The *NQO1* C609T polymorphism is associated with risk of secondary malignant neoplasms after treatment for childhood acute lymphoblastic leukemia: a matched-pair analysis from the ALL-BFM study group

In a matched-pair study, we analyzed the association of a phenotypically relevant *NQO1* polymorphism (C609T) with risk of secondary malignant neoplasms (SMN) after treatment for childhood acute lymphoblastic leukemia. Patients carrying a variant low-activity *NQO1* allele had a significantly increased risk of developing a SMN. The observed effect was restricted to solid tumors.

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One devastating late effect of treatment for childhood acute lymphoblastic leukemia (ALL) is a secondary malignant neoplasm (SMN) which is estimated to occur in at least 2% of the treated population within 15 years from diagnosis, but strongly depends on the type of treatment applied (1-3). Known risk factors for the development of a SMN after treatment for childhood ALL include, for example, age at diagnosis, cranial irradiation, etoposide treatment, and treatment for recurrent disease.[1-3]

The widely expressed detoxification enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) is involved in the cellular response to oxidative stress and irradiation and protects cells against the mutagenicity from free radicals and toxic oxygen metabolites.[4] NQO1 is subject to a genetic polymorphism (C609T) leading to a change in its amino acid sequence (P187S).[4] Heterozygous individuals (*C/T* or *NQO1*1/*2*) have intermediate activity and homozygotes (*T/T* or *NQO1*2/*2*) are NQO1 deficient.[4]

We investigated a potential association between NQO1 activity and the development of a SMN after treatment for childhood ALL on Berlin-Frankfurt-Münster (BFM) protocols, we searched our database for patients developing an SMN after treatment according to the multicenter trials ALL-BFM 79, 81, 83, 86, and 90. Treatment in these trials is described elsewhere and contained similar multidrug chemotherapeutic regimens and, over time, declining doses of cranial irradiation.[1,5] We identified 78 patients who developed a SMN (evaluation date June 2004), 49 (62.8%) had spare leukemic or remission bone marrow slides available for DNA isolation. Forty-one of these 49 SMN patients could be individually matched to a control patient with no SMN (minimum follow-up 10 years) according to the following criteria: gender, age at diagnosis (±6 months), white blood cell count (WBC) at diagnosis (±10,000/μL), immunophenotype, trial including risk-group and treatment branch, and dose of cranial irradiation. Only 2 of the 41 SMN patients that could be matched received treatment for recurrent disease previous to the SMN diagnosis; their controls received identical treatment. *NQO1* genotyping was as previously described.[6]

Table 1 shows the patient characteristics of our matched-pair group. When odds ratios were calculated for the overall group, we observed a 2.4-fold increased risk of an SMN for those carrying at least one *NQO1*2* allele (Table 2). Upon stratification, the association of at least one *NQO1*2* allele with the development of an SMN seemed to be restricted to patients with a solid tumor SMN. In these patients, the risk was increased 4.5-fold while no significant effects were observed for patients with a hematologic SMN (Table 2) or in an analysis restricted to the group of therapy-related acute myeloid leukemias (tAML) and myelodysplastic syndromes (MDS; *data not shown*).

Solid tumor SMN are strongly associated with irradiation.[1-3] This is supported by the observation that the strong reduction in the application of cranial irradiation in more recent treatment protocols for childhood ALL is paralleled by a declining incidence of solid tumor SMN.[2] By contrast, the incidence of hematologic SMN seems to be unaffected by this reduction.[2] Therefore, our findings suggest that in our treatment setting variant NQO1 activity may modulate irradiation-associated toxicity. Unfortunately, we cannot further investigate this hypothesis further because more than 90% of patients included in the present study underwent cranial irradiation, making a stratified analysis impossible.

Table 1. Characteristics of 41 individually matched patients developing a secondary malignant neoplasm (SMN) after treatment for childhood acute lymphoblastic leukemia and their control patients.

	Number of subjects and prevalence (%)	
	SMN cases	*Controls*
Gender		
Male	27 (65.9)	27 (65.9)
Female	14 (34.1)	14 (34.1)
Age at diagnosis (years)		
< 1	1 (2.4)	1 (2.4)
≥1-<6	24 (58.5)	25 (61.0)
≥6	16 (39.0)	15 (36.6)
Initial WBC (/μL)		
<10,000	19 (46.3)	18 (43.9)
≥10,000-< 50,000	12 (29.3)	13 (31.7)
≥50,000	10 (24.4)	10 (24.4)
Immunophenotype		
B	23 (56.1)	23 (56.1)
T	11 (26.8)	11 (26.8)
Other/not characterized	7 (17.1)	7 (17.1)
BFM study		
ALL-BFM 79	5 (12.2)	5 (12.2)
ALL-BFM 81	11 (26.8)	11 (26.8)
ALL-BFM 83	6 (14.6)	6 (14.6)
ALL-BFM 86	8 (19.5)	8 (19.5)
ALL-BFM 90	11 (26.8)	11 (26.8)
Cranial irradiation (Gray)		
No	4 (9.8)	4 (9.8)
12	12 (29.3)	12 (29.3)
18	20 (48.8)	20 (48.8)
>18	5 (12.2)	5 (12.2)
SMN[a]		
Hematologic	19 (46.3)	–
Solid tumors	22 (26.8)	–

[a]*Specific diagnoses were: hematologic SMN: Hodgkin's lymphoma (n=2), non-Hodgkin lymphoma (n=1), malignant histiocytosis (n=2), tAML/MDS (n=14); solid tumors: primitive neuroectodermal tumor of CNS (n=3), meningeoma (n=1), astrocytoma (n=3), glioblastoma (n=3), gliosarcoma (n=1); thyroid cancer (n=3), basal cell carcinoma (n=2), epithelial carcinoma (n=1), mucoepidermoid carcinoma (n=1), malignant teratoma (n=1), melanoma (n=1), nephroblastoma (n=1), unknown secondary tumor with lung metastasis (n=1).*

Table 2. Distribution of *NQO1* C609T polymorphism and its association with secondary malignant neoplasms (SMN) after treatment for childhood acute lymphoblastic leukemia.

	Number of subjects and prevalence (%)			
NQO1 genotype	*All SMN*	*Controls*	*OR[a] (95% CI[b])*	*p*
*1/*1	19 (46.3)	29 (70.7)	1.00[c]	
*1/*2	21 (51.2)	10 (24.4)	3.20 (1.23-8.31)	0.016
*2/*2	1 (2.4)	2 (4.9)	0.50 (0.01-55.56)	0.772
*1/*2 and *2/*2	22 (53.7)	12 (29.3)	2.43 (1.04-5.70)	0.041
	Hematological SMN	*Controls*		
*1/*1	10 (52.6)	12 (63.2)	1.00[c]	
*1/*2	8 (42.1)	6 (31.6)	n.c.d	
*2/*2	1 (5.3)	1 (5.3)	n.c.	
*1/*2 and *2/*2	9 (47.4)	7 (36.8)	1.00 (n.c.)[e]	
	Solid tumor SMN	*Controls*		
*1/*1	9 (40.9)	16 (72.7)	1.00[c]	
*1/*2	13 (59.1)	5 (22.7)	n.c.	
*2/*2	–	1 (4.5)	n.c.	
*1/*2 and *2/*2	13 (59.1)	6 (27.3)	4.50 (1.11-18.23)	0.035

[a]OR: odds ratio, based on discordant pairs; [b]CI: confidence interval; [c]reference category; [d]not calculated; [e]due to zero discordant pairs.

Previously, the *NQO1*2* allele had been associated with tAML/MDS in adults heterogeneously pretreated for different primary cancers.[6,7] Similar to our study, the only other published pediatric study analyzing NQO1 genetic variation in children after treatment for ALL did not detect an association with tAML/MDS.[8] An explanation for the non-concordant associations of low NQO1 with tAML/MDS observed in the above mentioned studies most likely lies in the different exposures primary to developing tAML/MDS.[6-8] Unfortunately, we are not aware of any other study analyzing the association of NQO1 genetic variation with solid SMN.

We have to acknowledge that our results may have been influenced by small sample size, selection bias, or may simply be due to chance. In addition, we cannot exclude the potential relevance of other phenotypically relevant NQO1 genetic variants associated with diminished enzyme activity (e.g., C465T, R139W).[9] However, it was recently suggested that in individuals with low NQO1 activity, exposure towards carcinogenic substrates of NQO1 could lead to increased genotoxic damage at lower p53 levels compared to wild-type NQO1 individuals.[10] Thus, childhood ALL patients with defective NQO1 and potentially lower p53 levels may experience enhanced genomic instability due to a decreased capacity to deal with chemotherapy-associated or, more likely, irradiation-associated oxidative stress and, therefore, are at an increased risk of developing a solid tumor SMN.

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