genetic abnormality (3, 4). In contrast, the most common leukemia in children, B-cell precursor or CALL, in the 2–5-year age peak of disease incidence (2), have other genetic abnormalities, most frequently TEL-AML1 gene fusion or hyperdiploidy (5–7). These subsets have been suggested to be the basis of the etiological mechanism for infant acute leukemia involving transplacental exposure to substances that form cleavable complexes with topoisomerase II-inhibiting substances (8, 9). Rearrangements and fusion of the MLL gene with various alternative partner genes occur in ~80% of infant leukemias and are acquired during fetal hemopoiesis in utero. Similar MLL gene recombinants also occur in topoisomerase II-inhibiting drug-induced leukemias. These data have led to the suggestion that some infant leukemia may arise via transplacental fetal exposures during pregnancy to substances that form cleavable complexes with topoisomerase II and induce illegitimate recombination of the MLL gene. A structural feature shared by many topoisomerase II-inhibiting drugs and other chemicals is the quinone moiety. We assayed, by PCR-RFLP, for a polymorphism in an enzyme that detoxifies quinones, NAD(P)H:quinone oxidoreductase (NQO1), in a series (n = 36) of infant leukemias with MLL rearrangements versus unselected cord blood controls (n = 100). MLL-rearranged leukemias were more likely to have genotypes with low NQO1 function (heterozygous CT or homozygous TT at nucleotide 609) than controls (odds ratio, 2.5; P = 0.015). In contrast, no significant allele bias was seen in other groups of pediatric leukemias with TEL-AML1 fusions (n = 50) or hyperdiploidy (n = 29). In the subset of infant leukemias that had MLL-AF4 fusion genes (n = 21), the bias increase in low or null function NQO1 genotypes was more pronounced (odds ratio, 8.12; P = 0.00013). These data support the idea of a novel causal mechanism in infant leukemia involving genotoxic exposure in utero and modulation of impact on a selective target gene by an inherited allele encoding a rate-limiting step in a carcinogenic detoxification pathway.

The Study is conducted by 12 teams of investigators (10 clinical and epidemiological and 2 biological) based in university departments, research institutes, and the Scottish health service. The United Kingdom Childhood Cancer Study is sponsored by a consortium of statutory bodies, cancer charities, and industrial sponsors. The United Kingdom Childhood Cancer Study is sponsored by a consortium of statutory bodies, cancer charities, and industrial sponsors.

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2 Laboratory studies were funded by the Kay Kendall Leukaemia Fund and the Leukaemia Research Fund. The United Kingdom Childhood Cancer Study is sponsored and administered by the United Kingdom Co-ordinating Committee on Cancer Research. The Study is conducted by 12 teams of investigators (10 clinical and epidemiological and 2 biological) based in university departments, research institutes, and the Scottish health service. The work is coordinated by a Management Committee and in Scotland by a Steering Group. It is supported by the United Kingdom Children’s Cancer Study Group of pediatric oncologists and by the National Radiological Protection Board. Funding is provided by a consortium of statutory bodies, cancer charities, and industrial sponsors.

3 The abbreviations used are: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CALL, common ALL; BCR, breakpoint cluster region; NQO1, NAD(P)H:quinone oxidoreductase; n, nucleotide; OR, odds ratio; CI, confidence interval.

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change at nt 609 yields a proline to serine substitution (32), and a T→C substitution at nt 464 results in a tryptophan replacement of arginine (33, 34). The first of these (C609T) effectively inactivates the enzyme due to decreased catalytic activity and stability of NQO1 protein (35, 36); the second (T464C) has not yet been completely characterized. We have analyzed these polymorphisms in three subgroups of infant and childhood leukemias with the hypothesis that: (a) MLL leukemia patients and/or their mothers will show a higher prevalence of low NQO1-inducing genotypes, reflecting a reduced ability to detoxify carcinogens that promote MLL translocations; and (b) other groups of childhood leukemias, including those cALLs with TEL-AML1 translocations and those exhibiting hyperdiploidy, that have not been epidemiologically associated with chemical exposure should not demonstrate a bias in allele frequency.

MATERIALS AND METHODS

Patient and Control Samples. All of the patients (<15 years old) were enrolled in the United Kingdom Childhood Cancer Epidemiology Study (UKCCS). Patient samples taken at the time of diagnosis of acute leukemia were screened and classified for common molecular subgroups of pediatric leukemia by banded karyotyping and by fluorescence in situ hybridization (for hyperdiploidy) and reverse transcription-PCR (for MLL fusions and TEL-AML1 fusions). Remission blood samples were also obtained. Controls consisted of umbilical cord blood samples obtained from healthy new-born infants. A small proportion (<10%) of patients was from minority ethnic groups in the United Kingdom (i.e., Asian, Afro-Caribbean black, and Oriental). No attempt was made to screen out such individuals, and we have no evidence that they are disproportionately represented in any leukemia subgroup.

NQO1 Genotyping. Genotyping was performed by PCR-RFLP analysis of DNA extracted from patient blood samples and controls. Infant (<24 months) leukemia patient samples used were taken either at diagnosis of leukemia (n = 16) or, when in remission, within 3 months of diagnosis (n = 20). All of the other patient DNA samples for genotyping were from blood taken during remission. Twenty nanograms of the primers NQO1609A, CCTCTCTGTGCTTTCTGTATCC with NQO1609B, GATGGACTTGCCCAAGTGATG (for the nt 609 polymorphism) or NQO1609A, CTGGTCTTACCTCAATGATGTC with NQO1464B, CCTGCATCAGTAGACACCAC (for the nt 464 polymorphism) were mixed with 60 ng of DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 pmol of each dNTP, and 1.25 units Taq polymerase in a total volume of 50 μl and subjected to 35 cycles (94°C for 1 min, 60°C for 1 min, and 72°C for 1 min) in an MJ Research thermal cycler (Watertown, MA) followed by an electrophoresis in 0.7% agarose with 2% Synergel (Diversified Biotech) and viewed by ethidium bromide staining/UV trans-illumination (Fig. 1).

Fig. 1. PCR-RFLP analysis of NQO1 polymorphisms in six individuals analyzed on a 0.7% agarose/2% Synergel electrophoresis gel. Lane 1, Marker (Life Technologies, Inc., 1 kb). Lanes 1–3, nt 464 PCR reactions digested with MspI; Lanes 4–6, nt 609 PCR reactions digested with HinFI. Lanes 1 and 4, homozygote normal individuals for the respective polymorphisms; Lanes 3 and 6, homozygote variant individuals for the respective polymorphisms; Lanes 2 and 5, heterozygote individuals for the polymorphisms.

Table 1 Subclassification of infant leukemia cases* with MLL gene fusions

<table>
<thead>
<tr>
<th>Fusion partner</th>
<th>Total n</th>
<th>AF4</th>
<th>ENL</th>
<th>AF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>ALL</td>
<td>30</td>
<td>21</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

*All of the patients were <18 months of age.

RESULTS

Of the 36 cases of MLL fusion gene-positive infant leukemias available, 30 were classified morphologically and by immunophenotype as ALL and 6 as AML. MLL fusion gene partners were as shown in Table 1. As anticipated, most of the cases were MLL-AF4-positive. Two hundred fifteen individuals were typed for NQO1 genotypes and are presented in Table 1. A 17% allele frequency was found for controls for the nt 609 variant allele, which is intermediate to the 13–25% allele frequency found in comparable studies in Caucasians in Europe and North America (34, 37–39). In the largest group to date, a 16% allele frequency was found among 575 individuals (34). A 5.5% allele frequency was found for controls at the nt 464 variant, which is similar to that found in Canadian Caucasians (5%; Ref. 34). Representative PCR gel results are illustrated in Fig. 1.

Patients with MLL translocations were found to harbor 2.5 times increased frequency of low NQO1 genotypes [heterozygous CT or homozygous TT at nt 609; OR, 2.5; 95% CI, 1.08–5.96; P = 0.015] than controls (Table 1). The allele frequency of the variant nt 609 allele in MLL+ leukemias was also significantly different (32% versus 17%; two-tailed P = 0.011). In addition, there was a significant “dose response” effect, homozygous TT having a higher risk of leukemia than heterozygous CT patients (test for trend, P = 0.0076). MLL+ infant leukemias did not demonstrate an allele bias compared with controls for nt 464 variants (OR = 1.12), nor did TEL-AML1 or hyperdiploid leukemias.

When NQO1 genotypes were analyzed for the major molecular subtype of MLL gene rearranged ALL, i.e., those with MLL-AF4 fusions, a more pronounced bias toward low-function genotypes (as nt 609) was evident [OR, 8.12; 95% CI, 2.29–31.48 (Table 2)]. Other subgroups of these infant leukemias, i.e., AML or ALL with MLL-
ENL or MLL-AF9 fusions had too few cases for a separate analysis of NQO1 allele frequency.

In contrast to MLL+ infant leukemias, childhood ALLs with TEL-AML1 translocations or hyperdiploidy did not demonstrate increased frequencies of NQO1 variant alleles. TEL-AML1 leukemias had a slightly higher odds of a low NQO1 nt 609 alleles (OR, 1.5), but the increase was not significant (Table 2).

**DISCUSSION**

NQO1 is a cytoplasmic, ubiquitously expressed enzyme that catalyzes an obligate two-electron reduction of a wide variety of quinones using NADH or NADPH as the reducing cofactor. NQO1 is generally known for its detoxifying properties, effective with such chemicals as menadione (40), benzene (41), and benzo(a)pyrene quinone (42). NQO1 is also known to be involved in the recycling of membrane antioxidants ubiquinone and vitamin E (43, 44). The simplest quinone, benzoquinone, is a metabolite of benzene produced in the bone marrow from hydroquinone via myeloperoxidase (45) and is also derived from arbutin, a glycoside conjugate of hydroquinone, which is common in the food supply (46). Studies suggest that the balance between myeloperoxidase and NQO1 levels in the bone marrow determines the level of toxic effects of hydroquinone and may ultimately determine the leukemogenicity of benzene (47, 48). NQO1 strongly inhibited a class of DNA adducts induced by hydroquinone (49), which suggests a potential mechanism for its protection of the hematopoietic system in benzene-exposed individuals (41). NQO1 has a wide substrate specificity and may play a similar role in detoxifying other environmentally derived quinones and quinone-imines.

Two polymorphic variants in NQO1 have been found, a C→T substitution at nt 609 and a T→C substitution at nt 464. The nt 609 polymorphism has been recently associated with specific leukemogenic changes, including clonal abnormalities in chromosomes 5 and/or 7, in therapy-related leukemias (50). This polymorphism is effectively completely inactivating, whereas the nt 464 polymorphism has not been characterized in mammalian cells. A higher prevalence of low NQO1-inducing genotypes (C609T), as we describe here for pediatric leukemia with MLL gene fusions, therefore, reflects a reduced ability to detoxify quinone-based carcinogens. Although the allele bias that we describe is statistically significant, especially for the small series of cases (n = 21) with MLL-AF4 gene fusions, it will be important to confirm this association in an independent series of patients. The prevalence of the C609T polymorphism varies among different ethnic groups. The highest reported allele frequency of the variant is approximately 40% in Asian populations (37, 41). It may be significant in this context that infant leukemia is more frequent in Oriental than in Western countries (analysis of data from Ref. 51). The lack of association of nt 464 variant alleles with MLL+ leukemias may indicate a low functional phenotype of this polymorphism.

Felix et al. (52) recently reported that an excess risk of secondary leukemias (both with and without MLL gene fusion) was associated with a reduced likelihood of inheritance of the CYP3A4-V allele of cytochrome P-450 CYP3A4 gene, which metabolizes epipodophyllotoxins (and other chemicals) to quinone metabolites. However, no such association was observed in de novo leukemias with MLL gene fusions, most of which were in infant cases. Taken at face value, these data seem to conflict with our own but need not necessarily do so. Firstly, cytochrome P-450 enzymes may be more critical or dose-limiting in the context of high-dose chemotherapeutic exposures generating genotoxic metabolites. Secondly, CYP3A4 is not expressed in fetal development (53, 54) and, therefore, cannot contribute to the risk of MLL gene fusions in the context of infant leukemia. NQO1 is expressed in fetal liver.6

There was no significant bias of allele frequency in other subsets of pediatric ALL with alternative acquired molecular abnormalities, hyperdiploidy, or TEL-AML1 fusion genes. The latter subtypes are members of the common (c) variant of childhood ALL in which an abnormal response to infection is postulated to be a major etiological factor (8). We, therefore, demonstrate a unique and hitherto undescribed link between an inherited genetic polymorphism and a specific acquired genetic abnormality in a cancer subtype. We hypothesize that the link is associated with suspected patterns of chemical exposure during pregnancy. The hypothesis is that substances that form cleavable complexes with topoisomerase II are prime candidates for the induction of MLL gene fusions (8, 23). This idea was prompted by the observation that MLL gene fusions characteristic of infant leukemia are also common in secondary leukemias associated with prior exposure to therapeutic drugs including epipodophyllotoxins or anthracyclines, which operate via topoisomerase II inhibition (16, 17).

Candidate substances that might generate infant leukemia with MLL gene fusions and that would be metabolized to quinones in the fetal liver include dietary flavonoids, podophyllin toxins, and benzene. Ongoing case/control studies are assessing via maternal, questionnaire-based data, exposure patterns during pregnancy. Epidemiological studies have implicated a number of different maternal exposures during pregnancy that are associated with infant leukemia, although cases were not analyzed for MLL gene status. These include

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5 Freda E. Alexander, unpublished observations.
6 Joseph Wiemels and Mel Greaves, unpublished observations.
diets rich in flavonoids (25), pesticides, marijuana, alcohol (55), and benzene or gasoline exposures (reviewed in Ref. 24). Some of these, if causally relevant, could operate via the quinone metabolic pathway. Benzene metabolites (in gasoline and in tobacco marijuana smoke, for example) as well as flavonoids are oxidized by peroxidases to yield semiquinones and quinones (31, 56). In the case of benzene metabolites, the oxidized products interact with topoisomerase II (57). A NQO1-deficient individual may be less able to cope with the quinone assault. Unoxidized flavonoids are excellent topoisomerase II inhibitors (26), and, therefore, the role of NQO1 is less clear.

It should be remembered that the ultimate mechanism for MLL breakage leading to translocation is unknown and could involve topoisomerase II inhibition or, alternatively, the formation of reactive metabolites or reactive oxygen species. Indeed, many topoisomerase II-inhibiting drugs form reactive oxygen species with great facility as do benzene metabolites (31) and flavonoids (56). The 3′ end of the MLL BCR has been shown to be a DNase-hypersensitive site and liable to breaks by a variety of apotic-inducing stimuli, which indicates that other mechanisms not involving topoisomerase II may play a role. The ultimate mechanism of NQO1-modified attack on the MLL gene in infant leukemia, therefore, remains to be uncovered. The objective of additional studies lies in uncovering the genetic-environmental interactions and pathway leading to the development of a highly malignant and clinically intractable leukemia in infants. Prevention is the long-term goal.

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REFERENCES


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