

# NAD(P)H:quinone oxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: A HuGE review

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NAD(P)H:quinone oxidoreductase (NQO1) catalyzes the two- or four-electron reduction of numerous endogenous and environmental quinones (e.g., the vitamin E  $\alpha$ -tocopherol quinone, menadione, benzene quinones). In laboratory animals treated with various environmental chemicals, inhibition of NQO1 metabolism has long been known to increase the risk of toxicity or cancer. Currently, there are 22 reported single-nucleotide polymorphisms (SNPs) in the *NQO1* gene. Compared with the human consensus (reference, "wild-type") *NQO1*\*1 allele coding for normal NQO1 enzyme and activity, the *NQO1*\*2 allele encodes a nonsynonymous mutation (P187S) that has negligible NQO1 activity. The *NQO1*\*2 allelic frequency ranges between 0.22 (Caucasian) and 0.45 (Asian) in various ethnic populations. A large epidemiologic investigation of a benzene-exposed population has shown that *NQO1*\*2 homozygotes exhibit as much as a 7-fold greater risk of bone marrow toxicity, leading to diseases such as aplastic anemia and leukemia. The extent of the contribution of polymorphisms in other genes involved in the metabolism of benzene and related compounds—such as the P450 2E1 (*CYP2E1*), myeloperoxidase (*MPO*), glutathione-S-transferase (*GSTM1*, *GSTT1*), microsomal epoxide hydrolase (*EPHX1*), and other genes—should also be considered. However, it now seems clear that a lowered or absent NQO1 activity can increase one's risk of bone marrow toxicity, after environmental exposure to benzene and benzene-like compounds. In cancer patients, the *NQO1*\*2 allele appears to be associated with increased risk of chemotherapy-related myeloid leukemia. Many other epidemiological studies, attempting to find an association between the *NQO1* polymorphism and one or another human disease, have now begun to appear in the medical literature. **Genet Med 2002;4(2):62–70.**

**Key Words:** NAD(P)H:quinone oxidoreductase (*NQO1*) gene polymorphism, benzene exposure, benzene metabolism, myelodysplastic syndrome, *CYP2E1*, therapy-related acute myeloid leukemia

## ENZYME AND GENE

NAD(P)H:quinone oxidoreductase (NQO1), originally referred to as DT-diaphorase,<sup>1</sup> is a flavoenzyme that plays an important role in protection against endogenous and exogenous quinones by catalyzing two- or four-electron reductions of these substrates.<sup>2</sup> Quinone compounds are present within our bodies (e.g., vitamin K) and in our natural environment (e.g., urushiol, the active chemical in poison ivy). The two- and four-electron reductions catalyzed by NQO1 are beneficial to the cell by preventing redox cycling, which leads to the generation of free radicals; therefore, NQO1 protects the cell from unwanted oxidative damage.<sup>3–5</sup>

The human *NQO1* gene (formerly called *DIA4*) is located on chromosome 16q22.1<sup>6</sup>; the gene spans approximately 17 kb and has six exons. Three of four polyadenylation sites in exon 6 can result in transcripts of 1.2 kb, 1.7 kb, and 2.7 kb in length.<sup>7</sup> A distantly related *NQO2* gene has seven exons and resides on chromosome 6p25; its gene product uses dihydronicotinamide riboside (NRH) instead of NAD(P)H as its electron donor.<sup>8</sup> Both *NQO1* and *NQO2* are induced by oxidative stress, dioxin, and polycyclic aromatic hydrocarbons such as those found in combustion processes (e.g., cigarette smoke, urban smog).

## Gene variants

MEDLINE and PubMed were searched by using the keywords "diaphorase, NQO1, NQO2, gene, polymorphism, benzene, metabolism, leukemia, CYP2E1, myeloperoxidase, glutathione-S-transferase, epoxide hydrolase, alcohol dehydrogenase, aldehyde dehydrogenase, aldoketoreductase, dihydrodiol dehydrogenase, muconic acid." The names of all genes encoding these enzymes were confirmed on UniGene. Everything thus identified, between 1975 and December of 2001, was downloaded and/or copied from library journals and then

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scrutinized. Web sites that we found to be especially useful are also listed at the end of this review.

In a study of the NQO1 cDNA in 10 human colon carcinoma cell lines,<sup>9</sup> a nucleotide substitution, c609C>T, leading to a nonsynonymous mutation (P187S) was found to be associated with a loss of enzyme activity. Homozygous patients having the defective NQO1\*2 allele show negligible NQO1 enzymic activity, whereas NQO1\*1/\*2 heterozygotes exhibit activities intermediate between that in the normal NQO1\*1/\*1 and mutant NQO1\*2/\*2 homozygotes. The frequency of NQO1\*2/\*2 homozygosity is now known to range between 1.5% and 20.3% in several ethnic populations<sup>2,10</sup>; given the Hardy-Weinberg Distribution ( $p^2 + 2pq + q^2$ ), the allelic frequency of NQO1\*2 thus ranges from 0.22 to 0.45 (Table 1). It has become increasingly appreciated (reviewed in Nebert and Menon<sup>11</sup>), however, that the use of ethnic classifications—such as “non-Hispanic White,” “Mexican-American,” and “African American”—are not “genetically sound,” because these groups reflect varying degrees of ethnic admixture, especially during the past five centuries.

## DISEASES

Because NQO1 has been associated with detoxification of numerous endogenous and foreign compounds, it seems likely that the lack of NQO1 activity might increase the risk of certain types of toxicity and cancer. Clinical evidence—summarized in this review—is accumulating that high NQO1 activity does indeed play a role in lowering the risk of toxicity associated with exposures to environmental chemicals (e.g., benzene, cigarette smoking, chemotherapy), and decreasing the risk of certain types of cancer. The *Nqo1*(-/-) knockout mouse has been shown to be more sensitive to quinone toxicity,<sup>12</sup> but no cancer studies with this animal have yet been reported.

### Toxicity of benzene

The metabolism of benzene<sup>13</sup> involves several key enzymes (Fig. 1). Benzene oxide and oxepin are formed in the liver by cytochrome P450 2E1 (CYP2E1). The oxide is converted non-enzymatically to phenol, which, in turn, may be further metabolized by CYP2E1 to di- and tri-hydroxybenzenes. Myeloper-

oxidase (MPO) can convert the intermediates to highly reactive and toxic free radical semiquinones and quinones. NQO1 reduces benzoquinones to hydroquinone and catechol, resulting in detoxification. Glutathione-S-transferases are also involved in detoxification, by converting the oxide in the first of four steps to the nontoxic S-phenylmercapturic acid; conversely, benzene oxepin can be converted by means of alcohol and aldehyde dehydrogenases to the toxic metabolite *trans*, *trans*-muconaldehyde.

Recent data suggest that normal NQO1 activity may protect individuals from benzene toxicity of the hematopoietic system. Benzene is a widely used industrial solvent and is a by-product of combustion (e.g., fuel exhaust and cigarette smoke). Benzene's toxicity in the bone marrow can lead to various forms of blood dyscrasias.<sup>18</sup> In particular, benzene and its metabolites target the bone marrow—causing progressive leukocytopenia, anemia, thrombocytopenia, and even pancytopenia.<sup>19,20</sup> The toxic process for benzene begins with the production of CYP2E1-mediated phenols in the liver. Subsequently, these benzene mono-, di-, and tri-hydroxy compounds are believed to travel to the bone marrow where MPO converts the phenols to several quinones, which are the ultimate toxic agents. However, NQO1, present in the bone marrow as well as most other tissues of the body, is protective in that this enzyme is able to convert quinone compounds to the less toxic hydroquinone. Most benzene metabolites are excreted in the urine within 48 hours after environmental exposure.<sup>13,21,22</sup>

Benzene has also been associated with several forms of leukemia known as the myelodysplastic syndrome (MDS).<sup>2</sup> MDS is a collective term that includes the diseases of acute myeloblastic leukemia (AML), acute nonlymphocytic leukemia (ANLL), and acute lymphocytic leukemia (ALL).

### Clinical studies of benzene exposure

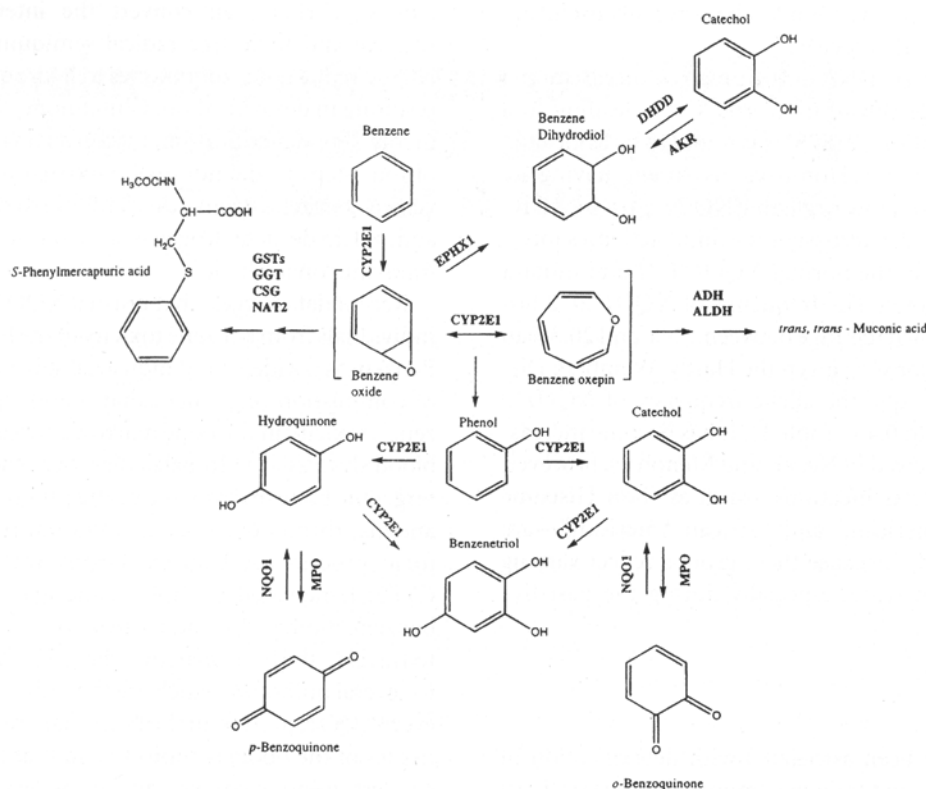
Most of the epidemiologic data to date have been collected from a single large cohort of 74,828 workers occupationally exposed to benzene in 672 factories in Shanghai, China.<sup>23</sup> Results from this study showed that ambient benzene levels in the work place as low as 10 parts-per-million (ppm) can produce toxic effects.

**Table 1**  
Frequency of NQO1 genotypes in population studies

Ethnic groups	NQO1*1/*1	NQO1*1/*2	NQO1*2/*2	N	Frequency of NQO1*2 allele
Non-Hispanic White	64 (56.1%)	45 (39.5%)	5 (4.4%)	114	0.22
Mexican-American	52 (32.3%)	84 (52.2%)	25 (15.5%)	161	0.39
African-American	83 (61.0%)	46 (33.8%)	7 (5.2%)	136	0.23
Asian <sup>a</sup>	37 (31.4%)	57 (48.3%)	24 (20.3%)	118	0.45

All these data are derived from Ref. 10.

<sup>a</sup> This refers to a combination of Korean and Chinese individuals.



**Fig. 1** Diagram of the metabolic pathways of benzene. GSTs, glutathione-S-transferases GSTM1 and GSTT1; GGT,  $\gamma$ -glutamyltransferase; CSG, cysteinylglycinase; NAT2, *N*-acetyltransferase-2; EPHX1, microsomal epoxide hydrolase; DHDD, dihydrodiol dehydrogenase dimeric form; AKR, one of several aldoketoreductases; ADH, one of several alcohol dehydrogenases; ALDH, one of several aldehyde dehydrogenases.<sup>14</sup> See text for other abbreviations. EPHX1,<sup>15</sup> DHDD,<sup>16</sup> and AKR<sup>14</sup> are known to participate in the benzene metabolic pathway, but their degree of importance in protection against benzene toxicity in bone marrow is unclear. Although catechol *O*-methyltransferase (COMT) activity is known to be inhibited by di- and tri-hydroxybenzenes,<sup>17</sup> it has not yet been established whether the benzene catechol might be further metabolized by COMT.

### Possible involvement of polymorphisms in genes other than *NQO1*

Polymorphisms have been described for the *MPO* gene,<sup>24</sup> many of the *GST* genes,<sup>25–27</sup> and the *EPHX1* gene,<sup>28</sup> but no studies of any of these polymorphisms in benzene-exposed workers have yet been reported. Individuals having variant alleles in the *MPO* gene that cause decreased or absent *MPO* activity would be expected to produce less of the benzene free-radical semiquinones and reactive quinones and thus should be at lower risk for benzene-induced hemotoxicity. Individuals having defective *EPHX1* alleles that cause decreased or absent *EPHX1* activity would be expected to produce more of the benzene free-radical semiquinones and reactive quinones (pushing the pathway more in the direction of *MPO*; Fig. 1) and, therefore, might be at greater risk for benzene-induced marrow toxicity.

The role of *GST* gene polymorphisms in susceptibility to benzene toxicity or MDS is currently unclear. The incidence of the deleted *GSTM1* or *GSTT1* gene (“null alleles” *GSTM1*\*0, *GSTT1*\*0) ranges between 20% and 50% in various ethnic populations; some associations between these null alleles and enhanced or diminished risk of cancer or toxicity have been reported, depending on the etiologic agent.<sup>29</sup> Interestingly, these same two *GST* genes appear to be especially involved in the detoxification of arene oxides such as benzene oxide.<sup>22</sup> One study reported a correlation between the *GSTT1*\*0 null allele

and an increased risk of MDS,<sup>30</sup> whereas another study, involving a greater sample size, found no such association.<sup>31</sup> Hypothetically, an individual with low or negligible *NQO1*, *EPHX1*, and *GST* activities, combined with extra-high *CYP2E1* and *MPO* activities, would be predicted to exhibit the greatest risk for benzene-induced toxicity. In animal model systems, although the mouse *Gstp* gene cluster has been knocked out and these animals show resistance to acetaminophen toxicity,<sup>32</sup> mouse lines with knockouts of their *Gstm* or *Gstt* gene clusters have not yet been generated.

### ASSOCIATIONS

In searching for associations between the *NQO1* polymorphism, benzene-induced toxicity, and other medical diseases, our search strategy was identical to that described under “Gene variants.” As described above, the majority of the epidemiologic data were collected from a large cohort of 74,828 workers occupationally exposed to benzene in Shanghai, China.<sup>23</sup> A follow-up study<sup>33</sup> then confirmed an association between an increased incidence of benzene-induced hemotoxicity in individuals having the mutant *NQO1*\*2 allele; these data strongly suggest a role for normal *NQO1* activity in both chemoprotection and chemoprevention.

Further genetic analysis of this cohort included a case-control study ( $N = 50$  exposed workers,  $N = 50$  unexposed controls) in which a possible link between benzene exposure and acute nonlymphatic leukemia (ANLL) was investigated.<sup>33</sup> Individuals were genotyped for the consensus and variant allele of the *NQO1* gene and the consensus versus one variant allele of the *CYP2E1* gene. Individuals were also phenotyped for *CYP2E1* metabolism by measuring urinary 6-chlorozoxazone formation. Persons with *CYP2E1* extensive metabolism (EM) and deficient *NQO1* activity (Fig. 1) would be expected to accumulate more toxic intermediates in their bloodstream and, therefore, be at greater risk for benzene poisoning than those with *CYP2E1* poor metabolism (PM) and normal *NQO1* activity. This is what was found (Table 2). Although the combined *CYP2E1* EM phenotype plus *NQO1*\*1/\*2 genotype showed a 2.7-fold increased risk, the combined *CYP2E1* EM phenotype plus *NQO1*\*2/\*2 genotype exhibited a 7.8-fold increased risk. Thus these data suggest that *CYP2E1* is involved in enhancing, and *NQO1* is involved in protecting against, benzene-induced hemotoxicity. In laboratory animal studies, the *Cyp2e1*(-/-) knockout mouse is very resistant to benzene toxicity,<sup>34</sup> confirming the importance of *CYP2E1* in this model system.

In this large retrospective cohort study, Rothman and coworkers<sup>33</sup> first calculated a 7.6-fold increased risk for the development of ANLL and other related MDS when a *CYP2E1* EM phenotype plus *NQO1*\*2 homozygote is occupationally exposed to benzene (Table 2). Rothman et al. analyzed this positive association further by using a case-control study design, nested within the original Shanghai cohort.<sup>23</sup> In a case-control study such as this, relative risk is estimated as the odds ratio (OR), comparing cases with controls. Individuals were genotyped for the *NQO1* mutant allele and for one of the *CYP2E1* mutant alleles, and they were phenotyped for *CYP2E1* efficient versus poor metabolism (EM, PM trait). *CYP2E1* enzymatic activity was determined by the fractional excretion of urinary 6-hydroxychlorzoxazone (Table 2). A correlation co-

efficient of 0.89 indicated a strong relationship between the *CYP2E1* genotype and the *CYP2E1* phenotype. Unfortunately, however, the study by Rothman et al.<sup>33</sup> examined only 1 (the *CYP2E1*\*5B allele) of the 12 *CYP2E1* variant alleles that have been characterized to date.<sup>35</sup>

As with any case-control study, the potential for misclassification bias must be addressed. Therefore, the white blood count was adjusted to  $\leq 3,500/\mu\text{L}$ , to decrease or remove all those individuals misclassified as benzene-poisoned. Controls and cases were matched by 5-year age intervals and sex. Associations were obtained using unconditional logistic regression. The data were adjusted for possible confounding factors—such as age, sex, and cumulative benzene exposure—and the standard two-tailed  $P$  value of  $<0.05$  was defined as statistically significant. The cases and controls were similar demographically. Regardless of whether the patients were of the *NQO1*\*1/\*2 or *NQO1*\*2/\*2 genotype (Table 2), the adjusted OR for the *CYP2E1* EM versus PM phenotype was the same, as to their influence on susceptibility to benzene toxicity. An approximate 2.7-fold risk for benzene toxicity was associated with either of these phenotypes,<sup>33</sup> implicating a contribution of *CYP2E1* metabolism to the process of benzene toxicity. When the two phenotypes were combined, however, the OR rose to 7.6 (CI, 1.8–31.2). This confidence interval is not as wide as that in the cohort study, but still suffers from a small sample size. It should also be noted that, although this increased risk was found in Asians, one might not be able to readily extrapolate these conclusions to relative risk determinations in other ethnic populations.

## INTERACTIONS

### *NQO1*\*2 allele and other diseases

Hematological toxicity and malignancies after benzene exposure are not the only phenotypes associated with the *NQO1* polymorphism. *NQO1*\*2 homozygosity appears to be correlated with an increased risk of renal cell cancer and urothelial cell carcinoma (Table 3), with OR values of 1.7 and 3.6, respectively<sup>36</sup>; there is also a heightened predisposition toward urolithiasis (kidney stones) with an OR of 2.97.<sup>37</sup>

For patients with renal cell carcinoma and urothelial carcinoma,<sup>36</sup> all 95% confidence intervals in this study contained the value of 1.0, however, except for the renal cell carcinoma *NQO1*\*1/\*2 heterozygotes compared with controls (Table 3). Again, a loss in power is likely due to the small study population (renal cell carcinoma patients,  $N = 131$ ; urothelial carcinoma patients,  $N = 99$ ; and controls,  $N = 260$ ). Moreover, these studies involved predominant German citizens (non-Hispanic White), so that these effects seen in Caucasians might not necessarily be able to be extrapolated to other ethnic groups.

The distribution of the *NQO1*\*2 allele in urolithiasis patient populations<sup>37</sup> was significantly higher, based on Mantel-Haenszel chi-squared analysis ( $P = 0.003$ ). Heterozygotes experienced a 1.8-fold increased risk of urolithiasis (95% CI, 1.17–2.86). The OR for the homozygotes was higher, at 2.97;

**Table 2**

Combined effects of the *NQO1* genotype and *CYP2E1* phenotype on the risk of benzene poisoning in the Chinese cohort

<i>CYP2E1</i> metabolism <sup>a</sup>	<i>NQO1</i> genotype <sup>b</sup>	OR (95% CI) <sup>c</sup> [no. of cases]	OR <sub>adj</sub> <sup>d</sup> (95% CI)
Poor	*1/*2	1.0 [8]	1.0
Poor	*2/*2	2.4 (0.6–9.7) [6]	2.7 (0.6–11.8)
Extensive	*1/*2	2.9 (1.0–8.2) [21]	2.7 (0.9–8.0)
Extensive	*2/*2	7.6 (1.8–31.2) [13]	7.8 (1.9–32.5)

All these data are derived from Ref. 33.

<sup>a</sup> *CYP2E1* metabolic phenotyping, as measured by the fractional excretion of urinary chlorzoxazone as 6-hydroxychlorzoxazone over an 8-hr period.

<sup>b</sup> Individuals who are heterozygous versus homozygous for the *NQO1*\*2 allele.

<sup>c</sup> Odds ratios and confidence intervals are given only for the matching variables of age and sex.

<sup>d</sup> Odds ratio adjusted for age, sex, body mass index, and alcohol and cigarette use. One additional control was deleted, due to missing data.

**Table 3**  
Frequency of *NQO1* genotypes in patient studies

Patient group	<i>NQO1</i> *1/*1	<i>NQO1</i> *1/*2	<i>NQO1</i> *2/*2	N	Frequency of <i>NQO1</i> *2 allele	Ref.
Controls	195 (75.0%)	61 (23.5%)	4 (1.5%)	260	0.039	36
Renal cell carcinoma	84 (64.1%)	44 (33.6%)	3 (2.3%)	131	0.048	36
Urothelial carcinoma	68 (68.7%)	26 (26.3%)	5 (5.1%)	99	0.071	36
Controls	202 (74.5%)	65 (24.0%)	4 (1.5%)	271	0.039	37
Urolithiasis	85 (60.7%)	50 (35.7%)	5 (3.6%)	140	0.06	37

All these data are derived from Northern European (German) populations.

however, the 95% CI (0.78–11.33) contains the value of 1.0. Small-sample size is likely to explain the lack of statistical significance of this OR, especially because an increased risk with the homozygous *NQO1*\*2 genotype is supported in other studies. These types of molecular epidemiologic association studies with borderline significance, and based on a single nucleotide substitution, must be interpreted, however, with a great deal of caution.<sup>24,38</sup>

Other very recent epidemiological studies of other diseases and their possible association with the *NQO1* polymorphism have been reported. A weak correlation ( $P = 0.04$ ) in a population study of 457 patients was noted between the combination of the *NQO1*\*2 and *GSTT1*\*0 alleles with basal cell carcinoma.<sup>39</sup> An increased risk of adult leukemia appears to be correlated with lowered *NQO1* activity<sup>40</sup> and presence of the *NQO1*\*2 allele.<sup>41</sup> Two studies<sup>42,43</sup> reported an association between the *NQO1*\*1 wild-type (consensus) allele and lung cancer risk; however, these studies did not stratify the population with regard to current or previous cigarette smokers. When this consideration was included in two recent epidemiological studies, the reverse correlation was established: in other words, an association between the *NQO1*\*2 allele and susceptibility to lung cancer,<sup>44</sup> specifically non-small cell lung carcinoma,<sup>45</sup> was found in cigarette smokers (and former smokers) but not in patients who had never smoked. These latter data would appear to be more consistent with high pulmonary *NQO1* activity being important in the detoxification of proximate carcinogens in cigarette smoke, i.e., protection against lung cancer.

The *NQO1*\*2 allele appears to have little<sup>46</sup> or no<sup>47</sup> association with Parkinson disease. No association has also been reported between the *NQO1*\*2 allele and adult glioma<sup>48</sup> or the *NQO1*\*2 allele and the response of human tumor xenografts to mitomycin C chemotherapy.<sup>49</sup>

The *NQO1* polymorphism may play a role in cancer prevention during chemotherapy, as well as carcinogenesis. Just as environmental compounds such as dinitropyrenes and heterocyclic amines are metabolically activated to carcinogens by *NQO1*, chemotherapeutic alkylating agents such as mitomycin C rely on *NQO1* for metabolic activation for therapeutic effectiveness.<sup>9,50</sup> There are numerous documented cases of individuals receiving chemotherapy with alkylating agents who develop secondary myeloid leukemia.<sup>51–53</sup> The *NQO1*\*2 allelic frequency was found to be higher in these individuals than in

patients who received chemotherapy but did not develop secondary malignancies,<sup>54,55</sup> implicating *NQO1* in some chemical-induced leukemias.

Larson and coworkers<sup>54</sup> compared the incidence of the *NQO1*\*2 allele with leukemias resulting from benzene exposure, as well as the incidence of this allele with hematopoietic disorders that originate as a side effect of chemotherapy—especially therapy-related acute myeloid leukemia (t-AML). A chi-squared distribution was used to compare expected-versus-observed means of the *NQO1*\*1/\*2 and *NQO1*\*2/\*2 genotypes, with levels of significance set at  $P \leq 0.05$ . As seen in Table 4, the incidence of the *NQO1*\*2 allele was statistically significant for t-AML ( $P = 0.036$ ) and borderline significant for the total number of leukemias observed. The 1.4-fold increase in patients lacking *NQO1* activity thus suggests that this enzyme might play a significant role in the induction of t-AML. This increase was not significant in primary MDS, AML de novo, or chronic myelogenous leukemia. A selection bias may have influenced the results of this study,<sup>54</sup> because the patients were recruited on the basis of diagnosis, they were referred to the University of Chicago, and they had clear karyotyping results. The role of the *NQO1* polymorphism should be explored further in this regard, because patients at increased risk for serious side effects of chemotherapy (such as t-AML) might possibly benefit from *NQO1* allelotyping before treatment with anticancer agents.

#### Other *NQO1* mutations recently discovered

In a study of 84 unrelated Japanese volunteers,<sup>56</sup> three new single-nucleotide polymorphisms (SNPs; nucleotide substitutions) in the *NQO1* gene—two in intron 1 and one in the 3' untranslated region (UTR) of exon 6—were recently reported, but no allelic frequency data were included (Table 5). As this review goes to press, the SNP database (dbSNP) at the University of Utah has recorded 18 additional SNPs (Table 5). In all studies to date, only 5' and 3' flanking sequences, exons, and portions of introns near exons of the 17-kb *NQO1* gene have been screened for SNPs. The only other mutation in the coding region (other than that as the subject of this review) is a G>A transition leading to a synonymous mutation (Glu at residue 24). In summary, the *NQO1* polymorphism now includes five SNPs in the 5' flanking region, 10 SNPs at nine sites in intron 1, a synonymous mutation in exon 2 and the nonsynonymous

**Table 4**  
Frequency of the NQO1\*2 allele in patients with primary and therapy-related myeloid leukemias

Diagnosis	No. of patients	No. (%) of patients with			P
		NQO1*1/*1	*1/*2	*2/*2	
Primary MDS, AML de novo, or CML	48				
Observed		24 (50%)	22 (46%)	2 (4%)	0.27
Expected <sup>a</sup>		28.56	16.72	2.72	
t-AML <sup>b</sup>	56				
Observed		27 (48%)	23 (41%)	6 (11%)	0.036
Expected <sup>a</sup>		34.42	18.93	2.66	
Total	104				
Observed		51 (49%)	45 (43%)	8 (8%)	0.05
Expected <sup>a</sup>		62.98	35.65	5.38	

All these data are derived from Caucasians studied in Ref. 54.

<sup>a</sup> Calculated from the frequency of the two NQO1 alleles reported within different ethnic groups and the racial composition of the observed group.

<sup>b</sup> t-AML denotes therapy-related acute myeloid leukemia.

mutation in exon 6, two SNPs in the 3' untranslated region, and three SNPs in the 3' flanking region of the gene. It is likely that some of the SNPs having no allelic frequencies reported in Table 5 are rare ( $q < 0.01$ ) rather than polymorphic ( $q \geq 0.01$ ) variants.

## LABORATORY TESTS

A simple method for NQO1 allelotyping, from genomic DNA or cDNA samples by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) methodology, has been developed for detecting the c609C>T nucleotide substitution.<sup>36</sup> Application of this method has allowed for rapid DNA screening in large human population studies; the success and ease of this assay has made it a valuable analytically valid test that is being used in dozens, if not hundreds, of laboratories worldwide. The point mutation c609C>T creates a new restriction site so that PCR products of genomic DNA from the NQO1\*1 consensus allele yields two bands of 218 and 22 bp, whereas that from the NQO1\*2 allele yields three bands of 165, 53, and 22 bp. Similarly, PCR products using cDNA (reverse-transcribed from the NQO1 mRNA) yield one 269-bp band for the NQO1\*1 allele and fragments of 217 and 52 bp for the NQO1\*2 allele transcripts. The advantage of this method enables the investigation of the NQO1 polymorphism in genomic DNA as well as mRNA without DNA sequencing. If no blood sample can be obtained, Le Marchand et al.<sup>57</sup> recently demonstrated that one can collect sufficient amounts of DNA in mouthwash samples that can be mailed from long distances to the laboratory and that can be successfully used for the allelotyping of NQO1 as well as other genes.

As a phenotyping assay, NQO1 enzyme activity can be measured in sonicated cell preparations, using the dicoumarol-sensitive reduction of 2,6-dichlorophenol-indophenol assay.<sup>1,36</sup> The amount of activity inhibited by dicoumarol is used as an indicator of NQO1 activity; this assay is relatively diffi-

cult, however, and one can expect 15% to 20% variability from one assay to the next on the same sample (D.W.N., unpublished observations). Human bone marrow cells appear to lack NQO1 constitutive expression, regardless of genotype; however, after exposure to benzene metabolites, NQO1 activity is inducible in NQO1\*1/\*1 and NQO1\*1/\*2 bone marrow cells but not in cells from NQO1\*2/\*2 individuals.<sup>58</sup>

## POPULATION TESTING

From <2% to >20% of the general population is homozygous for NQO1\*2 and would appear to have an increased risk of toxicity when exposed to benzene. As mentioned earlier, however, the large cohort study involved Asians,<sup>23,33</sup> and one must take care in extrapolating these OR values to those of other ethnic groups. The incidence of the NQO1\*2 allele is approximately double in Asian and Mexican-American populations compared with that in non-Hispanic White or African American populations (Table 1); these studies represent the straightforward determination of allelic frequencies and do not involve patient studies. At first glance, the benefit/cost ratio associated with general population testing would appear to be most helpful to populations having the highest NQO1\*2 allelic frequencies, if such measures were done to advise workers to avoid benzene exposure. The incredible admixture in almost all so-called racial or ethnic populations is being increasingly appreciated,<sup>11</sup> however, indicating that it would be unwise to test one ethnic group and exclude another, based on presumed differences in NQO1 allelic frequencies between the two groups because of their apparent "ethnic appearance."

Individuals might also be considered for susceptibility testing on the basis of risky occupational status (e.g., those known to be working in an environment containing benzene). As with most environmental exposure-genotype interaction studies, the data described herein<sup>33</sup> are based on a population with high exposure levels that are not commonly observed in the general

**Table 5**  
Position, mutation, and frequency of recent *NQO1* SNPs

SNP position	Nucleotide mutation	SNP frequency	No. of chromosomes	Ref.
5' flanking	-3348A>C <sup>a</sup>	<i>b</i>		<i>c</i>
5' flanking	-3347A>T	<i>b</i>		<i>c</i>
5' flanking	-784G>A	0.02	180	<i>c</i>
5' flanking	-668G>C	0.01	180	<i>c</i>
5' flanking	-230C>G	0.01	180	<i>c</i>
Intron 1	+107G>C	0.02	180	<i>c</i>
Intron 1	+137C>A	?? <sup>d</sup>	168	56
Intron 1	+646T>C	0.05	180	<i>c</i>
Intron 1	+1199G>T	<i>b</i>		<i>c</i>
Intron 1	+1993A>C	<i>b</i>		<i>c</i>
Intron 1	+2312A>G	<i>b</i>		<i>c</i>
Intron 1	+2560C>T	<i>b</i>		<i>c</i>
Intron 1	+2707A>G	<i>b</i>		<i>c</i>
Intron 1	+7924G>A	0.02	180	<i>c</i>
Intron 1	+7924C>G	?? <sup>d</sup>	168	56
Exon 2	+8015G>A <sup>e</sup>	0.021	180	<i>c</i>
Exon 6	(+13,140)C>T <sup>f</sup>	<i>s</i>	1,058	10, 56
Exon 6 3' UTR	(+14,529)C>T	?? <sup>d</sup>	168	56
Exon 6 3' UTR	(+14,733)A>C	<i>b</i>		<i>c</i>
3' flanking	(+16,669)A>G	<i>b</i>		<i>c</i>
3' flanking	(+17,109)C>G	<i>b</i>		<i>c</i>
3' flanking	(+20,072)G>T	<i>b</i>		<i>c</i>

<sup>a</sup> Numbering of nucleotides, relative to the 5'-most transcription start-site as "+1."

<sup>b</sup> Insufficient number of chromosomes so far screened to determine allelic frequency.

<sup>c</sup> University of Utah "genesnps" Web site.

<sup>d</sup> ?? Frequency not reported, although 84 individuals studied.

<sup>e</sup> Synonymous mutation (Glu-24).

<sup>f</sup> Parentheses denote equivocal numbering, because of a sequence gap of ~1.5 kb in intron 3.

<sup>g</sup> Nonsynonymous P187S mutation (609C>T in the cDNA), the main subject of this review.

population. Public health officials should, therefore, not determine policies that are predicated on the data from a single occupational cohort.

It would appear that *NQO1* allelotyping in cancer patients might also prove useful in determining the beneficial effects of various chemotherapeutic regimens. An individual's metabolic rate can differ, depending on the chemotherapy used, and the drug of choice (such as mitomycin C) will depend on how rapidly a chemotherapeutic agent is cleared, which of course is a reflection of the genetic makeup of each patient. *NQO1* enzyme activity can be highly induced by synthetic antioxidants and extracts of cruciferous vegetables,<sup>4,5</sup> which might suggest a possible role for *NQO1* in cancer chemoprevention; however, cigarette smoking also induces *NQO1*,<sup>2</sup> and most health officials would not condone smoking cigarettes to prevent cancer. If workers are exposed to ambient benzene in the work place, or if patients were given a particular chemotherapeutic agent,

there would be ethical, legal, and social issues surrounding the collection of DNA samples and *NQO1* allelotyping to assess one's risk. The pros and cons of these ethical, legal, and social issues have been examined and discussed recently (Nebert and Bingham and refs therein<sup>59</sup>) and are beyond the scope of this review.

In conclusion, as more knowledge becomes available about polymorphisms in the genes encoding *NQO1* and other drug-metabolizing enzymes, it is possible that preventive and therapeutic regimens might be introduced. Although modification of exposure limits of a chemical such as benzene (in the ambient air of a factory) is not only feasible but reasonable to carry out, it is extremely unlikely that biological-based interventions (e.g., gene therapy, large amounts of dietary antioxidants) would ever be proposed. If *NQO1*\*2/\*2 homozygotes were strongly advised against working in a benzene-exposed occupation, however, would this make them unemployed if no al-

ternative work were available? *NQO1* allelotyping might be regarded as a form of preventive toxicology; the decision about which chemotherapeutic agent to give cancer patients, based on their *NQO1* genotype, could save lives. Further studies are needed to assess the current 22 reported SNPs in the *NQO1* gene and to determine the evolution of haplotype patterns as they relate to phenotype (*NQO1* activity). Clearly, more studies are also needed to quantify the effects of the *NQO1*, *CYP2E1*, *MPO*, *GSTM1*, *GSTT1*, and *EPHX1* polymorphisms. Such studies need to be completed and corroborated—before one can begin to advise workers exposed to benzene and other quinone-containing occupationally hazardous chemicals, as well as to advise physicians who give chemotherapeutic agents to cancer patients.

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