



Human Genome Epidemiology (HuGE) Review

***NQO1* Polymorphisms and De Novo Childhood Leukemia: A HuGE Review and Meta-Analysis**

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Polymorphisms in *NQO1*, a gene coding for the phase II enzyme involved in the detoxification of quinone carcinogens, have been associated with childhood leukemia in some studies, although the observed direction and magnitude of effects have been inconsistent. Therefore, the authors systematically reviewed all published reports describing the effect of *NQO1* in de novo childhood leukemia and conducted a meta-analysis of 7 case-control studies that examined the association between *NQO1**2 and childhood leukemia. Although a family-based study previously demonstrated overtransmission of this allele among childhood acute lymphoblastic leukemia cases, the meta-analysis showed that the presence of a *NQO1**2 variant allele, which reduces the activity of the enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1), had no significant effect on childhood leukemia. However, there was an increased risk associated with having at least 1 copy of the *NQO1**2 allele in a subset of cases with *MLL* translocations (summary odds ratio = 1.39, 95% confidence interval: 0.98, 1.97). Heterogeneity between studies may be due to differences in population exposures to NQO1 substrates and small sample sizes, as well as potential population stratification in non-family-based studies. Therefore, further research is warranted on the role of *NQO1* polymorphisms in the etiology of childhood leukemia, especially among *MLL*-positive leukemias.

epidemiology; leukemia; meta-analysis; NAD(P)H dehydrogenase (quinone); *NQO1*; polymorphism, single nucleotide; systematic review

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CI, confidence interval; NQO1, NAD(P)H:quinone oxidoreductase 1; OR, odds ratio; SNP, single nucleotide polymorphism.

Editor's note: This paper is also available on the website of the Human Genome Epidemiology Network (<http://www.cdc.gov/genomics/hugenet/>).

GENE AND ENZYME

The human NAD(P)H:quinone oxidoreductase 1 gene (*NQO1*; DT-diaphorase, Enzyme Commission (EC) number 1.6.99.2) occupies 17 kilobase pairs (kb) within a gene-rich region on chromosome 16 at 16q22.1 (1). This cytosolic flavoenzyme detoxifies quinones (a large class of aromatic compounds found commonly in plants, benzene metabolites,

and chemotherapies) to hydroquinones or catechols (2–4). The enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) acts as an antioxidant by catalyzing a 2-electron reduction that bypasses the need for two 1-electron reductions that can result in the production of DNA and protein-damaging reactive oxygen species. In certain conditions (e.g., the presence of myeloperoxidase or autooxidants), NQO1 can contribute to the formation of reactive oxidation species via oxidative cycling and therefore can act as a prooxidant (2, 5). NQO1 is constitutively expressed in most tissues including the bone marrow, where expression is thought to be highly inducible by xenobiotics with quinone moieties, and is upregulated during times of oxidative or electrophilic stress (6). *NQO1* expression is also upregulated in tumor

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tissues as the result of hypoxia (7): The success of quinone-based chemotherapies to exert their cytotoxic effects depends on their bioactivation by elevated levels of NQO1.

GENE VARIANTS

There have been more than 93 single nucleotide polymorphisms (SNPs) identified in the *NQO1* gene. The most widely studied SNP of *NQO1* is a C to T change at nucleotide position 609 (rs1800566), also known as *NQO1**2. This results in a proline to serine amino acid change at codon 187 that is associated with a loss of enzyme activity due to instability of the protein product (8). Thus, the enzyme activity of the homozygous variant genotype (*NQO1**2/*2) is almost undetectable, and the enzyme activity of the heterozygous genotype (*NQO1**1/*2) is intermediate between the homozygous variant genotype and wild type (*NQO1**1/*1) (6). The *NQO1**2 allele frequency varies between different ethnic groups, ranging from 16% in Caucasians to 49% in Chinese populations. Prevalence of the *NQO1**2/*2 genotype is 4.4% in non-Hispanic whites, 5.2% in African Americans, 12.2% in Japanese, 15.5% in Mexican Hispanics, 17.9% in Native Americans, 18.8% in Koreans, and 22.4% in Chinese (6, 9–11).

Another genetic polymorphism of *NQO1* is a single nucleotide change from C to T at nucleotide position 465 (rs4986998), also known as *NQO1**3, which changes the amino acid at codon 139 from arginine to tryptophan. This SNP results in alternative messenger RNA splice sites that can lead to a deletion of exon 4 and create a protein lacking the quinone binding site (12–15) for which enzyme activity differs according to the substrate (14). The frequency of the *NQO1**3 polymorphism is generally low and ranges from 0% to 5% among different ethnic populations (9).

LABORATORY TESTS

Most publications describing *NQO1* genotyping utilized polymerase chain reaction followed by restriction fragment length polymorphism analysis or allele-specific oligonucleotide hybridization assays, as described previously (16, 17). The SNPs on *NQO1* are also amenable to genotyping by using more modern array-based methods.

DISEASE

Leukemia represents one-third of all cancer cases under the age of 15 years and is the most common pediatric cancer (18). A heterogeneous disease that disrupts normal hematopoiesis, it can present as either acute or chronic: Approximately 80% of childhood leukemias (<15 years of age) are acute lymphoblastic leukemia (ALL), less than 20% are acute myeloid leukemia (AML), and 2% are chronic myeloid leukemia, while chronic lymphocytic leukemia rarely occurs in children (19).

The incidence of childhood ALL varies by and within countries, with the lowest rates observed by the African countries (from 4.5 per million to 14.3 per million), followed by Asian countries (from 9.2 per million to 40.6

per million), with the highest rates observed by European, American (North, Central, and South), and Oceania (Australia and New Zealand) countries (from 16.1 per million to 48 per million) (20). In contrast, the differences in the incidence of childhood AML among different countries are not as distinct (20). In addition, the ratio of ALL to AML also varies by region, with the lower ratios observed in most African and Asian countries (i.e., more AML than ALL cases) and higher ratios observed in the European, American, and Oceania countries (20).

The most common cytogenetic changes in childhood leukemias include high hyperdiploidy (>50 chromosomes in karyotype) and specific recurrent chromosomal translocations; these are both believed to occur in utero after prenatal exposure to toxic agents. About 80% of infant leukemia cases (acute leukemia in children <12 months of age) harbor the *MLL* gene rearrangement at 11q23, and the most common translocation among children aged 2–5 years is the *TEL-AML1*. Despite our knowledge of tumor genetics, the causes of childhood leukemia are largely unknown: Currently, the only established risk factors are ionizing radiation and genetic syndromes such as Down syndrome (21). Accordingly, exogenous factors including history of infections, maternal diet and medication use during pregnancy, and benzene exposures from pesticides, parental smoking, parental occupation, and traffic emissions have been the subject of investigation. Several compounds from these exposures can be oxidized to semiquinones and quinones by peroxidases (e.g., myeloperoxidase), which then are substrates for NQO1 (5, 22).

The *NQO1**2 variant allele has been associated with infants' leukemias harboring the *MLL* rearrangement (17, 23), childhood ALL (16), and therapy-related leukemias (24, 25). These observations are supported by a large epidemiologic study that showed that presence of the *NQO1**2 allele increased the risk of hematotoxicity more than 2-fold among a large cohort of Chinese workers exposed to benzene, an established risk factor for adult AML (26). The *NQO1**3 allele has been less studied, although Eguchi-Ishimae et al. (27) showed an association with Japanese infant leukemia cases that harbored the *MLL-AF4* [t(4;11)] translocation. In contrast, in a comparison of childhood leukemia cases with controls in a British population, there was no evidence of association of the *NQO1**3 allele in *MLL*-positive leukemias in infants (odds ratio (OR) = 1.12) or in *TEL-AML1* or hyperdiploid leukemias compared with controls (17).

REVIEW AND METHODS—IDENTIFICATION AND ELIGIBILITY OF RELEVANT STUDIES

Our objective was to identify all published articles examining the association between *NQO1* polymorphisms and childhood leukemia. We searched PubMed for all articles published up to September 24, 2007. We identified 56 articles with the search terms (“NAD(P)H Dehydrogenase (Quinone)” [MeSH] or “NQO1”) and “leukemia” and limiting the search to studies in human populations. Articles with the following characteristics were excluded from the review: 1) non-English articles; 2) review articles; 3) non-epidemiologic studies (e.g., studies on animals or cell culture); 4) treatment outcome studies; 5) studies with

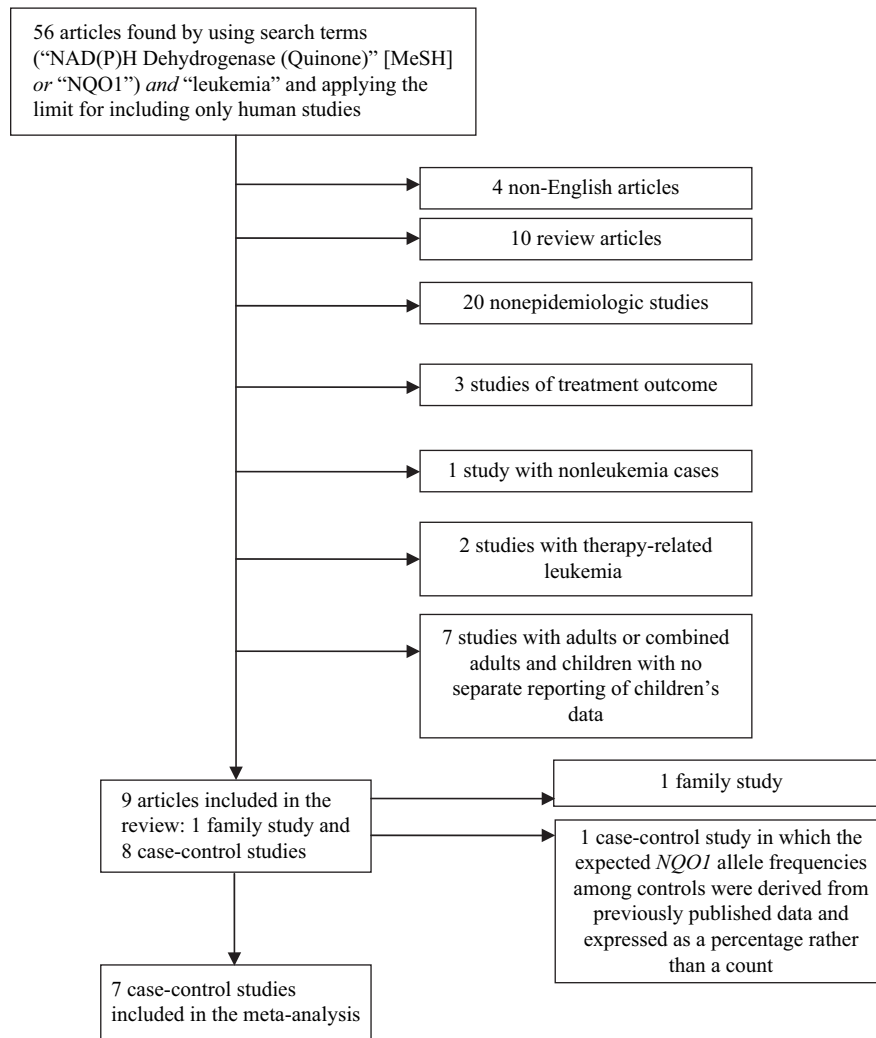


Figure 1. Process of article exclusion. MeSH, Medical Subject Headings; NQO1, NAD(P)H:quinone oxidoreductase 1.

therapy-related leukemia; 6) studies that included nonleukemia cases (e.g., non-Hodgkin's lymphoma) without reporting separate results for leukemia; and 7) studies with only adults or studies that combined adult and childhood leukemia but did not report results separately by age group (Figure 1). As of September 24, 2007, we had identified 9 published studies describing the association between *NQO1* polymorphisms and de novo childhood leukemia: 1 family-based study (28) and 8 "case-control" studies (16, 17, 23, 27, 29–32) for which 2 of these studies (23, 31) included case-only analyses.

ASSOCIATIONS

Family-based studies

One study with a family-based design of 657 case-parent trios and case-parent-grandparent clusters found that the *NQO1**2 allele was transmitted to childhood ALL cases more frequently than expected, with a relative risk (dominant

model) of 1.39 (95% confidence interval (CI): 1.07, 1.79) (28). This study also examined transmission of the *NQO1**2 allele among 21 trios of infants (aged ≤ 12 months) with leukemia and found that there was a large, nonstatistically significant increased risk with carrying the variant allele (relative risk = 4.29, 95% CI: 0.58, 31.68). The family-based design is a powerful approach to study the effects of genetic variation in disease susceptibility, because it is immune to the effects of population stratification that could bias the results of case-case and case-control studies.

Case-case studies

Two studies used a case-only approach to examine the association between *NQO1**2 and *MLL*-positive leukemias in infants (23, 31). Both studies used patients with de novo B-lineage ALL without the *MLL* gene rearrangement as a comparison group. The *NQO1**2 allele significantly increased the risk of *MLL*-negative leukemia in infants in both the Italian (OR = 5.55, 95% CI: 1.81, 16.98) (31) and

American (OR = 2.47, 95% CI: 1.08, 5.68) populations (23).

Case-control studies

Among the 5 traditional case-control studies that examined the association between *NQO1**2 and acute childhood leukemia, the results were inconclusive (Table 1) (16, 27, 29, 31, 32). In a Canadian population, the presence of at least 1 variant *NQO1**2 allele increased the risk of childhood ALL in cases compared with controls by approximately 50% (OR = 1.49, 95% CI: 1.02, 2.19) (Figure 2) (16). In contrast, a decreased risk of childhood ALL associated with the *NQO1**2 allele was observed in a Turkish population (OR = 0.79, 95% CI: 0.58, 1.08) (32). The other case-control studies showed essentially null results (27, 29, 31). In addition, Wiemels et al. (17) did not detect any association of the *NQO1**2 allele in a subset of *TEL-AMLI*-positive and hyperdiploid childhood ALL cases. There was no evidence of association between *NQO1**2 and childhood AML among the 3 case-control studies that examined this outcome (27, 29, 32).

Four case-control studies examined the association between *NQO1**2 and *MLL*-positive childhood leukemia (17, 27, 30, 31). A significantly increased risk of *MLL*-positive leukemia associated with the *NQO1**2 allele was observed in a British population (OR = 2.5, 95% CI: 1.08, 5.96) (17), while results from 2 other studies were also suggestive of an increased risk (27, 31). Additionally, in a case-control analysis comparing allele frequencies in cases with the expected population distribution of *NQO1* allele frequencies at nucleotide position 609 derived from the published literature, Smith et al. (23) observed that *MLL*-positive leukemia cases were significantly more likely to have at least 1 copy of the *NQO1**2 allele compared with ethnically matched, disease-free subjects (OR = 2.50; $P = 0.02$). Three of these studies further classified *MLL*-positive cases and identified a small subset ($n \leq 35$) of cases harboring the *MLL-AF4* translocation [t(4;11)] (17, 27, 30). Wiemels et al. (17) authored the only case-control study to show a significantly increased risk of *MLL-AF4*-positive leukemias associated with the *NQO1**2 allele (OR = 8.63, 95% CI: 2.45, 33.22). It should be noted that this study (17) had the smallest sample size (21 *MLL-AF4*-positive cases) among all 3 studies. In summary, the results of 4 case-control studies suggest that the *NQO1**2 allele may be associated with *MLL*-positive leukemia (17, 27, 30, 31) and possibly with the *MLL-AF4*-positive subtype (17).

INTERACTIONS

The presence of a functional polymorphism in *NQO1* does not appear to confer a strong increased risk of childhood leukemia in general but may do so in the presence of an environmental exposure to quinones. Furthermore, this effect may be modified by polymorphisms in other carcinogen-metabolizing genes such as the *GSTs*, *CYP2E1*, and *MPO*. Therefore, it is important to study gene-gene and gene-environment interactions. In a French study, Clavel et al. (29) examined interactions among the *NQO1**2 polymorphism, maternal tobacco smoking, and alcohol and coffee consumption during pregnancy. Using a case-control

analysis, they observed that maternal coffee consumption negatively interacted with the *NQO1**2 allele to reduce the risk of childhood acute leukemia (OR = 0.3, 95% CI: 0.1, 1.0). However, they found no significant interactions between the *NQO1**2 polymorphism and either maternal smoking or alcohol consumption during pregnancy. A case-only analysis produced similar results.

Using a trio design (biologic parents and the affected child) to study a French-Canadian population, Infante-Rivard et al. (28) did not observe a multiplicative interaction between prenatal exposure to mononuclear aromatic hydrocarbons and the child's (*NQO1*) genotype (likelihood ratio test: $P = 0.33$) but did find weak evidence for a "supermultiplicative" interaction between maternal smoking during pregnancy and the child's (*NQO1*) genotype (likelihood ratio test: $P = 0.15$). In a small sample of study subjects, Krajnovic et al. (16) found a suggestion of a combined 3-way interaction between *NQO1* and *CYP2E1* risk-elevating genotypes and the wild-type *MPO* allele in increasing the risk of childhood ALL (OR = 5.4, 95% CI: 1.2, 23.4).

META-ANALYSIS

Identification and eligibility of relevant studies for meta-analysis

We decided that a quantitative summary of the role of *NQO1* in childhood leukemia would be useful and therefore conducted a meta-analysis using the results of published studies. It was only possible to include case-control studies from the review into the meta-analysis because of the need for exposed and unexposed groups and diseased and non-diseased groups. Therefore, 2 of the studies included in the review were excluded from the meta-analysis because of 1) a family-based design (28) and 2) a case-only analysis of *MLL*-positive versus *MLL*-negative cases (23) (Figure 1). Although the latter study (23) included a second comparison group of disease-free, ethnically matched controls, the expected *NQO1* allele frequencies were derived from previously published data and expressed as a percentage, rather than a count, and therefore could not be included in our meta-analysis. Table 1 describes the 7 case-control studies included in the meta-analysis.

Data abstraction for meta-analysis

All articles were assessed independently by two reviewers (N. G. and J. S. C.), who extracted data from each article that included authors, year of publication, country of origin, case and control selection, demographics of the study subjects, and the number of cases and controls for the genotypes of each *NQO1* polymorphism. Any discrepancies between the two reviewers were resolved by discussion and consultation with a third reviewer (A. P. C.).

Statistical methods for meta-analysis

Four childhood leukemia outcomes were assessed in this meta-analysis: 1) ALL, 2) AML, 3) *MLL*-positive leukemia (ALL or AML), and 4) *MLL-AF4*-positive leukemia (ALL

or AML). We calculated summary odds ratios for studies that assessed the relation between *NQO1**2 and each outcome. Because *NQO1**3 was assessed in only 2 studies (16, 27), we did not include this marker in the current meta-analysis. Forest plots were used to display the results from individual studies on a common scale, as well as the summary odds ratios, and further allowed for a visual assessment of the degree of heterogeneity among studies (Figures 2–5) (33). We used both random and fixed effect models with weights equal to the inverse of the variance to calculate a summary odds ratio for the effect of *NQO1**2 on the different subtypes of childhood leukemia. Results from random effects models, which account for heterogeneity among studies, are presented when Mantel-Haenszel chi-squared (χ^2) tests for heterogeneity showed significant differences across studies (conservatively defined as $P < 0.20$).

The I^2 statistic measured the extent of inconsistency among the studies (34). I^2 values of 25%–50% indicate moderate inconsistency, while values larger than 50% reflect large inconsistencies among studies. We assumed a dominant mode of inheritance (the presence of at least 1 variant allele vs. wild-type), because only 1 copy of the variant *NQO1**2 allele is needed to reduce enzyme activity. However, to explore the possibility of a “threshold effect” of enzyme activity, we also explored the analyses for the ALL and AML subsets assuming a recessive mode of inheritance (homozygous variant vs. heterozygote + wild-type); the results were similar to those assuming a dominant model (data not shown). It should be noted that only 1 study did not separate heterozygote and homozygote variants and therefore was not amenable to assessing a recessive mode of inheritance (31).

We evaluated publication bias by using funnel plots (35); however, this assessment was uninformative because of the limited number of studies. The results of the meta-analysis were generated using RevMan software (Review Manager, version 4.2 for Windows; The Nordic Cochrane Centre, Copenhagen, Denmark, 2003). Forest plots were generated using the “plot.meta” function in R software (<http://www-ice.iarc.fr/~martyn/software/meta/>).

META-ANALYSIS RESULTS

Overall effects

Figure 2 presents the results of the 5 previous studies examining the association between the *NQO1**2 polymorphism and risk of childhood ALL by using a random effects model. Only 1 of the 5 studies showed a significant positive association between childhood ALL and the presence of at least one *NQO1**2 allele (16). Overall, the summary odds ratio showed no evidence of association of the *NQO1**2 variant and risk of childhood ALL (OR = 1.08, 95% CI: 0.83, 1.40).

Results of the 3 studies that examined the association between the *NQO1**2 polymorphism and childhood AML are shown in Figure 3. None of the 3 individual studies showed a statistically significant association (27, 29, 32). The combined analysis, using a random effects model showed no effect of the *NQO1**2 variant on the development of childhood AML (OR = 0.90, 95% CI: 0.50, 1.62). Of the

studies that evaluated the relation between the *NQO1**2 polymorphism and *MLL*-positive leukemia (ALL or AML), 3 of 4 showed a positive association (17, 27, 31) (Figure 4), but only 1 study had statistically significant results (17). The combined odds ratio of these 4 studies suggested an increased risk of *MLL*-positive childhood leukemia associated with the presence of at least 1 *NQO1**2 allele (fixed effects model: OR = 1.39, 95% CI: 0.98, 1.97; χ^2 test for heterogeneity: $P = 0.23$). This result was also supported by that of Smith et al. (23) (not included in this meta-analysis), who showed that the *NQO1**2 allele increased risk of *MLL*-positive leukemia (OR = 2.47, 95% CI: 1.08, 5.68).

Results of the 3 studies that examined the associations between risk of childhood ALL or AML with the *MLL-AF4* translocation and the presence of the *NQO1**2 allele were inconsistent (Figure 5). The combined odds ratio for these 3 studies (OR = 1.79, 95% CI: 0.49, 6.55) suggested that the presence of at least 1 *NQO1**2 allele confers an increased risk of *MLL-AF4*-positive childhood leukemia; however, this result must be interpreted with caution as the confidence interval of the combined odds ratio is very wide and driven mainly by the results of 1 study (17).

DISCUSSION

Overall, we did not find any statistically significant association of the *NQO1**2 polymorphism and the risk of childhood ALL. However, a family-based study (28) using trios (biologic parents and the affected child) found that there was excess transmission of the *NQO1**2 variant allele from parents to affected offspring, suggesting that the *NQO1**2 variant was associated with developing childhood ALL.

When restricting to a subset of studies with *MLL*-positive leukemia as the outcome, we observed an elevated risk with borderline statistical significance with the presence of at least 1 variant *NQO1**2 allele (17, 27, 30, 31). Smith et al. (23) also reported that infants with de novo *MLL*-positive leukemias were significantly more likely to be heterozygous for the *NQO1**2 allele (OR = 2.77, 95% CI: 1.17, 6.57; $P = 0.02$) and also significantly more likely to have low/null activity *NQO1* genotypes (*NQO1**1/*2 or *NQO1**2/*2) than patients with de novo B-lineage ALL without *MLL* translocations (OR = 2.47, 95% CI: 1.08, 5.68; $P = 0.03$). This subgroup of infants with de novo *MLL*-positive leukemias were also significantly more likely to have low/null activity *NQO1* genotypes (*NQO1**1/*2 or *NQO1**2/*2) than expected in an ethnically matched population of disease-free subjects (OR = 2.50; $P = 0.02$) (23). The lack of statistically significant results among the *MLL*-positive subgroup in our meta-analysis may be due to the rare occurrence of *MLL*-positive leukemias in infants, but these results are biologically plausible.

A rearrangement in the *MLL* gene at 11q23 is common in leukemias of both infants (<2 years) and adults (as secondary leukemias that arise after chemotherapy with topoisomerase II inhibitors), suggesting that topoisomerase II inhibition may be a common mechanism (36). Many of the compounds that are metabolized by *NQO1* also inhibit

Table 1. Case-Control Studies Reporting the Association Between *NQO1* and Incident Childhood Leukemia That Were Included in the Meta-Analysis, 1999–2005

| First Author, Year, Location (Reference No.) | Study Population | Case Details ^a | Odds Ratio ^b | 95% Confidence Interval | MAF and Genotyping Method | Comments, Concerns, and Bias | |
|--|--|--|-------------------------|-------------------------|--|---|---|
| Clavel, 2005, France (29) | Cases: 219 incident acute leukemia (ALL, $n = 191$; ANLL, $n = 28$) cases diagnosed in Paris, Lyon, Lille, and Nancy from January 1995 to December 1999; aged <15 years; 129 males, 90 females. Controls: 105 frequency matched to cases by age, gender, hospital, and ethnicity and hospitalized for diseases other than cancer or birth defects (mainly from orthopedic department); aged <15 years; 57 males, 48 females. | Acute leukemia ($n = 219$) | | | | *2: 19%; DNA extracted from lymphocytes, and PCR-RFLP was used for genotyping. | Genotypes were tested and found to be in HWE. Population stratification possible but unlikely in this 92% white population. Odds ratios should not have been adjusted for parental socioprofession because it does not affect the <i>NQO1</i> genotype. Selection bias minimized by including different diagnostic categories for controls in case a particular condition was related to the exposures of interest. |
| | | *1/*2 | 0.9 | 0.5, 1.6 | | | |
| | | *2/*2 | 1.7 | 0.4, 6.6 | | | |
| | | ALL ($n = 191$) | | | | | |
| Eguchi-Ishimae, 2005, Japan (27) | Cases: 103 infants diagnosed with acute leukemia according to FAB classification, Southern blotting, cytogenetics, and RT-PCR and registered by the Japan Infant Leukemia Study Group between December 1995 and December 1998; aged <18 months; gender distribution not reported. Controls: 185 healthy newborn Japanese infants' umbilical cord samples; gender distribution not reported. | MLL-positive total ($n = 64$) | 1.44 | 0.80, 2.58 | *2: 34%; *3: 1.9%; DNA extracted from blood, and PCR-RFLP was used for genotyping. | Authors did not report HWE testing, but our calculations show that all genotype frequencies were in HWE among controls. Population stratification unlikely in this Japanese population. Unknown whether controls arose from the same source population as the cases and thus could be prone to selection bias. No replication of genotyping, and thus bias could occur from genotyping error; however, control genotypes were in HWE. Not reported if genotyping calls were blinded to case-control status and could be prone to observer bias. | |
| | | ALL ($n = 49$) | 1.17 | 0.62, 2.21 | | | |
| | | MLL- <i>AF4</i> ($n = 25$) | 1.03 | 0.44, 2.38 | | | |
| | | AML ($n = 15$) | 3.23 | 0.88, 11.80 | | | |
| | | MLL-negative total ($n = 39$) | 1.16 | 0.58, 2.33 | | | |
| | | ALL ($n = 23$) | 1.26 | 0.52, 3.04 | | | |
| | | AML ($n = 16$) | 1.04 | 0.37, 2.90 | | | |
| | | MLL-positive total ^c ($n = 64$) | 2.63 | 0.85, 8.14 | | | |
| | | ALL ^c ($n = 49$) | 3.55 | 1.13, 11.10 | | | |
| | | MLL- <i>AF4</i> ^c ($n = 25$) | 6.36 | 1.84, 21.90 | | | |
| | | MLL-negative total ^c ($n = 37$) | 0.71 | 0.08, 5.92 | | | |
| | | ALL ^c ($n = 21$) | 1.27 | 0.15, 10.87 | | | |
| Kraht, 2004, Austria, Germany, Czechoslovakia (30) | Cases: 138 incident childhood ALL cases diagnosed by FAB criteria, cytochemistry, and multiplex PCR from October 1986 to December 2000 through German, Austrian, and Czech study centers; aged <19 years; 78 males, 60 females. Controls: 190 healthy blood donors with no history of cancer from a German center; 18–68 years; 130 males, 60 females. Restricted to individuals of Caucasian descent. | MLL- <i>AF4</i> ^d ($n = 35$) | 0.79 | 0.36, 1.74 | *2: 17.6%; DNA extracted from blood (controls) or leukemic bone marrow (cases), and SNPs identified by PCR-RFLP. | Genotypes were tested and found to be in HWE. Population stratification unlikely as the subjects were restricted to Caucasians. Controls and cases don't have the same age distribution; thus, this could introduce bias. Controls are adults and composed of volunteer blood donors; therefore, they may not be representative of the source population. Controls were recruited from one medical center in Germany, but the case children were from sites from 3 countries. | |
| | | MLL- <i>AF4</i> , <18 months ^d ($n = 22$) | 0.44 | 0.14, 1.35 | | | |
| | | TEL- <i>AML1</i> ^d ($n = 72$) | 0.92 | 0.52, 1.65 | | | |
| | | BCR- <i>ABL</i> ^d ($n = 31$) | 1.42 | 0.38, 3.78 | | | |
| Krajinovic, 2002, Quebec, Canada (16) | Cases: 174 incident ALL cases (134 pre-B, 23 T-cell, 17 undetermined lineage) diagnosed in a single Montreal hospital from August 1988 to September 1998; 97 males, 77 females; age range, 1–21 years; median age, 5.2 years. Controls: 323 anonymous, healthy, unrelated white individuals recruited from a large, institutional DNA bank serving the same hospital as cases successfully genotyped for <i>NQO1</i> ; not reported if controls were recruited during the same time period as cases; age and gender distribution not reported. Subjects restricted to white, French-Canadian origin and residing in the Province of Quebec as judged by their names, languages, and places of birth. | ALL ($n = 174$) | 1.49 ^e | 1.02, 2.19 ^e | *2: 17.8%; *3: 5%; DNA from buccal cells, peripheral blood, or bone marrow was used to investigate SNPs by PCR-ASO hybridization assays. | Authors did not report HWE testing, but our calculations show that all genotype frequencies were in HWE among controls. Age and health status for controls unknown; this could introduce bias. Population stratification unlikely because there is a founder effect in Quebec that indicates a relative genetic homogeneity. Not reported if genotyping calls were blinded to case-control status and could be prone to observer bias. | |
| | | *1/*2 | 1.54 ^e | 1.04, 2.29 ^e | | | |
| | | *2/*2 | 1.34 ^e | 0.53, 3.37 ^e | | | |

| | | | | | | |
|------------------------------------|--|---|------|-------------|---|--|
| Lanciotti, 2005, Italy (31) | Cases: 50 infant ALL cases diagnosed by banded karyotyping, RT-PCR, and FISH and enrolled in the AIEOP protocol between 1995 and 2003; aged ≤ 12 months; gender distribution not reported. Healthy controls: 147 healthy control children admitted for traumas, acute infections, or minor surgical procedures; gender distribution not reported. | ALL total ($n = 50$) | 1.91 | 1.00, 3.65 | *2 ^d ; DNA was extracted from blood (controls) or bone marrow (cases), and SNPs were identified by PCR-RFLP. | Genotypes were tested and found to be in HWE. Population stratification unlikely in this Italian population. Selection bias possible because it is not reported if cases and controls were admitted to the same hospital or came from the same source population. |
| | | <i>MLL</i> -negative ($n = 18$) | 4.22 | 1.43, 12.49 | | |
| | | <i>MLL</i> -positive ($n = 32$) | 1.26 | 0.58, 2.74 | | |
| Sirma, 2004, Turkey (32) | Cases: 273 (189 ALL, 84 AML) cases diagnosed between 1996 and 2001 in 2 different medical faculties in Istanbul, Turkey, according to standard morphologic, cytochemical, and immunophenotypic criteria; aged 1–16 years (median ages, 8 (ALL) and 9 (AML) years); 128 females, 145 males. Controls: 286 healthy volunteers from different geographic areas of Turkey; aged 2–60 years (median, 19 years); 141 females, 145 males. | ALL | 0.75 | 0.25, 2.23 | *2: 24.8%; DNA was extracted from blood, and SNPs were identified by using PCR-RFLP genotyping. | Genotypes were tested and found to be in HWE. Controls and cases don't have the same age distribution; thus, this could introduce bias. Cases and controls are drawn from different source populations and could have very different exposure histories. Population stratification unlikely in this Turkish population. |
| | | ALL *2 allele | 0.79 | 0.58, 1.08 | | |
| | | AML | 0.67 | 0.15, 3.13 | | |
| | | AML *2 allele | 0.71 | 0.46, 1.09 | | |
| | | ALL + AML | 0.73 | 0.27, 1.94 | | |
| ALL + AML *2 allele | 0.76 | 0.58, 1.01 | | | | |
| Wiemels, 1999, United Kingdom (17) | Cases: 115 pediatric leukemia cases diagnosed by banded karyotyping, FISH, and RT-PCR and enrolled in the UKCCS between 1991 and 1996; aged < 15 years; gender distribution not reported. Controls: 100 umbilical cord samples from healthy newborn infants; gender distribution not reported. Subjects were $> 90\%$ white and $< 10\%$ from minority groups (Asian, Oriental, Afro-Caribbean, black). | <i>MLL</i> -positive total ($n = 36$) | 2.54 | 1.08, 5.96 | *2: 17%; *3: 5.5%; DNA was extracted from blood, and SNPs were identified by using PCR-RFLP genotyping. | Authors did not report HWE testing, but our calculations show that all genotype frequencies were in HWE among controls. Unknown whether controls arose from the same source population as the cases and thus could be prone to selection bias. No replication of genotyping, and bias could occur from genotyping error; however, control genotypes were in HWE. Not reported if genotyping calls were blinded to case-control status and could be prone to observer bias. |
| | | <i>MLL-AF4</i> ($n = 21$) | 8.63 | 2.45, 33.22 | | |
| | | <i>TEL-AML1</i> ($n = 50$) | 1.52 | 0.71, 3.25 | | |
| | | Hyperdiploid ($n = 29$) | 0.91 | 0.33, 2.38 | | |

Abbreviations: AIEOP, Italian Pediatric Hematology and Oncology Group; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ANLL, acute nonlymphocytic leukemia; ASO, allele-specific oligoprimers; FAB, French-American-British; FISH, fluorescence in situ hybridization; HWE, Hardy-Weinberg equilibrium (calculated among controls); MAF, minor allele frequency (among controls); PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RT, reverse transcription; SNP, single nucleotide polymorphism; UKCCS, United Kingdom Childhood Cancer Study.

^a The referent is *1/*1. The odds ratio is for *1/*2 + *2/*2 genotypes combined unless otherwise specified.

^b All odds ratios were unadjusted except those for the study by Clavel et al. (29) that were adjusted for parental socioprofessional category and matching factors (age, gender, hospital, ethnicity).

^c Odds ratio for *1/*3 + *3/*3 genotypes combined.

^d There were insufficient numbers for the *2/*2 genotype, and therefore it was not included in calculations.

^e Odds ratios and 95% confidence intervals were calculated by using genotype frequencies extracted from haplotype frequencies presented in Table III of Krajinovic et al. (16).

^f Not able to calculate the frequency of the *NQO1**2 allele as the authors did not present separate genotype frequencies for heterozygote and homozygote variants.

topoisomerase II, an enzyme involved in maintaining DNA structure through cleavage and subsequent repair of double-stranded DNA. Inhibition of topoisomerase II activity can ultimately result in chromosomal translocations, as has been evidenced by the ability of dietary flavonoids to cleave the *MLL* gene (37).

However, the mechanism by which *MLL* breakage and subsequent translocation occur still remains to be discovered: Topoisomerase II inhibition, as well as bioactivation of reactive metabolites, and formation of reactive oxygen species have been suggested. The double-stranded DNA breaks created by topoisomerase II inhibition can stimulate apoptosis, which leads to the activation of endonucleases that cause further DNA breaks. Wild-type *NQO1* has been shown to sensitize cells to undergo apoptosis by tumor necrosis factor- α and also to stabilize the p53 tumor suppressor protein, whereas *NQO1**2 does not (38, 39). Therefore, it is also possible that the *NQO1**2 variant can enable cells to survive DNA damage, thereby increasing the likelihood of survival with DNA breaks and translocations.

The overall results of the meta-analysis may be biased because of confounding and population stratification that may be present in the individual case-control studies included. However, many of the case-control studies included in this meta-analysis had relatively homogeneous ethnic/racial populations (16, 17, 27, 30–32) and/or were matched for ethnicity (29).

The largely negative results among childhood leukemias (outside the *MLL* group) may be a reflection of low bioactivation in precursor B-cells (i.e., low myeloperoxidase) and also low relevant exposures in the pediatric population. There may also be unrecognized gene-gene and gene-environment interactions that have yet to be elucidated. The elevated risk in the *MLL* group and in particular the *MLL-AF4* group (17, 23) suggests that the *MLL* gene or the *AF4* gene is particularly sensitive to reactive quinones in the bone marrow. This result was not upheld in the Japanese population (27), leaving open the suggestion that the *NQO1**2 allele may be linked to a second causal allele within this gene-rich region of chromosome 16 in non-Japanese populations.

Furthermore, the differences in worldwide incidence rates of childhood leukemia and the worldwide differences in the observed ratios of ALL to AML could be due to the variation of environmental exposures among regions; for example, differences in exposure profiles to infectious agents have also been suggested (40). As suggested by Kracht et al. (30), the specific exposure histories for leukemia cases may reflect differences in exposure profiles among countries.

BIAS

The results of the meta-analysis were most suggestive of the role of low/null activity *NQO1**1/*2 and *NQO1**2/*2 genotypes in *MLL*-positive leukemia. Among the 4 studies included in this subgroup analysis, the study by Wiemels et al. (17) was the most influential. Therefore, we also re-assessed the results by dropping this study and found weak evidence of association between *NQO1**1/*2 and *NQO1**2/*2

variant genotypes and *MLL*-positive infant leukemia (OR = 1.19, 95% CI: 0.80, 1.77).

The different choice of control groups (Table 1) could bias the results of the individual studies and lead to inconsistencies between studies; however, the direction and magnitude of this bias are uncertain. For example, hospital-based controls may not necessarily be representative of the underlying source population, and therefore population-based controls may be a better comparison group. Use of controls that are older than cases can introduce a bias because older subjects could have a very different exposure history and also a different response to toxic exposures (because of differences in body size) than younger subjects. Controls selected from different geographic areas than those of the cases can also introduce bias because the cases and controls could have different genetic ancestries and exposure histories. Lack of quality control measures used during genotyping could introduce misclassification bias from genotyping error. Furthermore, the genotyping calls could be prone to bias if the observer was not blinded to case-control status. Although we used random effects models to account for heterogeneity between studies, results from fixed effects models were similar.

We also evaluated bias in our results using 3 criteria (amount of evidence, replication, and protection from bias) published in the interim guidelines for the assessment of cumulative evidence on genetic associations (41). For the amount of evidence, all subgroup analyses (Figures 2–5) contained a moderate number of subjects studied with the *NQO1**2 allele. Furthermore, there was no obvious bias incurred from phenotype misclassification or population stratification; however, there was insufficient information on quality control procedures for genotyping (Figures 2–5; Table 1). To assess replication of the associations, we used the I^2 values that ranged from 30.9% to 83.3%. The totality of evidence for the association of the *NQO1**2 allele with childhood leukemia, as suggested by these guidelines, is moderate for acute lymphoblastic and *MLL*-positive leukemia but weak for acute myeloid and *MLL-AF4*-positive leukemia.

POPULATION TESTING

Currently there is insufficient evidence to establish the role of *NQO1* in childhood leukemia and, thus, population testing for polymorphisms in this gene has not been implemented.

OTHER POTENTIAL PUBLIC HEALTH APPLICATIONS

There are currently not enough data to support any public health applications.

CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

Results of studies examining the effect of polymorphisms in *NQO1* on childhood leukemia have been inconsistent, and this is the first meta-analysis to systematically summarize

| First Author, Location (Reference No.) | *1/*2 + *2/*2 Cases | Total Cases | *1/*2 + *2/*2 Controls | Total Controls | Weight, % | Odds Ratio | 95% CI |
|--|---------------------|-------------|------------------------|----------------|-----------|------------|------------|
| Clavel, France (29) | 69 | 105 | 122 | 190 | 15.5 | 1.07 | 0.65, 1.76 |
| Eguchi-Ishimae, Japan (27) | 43 | 152 | 29 | 117 | 17.5 | 1.20 | 0.69, 2.07 |
| Krajinovic, Canada (16) | 71 | 173 | 103 | 324 | 19.2 | 1.49 | 1.02, 2.19 |
| Lanciotti, Italy (31) | 62 | 118 | 94 | 185 | 23.6 | 1.07 | 0.68, 1.70 |
| Sirma, Turkey (32) | 73 | 205 | 116 | 270 | 24.2 | 0.73 | 0.51, 1.07 |
| Overall | 318 | 753 | 464 | 1,086 | 100.0 | 1.08 | 0.83, 1.40 |

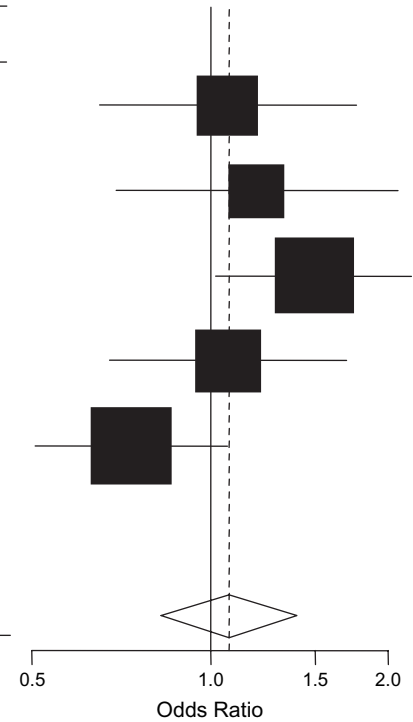


Figure 2. Forest plot of *NQO1* and childhood acute lymphoblastic leukemia, random effects model. Test for heterogeneity: $\chi^2 = 6.98$, $df = 4$ ($P = 0.14$), $I^2 = 42.7\%$; test for overall effect: $Z = 0.54$ ($P = 0.59$). The odds ratio for each study is represented by a black square that is proportional to the sample size; the horizontal line shows the corresponding 95% confidence interval (CI). A dashed line marks the combined estimate, while the vertical solid line represents the null result.

| First Author, Location (Reference No.) | *1/*2 + *2/*2 Cases | Total Cases | *1/*2 + *2/*2 Controls | Total Controls | Weight, % | Odds Ratio | 95% CI |
|--|---------------------|-------------|------------------------|----------------|-----------|------------|------------|
| Clavel, France (29) | 8 | 44 | 20 | 88 | 25.6 | 0.76 | 0.30, 1.88 |
| Eguchi-Ishimae, Japan (27) | 21 | 130 | 10 | 98 | 29.7 | 1.70 | 0.76, 3.79 |
| Sirma, Turkey (32) | 30 | 162 | 54 | 208 | 44.7 | 0.65 | 0.39, 1.07 |
| Overall | 59 | 336 | 84 | 394 | 100.0 | 0.90 | 0.50, 1.62 |

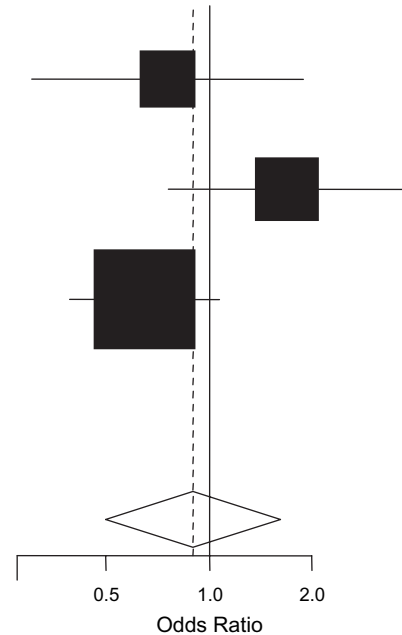


Figure 3. Forest plot of *NQO1* and childhood acute myeloid leukemia, random effects model. Test for heterogeneity: $\chi^2 = 4.00$, $df = 2$ ($P = 0.14$), $I^2 = 50.0\%$; test for overall effect: $Z = 0.36$ ($P = 0.72$). The odds ratio for each study is represented by a black square that is proportional to the sample size; the horizontal line shows the corresponding 95% confidence interval (CI). A dashed line marks the combined estimate, while the vertical solid line represents the null result.

| First Author, Location (Reference No.) | *1/*2 + *2/*2 Cases | Total Cases | *1/*2 + *2/*2 Controls | Total Controls | Weight, % | Odds Ratio | 95% CI |
|---|------------------------|----------------|---------------------------|-------------------|--------------|---------------|------------|
| Eguchi-Ishimae, Japan (27) | 41 | 150 | 23 | 111 | 36.6 | 1.44 | 0.80, 2.58 |
| Kracht, Austria, Germany, Czech (30) | 10 | 74 | 25 | 151 | 27.1 | 0.79 | 0.36, 1.74 |
| Lanciotti, Italy (31) | 14 | 70 | 18 | 109 | 21.5 | 1.26 | 0.58, 2.74 |
| Wiemels, United Kingdom (17) | 20 | 53 | 16 | 83 | 14.8 | 2.54 | 1.17, 5.53 |
| Overall | 85 | 347 | 82 | 454 | 100.0 | 1.39 | 0.98, 1.97 |

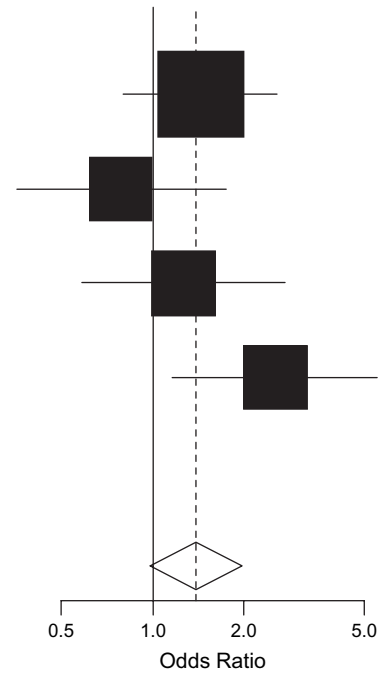


Figure 4. Forest plot of *NQO1**2 and *MLL*-positive leukemia (acute lymphoblastic leukemia or acute myeloid leukemia), fixed effects model. Test for heterogeneity: $\chi^2 = 4.34$, $df = 3$ ($P = 0.23$), $I^2 = 30.9\%$; test for overall effect: $Z = 1.83$ ($P = 0.07$). The odds ratio for each study is represented by a black square that is proportional to the sample size; the horizontal line shows the corresponding 95% confidence interval (CI). A dashed line marks the combined estimate, while the vertical solid line represents the null result.

| First Author, Location (Reference No.) | *1/*2 + *2/*2 Cases | Total Cases | *1/*2 + *2/*2 Controls | Total Controls | Weight, % | Odds Ratio | 95% CI |
|---|------------------------|----------------|---------------------------|-------------------|--------------|---------------|-------------|
| Eguchi-Ishimae, Japan (27) | 14 | 123 | 11 | 99 | 34.5 | 1.03 | 0.44, 2.38 |
| Kracht, Austria, Germany, Czech (30) | 10 | 74 | 25 | 151 | 35.1 | 0.79 | 0.36, 1.74 |
| Wiemels, United Kingdom (17) | 17 | 50 | 4 | 71 | 30.4 | 8.63 | 2.69, 27.70 |
| Overall | 41 | 247 | 40 | 321 | 100.0 | 1.79 | 0.49, 6.55 |

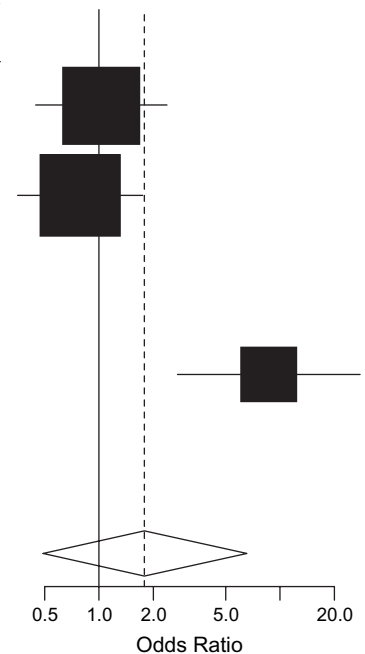


Figure 5. Forest plot of *NQO1**2 and *MLL-AF4* leukemia (acute lymphoblastic leukemia or acute myeloid leukemia), random effects model. Test for heterogeneity: $\chi^2 = 11.98$, $df = 2$ ($P = 0.002$), $I^2 = 83.3\%$; test for overall effect: $Z = 0.88$ ($P = 0.38$). The odds ratio for each study is represented by a black square that is proportional to the sample size; the horizontal line shows the corresponding 95% confidence interval (CI). A dashed line marks the combined estimate, while the vertical solid line represents the null result.

the association of the *NQO1**2 polymorphism and the risk of childhood leukemia by immunophenotypic and cytogenetic subtype, when possible. A more homogeneous and consistent classification of the outcome would increase statistical power to detect any significant associations. Therefore, future studies should also cytogenetically classify leukemia cases. Such efforts will understandably require large numbers of patients to assess statistically meaningful cytogenetic subgroups, requiring coordinated collaboration among epidemiology study groups.

We conclude that the *NQO1**2 variant allele appears to have no strong association with childhood ALL or AML, but it may be associated with *MLL*-positive childhood leukemias. However, these results should be interpreted with caution, as this meta-analysis included only a few studies with small sample sizes. Furthermore, the suggested increase in risk of *MLL*-positive childhood leukemia among the *NQO1**2 allele carriers was driven mainly by the results of 1 case-control study but was also supported by 1 additional case-only study. This emphasizes the need for further replication of these findings. Because this is a rare subtype, this would be a prime candidate for a consortium-based effort.

Because the presence of a functional polymorphism in *NQO1* may not confer an increased risk of childhood leukemia alone, but rather in the presence of an environmental exposure to quinones, in future studies it would also be important to incorporate both the effects of environmental agents and genotypes of other enzymes (e.g., *GSTs*, *CYP2E1*, *MPO*) that metabolize these carcinogenic substances to toxic quinones and reactive oxygen species (22). Such investigations will require larger sample sizes to permit assessment of first- and, possibly, second-order interactions.

Some of the heterogeneity between the risk ratios of individual studies may simply be a result of unmeasured cofactors that vary among the populations studied here; therefore, inconsistent results between studies warrant detailed exploration. Because *NQO1**2 is a highly penetrant marker and metabolizes a number of putative leukemogens, it remains an important candidate risk factor for leukemia and deserves further analysis.

Our meta-analysis of case-control studies did not provide evidence for a statistically significant effect of *NQO1**2 in childhood ALL, yet a family-based study (28) did demonstrate an overtransmission of *NQO1**2 among ALL cases. These conflicting results underscore the need to incorporate genetic markers of ancestry for addressing the bias that may result from potential population stratification in non-family-based study designs (i.e., case-control studies), especially given the wide population differences in *NQO1**2 allele frequencies (42–44). In addition, family-based designs such as case-parent trios should be encouraged (44, 45). Future studies are needed in Asians and Hispanics, populations that have a higher occurrence of the *NQO1**2 polymorphism, in order to better understand its role in childhood leukemia in these populations. In addition, other SNPs in *NQO1*—such as the less-studied C465T functional variant (*NQO1**3), other haplotype-tagging SNPs, and SNPs in neighboring genes—should be further evaluated to comprehensively assess the importance of *NQO1* in the development of childhood leukemia.

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