The frequencies of NAD(P)H quinone oxidoreductase (*NQO1*) variant allele in Israeli ethnic groups and the relationship of NQO1*2 to adult acute myeloid leukemia in Israeli patients

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NAD(P)H:quinone oxidoreductase (NQO1) detoxifies quinones. The NQO1*2 variant enzyme (codon 609 C→T, encoding a proline to serine substitution) with greatly reduced activity has been reported to predispose to acute myeloid leukemia (AML). Our aim was to examine the relationship between NQO1*2 and AML in Israeli patients. We analyzed for NQO1*2 in 262 adult Israeli patients with *de novo* AML and 688 controls of the same ethnic groups (Arabs, and Caucasian and Ethiopian Jews). Our analysis showed significant differences in the frequencies of NQO1*2 by ethnic group (p=0.000068). However, NQO1*2 frequencies did not differ between AML patients and controls. Karyotype was not found to be associated with NQO1*2. In Israeli patients, NQO1*2 does not predispose to *de novo* AML.

Key words: NAD(P)H:quinone oxidoreductase, myeloid leukemia, predisposing polymorphisms, chromosomal abnormalities.

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NAD(P)H:quinone oxidoreductase (NQO1) is a flavoenzyme that has an important role in detoxifying numerous endogenous and environmental quinones and their derivatives. These compounds are widespread in the environment in natural (fruits and vegetables) and man-made (organic solvents such as benzene, cigarette smoke, air pollution) compounds. A polymorphic variant of *NQO1* (C609T, codon 187 proline to serine) referred to as *NQO1*2*, leads to low levels of mutant protein and greatly reduced enzyme activity.[1] The normal allele is referred to as *NQO1*1*.[2] Heterozygotes for the variant allele (CT, *NQO1*1/*2*) have intermediate enzymatic activity and homozygotes for the mutant allele (TT, *NQO1*2/*2*) have essentially no NQO1 activity. NQO1 can mitigate the toxic damage of benzene to the bone marrow, which can occur even at minute levels of exposure.[3] The *NQO1*2* allele would therefore be expected to predispose the bone marrow to toxic insults, due to inadequate protection of stem cells. Accordingly, *NQO1*2* has been shown to be associated with benzene toxicity and hematologic malignancy.[4] Furthermore, *NQO1*2* homozygotes were found to have increased t8;21 on exposure to benzene.[5] In addition, NQO1-deficient mice have increased susceptibility to benzene-induced toxicity.[6] Thus, NQO1*2 has been investigated as a predisposing element in leukemogenesis in pediatric and adult leukemias, including *de novo* and therapy-related leukemia. Four previous studies have analyzed *NQO1* genotypes in adult AML. Larson and colleagues analyzed 104 adult patients from the United States with myeloid leukemias[7] including *de novo* AML, myelodysplastic syndromes (MDS) and therapy-related AML. This study found a significantly increased allele frequency for *NQO1*2* in patients with myeloid malignancies, particularly those with abnormalities of chromosomes 5 and 7[7] however, only nine *de novo* AML patients were included in the study. Naoe and colleagues analyzed 469 Japanese patients including 411 with *de novo* AML.[8] This study found that *NQO1*2* was more frequent in therapy-related leukemia patients[8] but not in *de novo* AML, and no correlation was found with karyotype. The third and largest of these studies[9] was performed on 493 adults with *de novo* acute leukemia (420 AML and 67 acute lymphoblastic leukemia) and 838 matched controls, all from the United Kingdom. This study found an increased allele frequency for *NQO1*2* in acute leukemia patients, in particular in those with balanced chromosomal translocations or inversions.[9] Lastly, another study from the United Kingdom found no significant predisposing effect of *NQO1*2* to either therapy-related or *de novo* AML (34 and 168 patients, respectively).[10] Our study (46 patients) did not show an association of *NQO1*2* with therapy-related leukemia.[11]

To further clarify the association between *NQO1*2* and *de novo* AML and karyotypic abnormalities, we performed an analysis of 262 adult patients with *de novo* AML. We

compared the allele frequencies with those of nearly 700 controls of similar ethnic backgrounds (Arabs, Caucasian Jews and recently immigrated Ethiopian Jews). In Israel, these ethnic groups are culturally distinct and intermarriage among them is uncommon. The designation Caucasian Jews includes both Ashkenazi and Sepharadic Jews.

Methods

DNA samples were prepared in accordance with the guidelines of the Helsinki Committee of Hadassah Medical Organization. DNA samples were prepared from 262 consecutive AML patients (males: Jews 93, Arabs 66; females: Jews 63, Arabs 40) with sufficient cells for preparation of DNA. These samples were accumulated during the years 1993-2004. The hematologic diagnosis had been made using standard morphologic, immunophenotypic and karyotypic criteria. Karyotyping was performed by standard G-banding techniques and/or fluorescent *in situ* hybridization (FISH). Anonymous control DNA samples from healthy individuals were either purchased from the National Laboratory for Genetics of the Sackler School of Medicine of Tel Aviv University, which has an ethnic group-specific bank of anonymous DNA samples, or prepared from anonymized samples of individuals referred for various non-malignant hematologic conditions. Analysis of the *NQO1* variant allele was performed as described by Smith *et al.*[9] using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Statistical methods

Statistical analyses was performed using Pearson's χ^2 test (two-sided).

Results and Discussion

Our results are shown in Tables 1 and 2. Table 1 demonstrates that the allele frequency of *NQO1*2* is significantly different among the three control groups (Arabs compared to Caucasian Jews, p=0.022; all three ethnic groups (Arabs, Caucasian Jews and Ethiopian Jews) compared to each other, p=0.000068). The distribution of the three genotypes (homozygous normal, heterozygous and homozygous *NQO1*2*) is also different among the various controls (Arabs compared to Caucasian Jews, p=0.011; the three ethnic groups compared to each other, p=0.000056). Since the genotypes varied significantly among the control ethnic groups, we performed the analysis of allele frequencies of the patients according to their ethnic group. However, since there were very few AML patients of Ethiopian origin, this analysis could not be performed for this group. Table 2 demonstrates the allele frequencies and *NQO1* genotypes for AML patients of Arab and Caucasian Jewish origin. It can be seen that the frequencies are virtually identical to the frequencies in the respective controls (Arab controls versus Arab AML patients, p=0.767, Caucasian Jewish controls versus Caucasian Jewish AML patients, p=0.714). Karyotypic data were available for 125 of the *de novo* AML patients (Table 3). The *NQO1* genotype was not found to be associated with a particular type of kary-

Table 1. Normal and variant *NQO1* allele frequencies in Israeli controls of different ethnicities.

	Homozygous normal n (%)	Heterozygous variant n (%)	Homozygous variant n (%)	Variant allele frequency
Arab controls n=270	146 (54.1)	104 (38.5)	20 (7.4)	0.267#
Caucasian Jewish controls n=250	152 (60.8)	93 (37.2)	5 (2)	0.206#
Ethiopian Jewish controls n=168	122 (72.6)	44 (26.2)	2 (1.2)	0.143#

#Significance: Arab controls compared to Jewish controls, p=0.02; all three ethnic groups compared to each other: p=0.000068.

Table 2. Normal and variant *NQO1* allele frequencies in Israeli AML patients.

	Disease	Homozygous normal n (%)	Heterozygous variant n (%)	Homozygous variant n (%)	Variant allele frequency
Arabs	AML n=106	56 (52.8)	44 (41.5)	6 (5.7)	0.264#
Jews	AML n=156	96 (61.5)	55 (35.3)	5 (3.2)	0.208#

#Significance comparing patients to controls of the same ethnic groups: Arab AML patients compared to Arab controls (Table 1): p=0.767; Jewish AML patients compared to Jewish controls (Table 1): p=0.714.

Table 3. Chromosomal abnormalities and *NQO1* genotype.

Genotype	Abnormal karyotype					Normal karyotype	Total
	Balanced translocations n (%)			Non-balanced translocations, gains, losses: n (%)			
	Inv16	t8;21	t15;17	Monosomy 7	All others		
*NQO1*1/ NQO1*2*	1 (2)	5 (10)	5 (10)	3 (6)	14 (29)	21 (43)	49
*NQO1*2/ NQO1*2*	1 (20)	1 (20)	1 (20)	0	1 (20)	1 (20)	5
*NQO1*1/ NQO1*1*	3 (4)	5 (7)	8 (12)	4 (6)	17 (25)	31 (46)	68

otypic abnormality. The percentage of patients with a normal karyotype did not differ according to *NQO1* genotype; 46% of *NQO1*1* homozygotes versus 43% of heterozygotes for *NQO1*2* had a normal karyotype (p=0.8). Furthermore, there was no difference in the *NQO1* genotypes of patients who had either unbalanced translocations, or gains/losses, as compared to balanced translocations (t8;21, t15;17) and inversion 16 (p=0.632).

The age of patients treated at Hadassah Hospital is lower than the mean age of AML patients reported in the literature and the Arab patients are younger than the Jewish patients. The mean age at diagnosis for Arab patients was 41.4±16 years and that for Jewish patients (48.7±18.7).[12] This raised the question of whether genetic background contributes to the predisposition to develop AML in young patients of either or both of these ethnic groups.

In our analysis of a large number of Israeli patients with AML, the frequencies of the *NQO1* null allele were similar to those found in other populations.[2,13] The frequencies were in accordance with the Hardy-Weinberg equilibrium. However, we did not find any increased risk of *de novo* adult AML in those carrying *NQO1*2*. This lack of association may be due to a number of factors. First it is possible that in our environment, *NQO1*2* is not a predisposing factor and that other genetic factors are more important. For instance, high *CYP2E1* activity may be able to compensate for the *NQO1* null allele.[4]

We did not measure CYP2E1 activity in our patients, and polymorphisms in this gene have not been accurately linked to enzyme activity.[4] Furthermore, it is possible that variations in myeloperoxidase, which is in the same metabolic pathway, may contribute to altered predisposition[14] though most studies have not shown this enzyme to be important.[15] Alternately, it is possible that the deleterious effect of *NQO1*2* may be seen only in Israeli individuals with particular exposure to carcinogens or toxins, but unfortunately our retrospective data did not include detailed information on exposure (occupational or environmental) to enable us to investigate such a hypothesis. In future studies, it will be important to study not just *NQO1*2* but also the *NQO1* C465T variant which also reduces enzyme activity due to alternative splicing and reduced normal protein product.[1] This variant has recently been found to predispose to infant leukemia with *MLL* translocations.[16]

The genetic epidemiology of *NQO1*2* has been studied, and the allele frequency has been reported to be 0.25 in non-Hispanic Caucasians in the United States which is similar to the frequency that we found in Caucasian Jews (0.206). Ethiopian Jews have a lower frequency (0.143) than that reported for African Americans (0.22).[13] We also found small but significant differences in *NQO1*2* frequency when comparing Arabs and Caucasian Jews. These groups are genetically similar, but not identical, as was found in studies of Y chromosome polymorphisms.[17]

In summary, our findings demonstrate that there are significant differences in *NQO1* genotypes in different ethnic groups in Israel. However, our data do not support a role for *NQO1*2* as a predisposing polymorphism for *de novo* adult AML. Our results suggest the need for studies in many populations to verify associations of genetic variation data with predisposition to disease. Polymorphisms which may have an impact in one geographic area or ethnic group may not be contributory in others.

All authors qualified for authorship according to the World Association of Medical Editors (WAME) criteria. Specific responsibilities were as follows; EM: performed all the experiments and data analysis, and wrote the first draft of the manuscript; SBC: developed the laboratory methods and supervised EM in all aspects of the laboratory experiments; DS: supervised clinical specimen collection and preparation in Rambam Hospital; EJD: assembled clinical data from Rambam Hospital and recruited patients for study; DR: responsible for the conception of the study, wrote the subsequent drafts of the manuscript, assembled clinical data from Hadassah. The authors declare that they have no potential conflicts of interest.

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References

1. Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, et al. Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase). Br J Cancer 1997; 75: 69-75.
2. Nebert DW, Roe AL, Vandale SE, Bingham E, Oakley GG. NAD(P)H: quinone oxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: a HuGE review. Genet Med 2002;4:62-70.
3. Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, et al. Hematotoxicity in workers exposed to low levels of benzene. Science 2004; 306:1774-6.
4. Rothman N, Smith MT, Hayes RB, Traver RD, Hoener B, Campleman S, et al. Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609C→T mutation and rapid fractional excretion of chlorzoxazone. Cancer Res 1997;57:2839-42.
5. Kim SY, Choi JK, Cho YH, Chung EJ, Paek D, Chung HW. Chromosomal aberrations in workers exposed to low levels of benzene: association with genetic polymorphisms. Pharmacogenetics 2004;14:453-63.
6. Bauer AK, Faiola B, Abernethy DJ, Marchan R, Pluta LJ, Wong VA, et al. Genetic susceptibility to benzene-induced toxicity: role of NADPH: quinone oxidoreductase-1. Cancer Res 2003;63:929-35.
7. Larson RA, Wang Y, Banerjee M, Wiemels J, Hartford C, Le Beau MM, et al. Prevalence of the inactivating 609C→T polymorphism in the NAD(P)H:quinone oxidoreductase (NQO1) gene in patients with primary and therapy-related myeloid leukemia. Blood 1999;94:803-7.
8. Naoe T, Takeyama K, Yokozawa T, Kiyoi H, Seto M, Uike N, et al. Analysis of genetic polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4 in 469 Japanese patients with therapy-related leukemia/ myelodysplastic syndrome and de novo acute myeloid leukemia. Clin Cancer Res 2000;6:4091-5.
9. Smith MT, Wang Y, Kane E, Rollinson S, Wiemeis JL, Roman E, et al. Low

NAD(P)H:quinone oxidoreductase I activity is associated with an increased risk of acute leukemia in adults. Blood 2001;97:1422-6.
10. Seedhouse C, Bainton R, Lewis M, Harding A, Russell N, Das-Gupta E. The genotype distribution of the XRCC1 gene indicates a role for base excision repair in the development of therapy-related acute myeloblastic leukemia. Blood 2002;100:3761-6.
11. Rund D, Krichevsky S, Bar-Cohen S, Goldschmidt N, Kedmi M, Malik E, et al. Therapy-related leukemia: clinical characteristics and analysis of new molecular risk factors in 96 adult patients. Leukemia. 2005;19:1919-28.
12. Kedmi M, Cohen SB, Rund D. Polymorphisms in drug metabolism/disposition genes and increased susceptibility to adult de novo AML: MDR1 and CYP3A4. Blood 2004;104 Suppl 1:572a[abstract].
13. Kelsey KT, Ross D, Traver RD, Christiani DC, Zuo ZF, Spitz MR, et al. Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy. Br J Cancer 1997; 76:852-4.
14. Reynolds WF, Chang E, Douer D, Ball ED, Kanda V. An allelic association implicates myeloperoxidase in the etiology of acute promyelocytic leukemia. Blood 1997;90:2730-7.
15. Morgan GJ, Smith MT. Metabolic enzyme polymorphisms and susceptibility to acute leukemia in adults. Am J Pharmacogenomics 2002;2:79-92.
16. Eguchi-Ishimae M, Eguchi M, Ishii E, Knight D, Sadakane Y, Isoyama K, et al. The association of a distinctive allele of NAD(P)H:quinone oxidoreductase with pediatric acute lymphoblastic leukemias with MLL fusion genes in Japan. Haematologica 2005; 90:1511-5.
17. Nebel A, Filon D, Weiss DA, Weale M, Faerman M, Oppenheim A, Thomas MG. High-resolution Y chromosomal haplotypes of Israeli and Palestinian Arabs reveal geographic substructure and substantial overlap with haplotypes of Jews. Hum Genet 2000; 107:630-41.