Anthracycline-Related Cardiomyopathy After Childhood Cancer: Role of Polymorphisms in Carbonyl Reductase Genes—A Report From the Children's Oncology Group

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S.B. has full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose

Carbonyl reductases (CBRs) catalyze reduction of anthracyclines to cardiotoxic alcohol metabolites. Polymorphisms in *CBR1* and *CBR3* influence synthesis of these metabolites. We examined whether single nucleotide polymorphisms in *CBR1* (*CBR1* 1096G>A) and/or *CBR3* (*CBR3* V244M) modified the dose-dependent risk of anthracycline-related cardiomyopathy in childhood cancer survivors.

Patients and Methods

One hundred seventy survivors with cardiomyopathy (patient cases) were compared with 317 survivors with no cardiomyopathy (controls; matched on cancer diagnosis, year of diagnosis, length of follow-up, and race/ethnicity) using conditional logistic regression techniques.

Results

A dose-dependent association was observed between cumulative anthracycline exposure and cardiomyopathy risk (0 mg/m²: reference; 1 to 100 mg/m²: odds ratio [OR], 1.65; 101 to 150 mg/m²: OR, 3.85; 151 to 200 mg/m²: OR, 3.69; 201 to 250 mg/m²: OR, 7.23; 251 to 300 mg/m²: OR, 23.47; > 300 mg/m²: OR, 27.59; *P*_{trend} < .001). Among individuals carrying the variant A allele (*CBR1*:GA/AA and/or *CBR3*:GA/AA), exposure to low- to moderate-dose anthracyclines (1 to 250 mg/m²) did not increase the risk of cardiomyopathy. Among individuals with *CBR3* V244M homozygous G genotypes (*CBR3*:GG), exposure to low- to moderate-dose anthracyclines increased cardiomyopathy risk when compared with individuals with *CBR3*:GA/AA genotypes unexposed to anthracyclines (OR, 5.48; *P* = .003), as well as exposed to low- to moderate-dose anthracyclines (OR, 3.30; *P* = .006). High-dose anthracyclines (> 250 mg/m²) were associated with increased cardiomyopathy risk, irrespective of *CBR* genotype status.

Conclusion

This study demonstrates increased anthracycline-related cardiomyopathy risk at doses as low as 101 to 150 mg/m². Homozygosity for G allele in *CBR3* contributes to increased cardiomyopathy risk associated with low- to moderate-dose anthracyclines, such that there seems to be no safe dose for patients homozygous for the *CBR3* V244M G allele. These results suggest a need for targeted intervention for those at increased risk of cardiomyopathy.

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INTRODUCTION

Anthracyclines are an essential component of childhood cancer therapy, as evidenced by their incorporation into more than 50% of front-line therapeutic regimens.¹ However, cardiomyopathy is a dose-limiting complication; cardiac abnormalities develop in up to 60% of patients exposed to high doses of anthracyclines.² The

dose-dependent increase in cardiomyopathy risk³⁻⁵ is modified by younger age at exposure and chest radiation.⁶ However, doses as low as 150 mg/m² result in cardiomyopathy in some patients,³ suggesting a role for interindividual variability in anthracycline pharmacodynamics.

The main route for anthracycline metabolism is a two-electron reduction of the C-13 carbonyl group in the anthracycline side chain, resulting in

the formation of alcohol metabolites (eg, doxorubicinol, daunorubicinol).^{7,8} Development of cardiomyopathy correlates with myocardial accumulation of anthracycline alcohol metabolites.^{9,10} Variability in the formation of these metabolites could influence the risk of cardiomyopathy.⁷ Synthesis of cardiotoxic alcohol metabolites is catalyzed by myocardial cytosolic carbonyl reductases (CBRs).^{7,11} In humans, two monomeric CBRs (CBR1 and CBR3) are encoded for by genes located on chromosome 21. A single nucleotide polymorphism (SNP) in the 3'-untranslated region of *CBR1* (*CBR1* 1096G>A) and in *CBR3* (*CBR3* V244M) impacts catalytic activity for anthracycline substrates.¹²⁻¹⁴ The goal of the current study was to understand the contribution of functional polymorphisms in *CBR1* and *CBR3* to the dose-dependent risk of anthracycline-related cardiomyopathy in survivors of childhood cancer.

PATIENTS AND METHODS

Study Design

Children's Oncology Group (COG) ALTE03N1 is a case-control study aimed at understanding the pathogenesis of cardiomyopathy in survivors of childhood cancer. One hundred twenty-one COG member institutions contributed patients to the study (Appendix, online only) after obtaining approval from local institutional review boards. Written informed consent/assent was obtained from all patients and/or their parents or legal guardians.

Participants

Prevalent patient cases and matched controls were identified from patients diagnosed with a primary cancer at age 21 years or younger and observed at a COG institution. Patient cases consisted of individuals who developed cardiomyopathy after completion of cancer therapy and were alive at study participation. For each patient case, one to four controls were randomly selected from childhood cancer survivors without cardiomyopathy, using the following matching criteria: cancer diagnosis, year of diagnosis (± 5 years), race/ethnicity, and duration of follow-up for controls to exceed time from cancer diagnosis to cardiomyopathy for index patient case. All participants provided a biologic specimen (blood, 85%; buccal cells/saliva, 15%). One hundred seventy patient cases with cardiomyopathy and 317 matched controls participated in this study.

Cancer Therapy

Detailed information regarding therapeutic exposures was abstracted from medical records. Total anthracycline exposure was calculated by multiplying the cumulative dose per meter square of body surface area of individual anthracyclines (doxorubicin, daunomycin, epirubicin, and idarubicin) by a factor that reflects the drug's cardiotoxic potential (Data Supplement)¹⁵ and then summing the result. Cumulative anthracycline exposure was treated as a categorical variable. Radiation therapy with heart in the radiation field was designated as chest radiation and summarized as a yes or no variable.

Validation of Cardiomyopathy

All anthracycline-exposed individuals had normal cardiac function before anthracycline initiation. Clinical and echocardiographic documentation of cardiomyopathy was provided by participating sites (Data Supplement). Patient cases were considered eligible if they fulfilled the American Heart Association criteria for cardiac compromise by presenting with symptoms (eg, dyspnea, orthopnea, fatigue) and/or signs (eg, edema, hepatomegaly, rales) of cardiac decompensation or, in the absence of symptoms/signs, if they had echocardiographic features of left ventricular dysfunction as evidenced by ejection fraction (EF) $\leq 40\%$ and/or fractional shortening (SF) $\leq 28\%$. Controls had no symptoms or signs of cardiac compromise (all 317 controls) and had normal echocardiographic features ($n = 203$; EF: median, 65%; range, 53% to 84%; SF: median, 36%; range, 29% to 61%) or no clinical indication for echocardiographic examination because of lack of exposure to anthracyclines or chest radiation ($n = 63$). In addition, echocardiograms were unavailable for

51 anthracycline-exposed ($n = 41$) or chest radiation-exposed ($n = 10$) controls. Thus, echocardiograms were unavailable for 114 controls. Exclusion of these 114 controls did not materially alter the associations (Data Supplement); therefore, we opted to include the 114 controls in the analysis.

DNA Isolation and Genotyping

Genomic DNA was isolated from peripheral blood or buccal cells/saliva by using QIAamp or Qiagen kits (Qiagen, Valencia, CA) and Puregene (Qiagen) or Oragene (DNA Genotek, Kanata, Ontario, Canada) kits, respectively. The *CBR3* V244M and *CBR1* 1096G>A polymorphisms (rs1056892, rs9024) were analyzed using validated assays for allelic discrimination with specific fluorescent probes (Applied Biosystems, Foster City, CA).^{13,14} Laboratory personnel were blinded to case-control status. Nine samples had noninformative results.

Statistical Analysis

Univariate conditional logistic regression and generalized linear models were used to compare patient cases and controls for categorical and continuous characteristics, respectively. χ^2 tests were used to test for deviation from Hardy-Weinberg equilibrium.

Anthracycline exposure and cardiomyopathy. Association between anthracycline exposure and cardiomyopathy was measured by estimating the odds ratio (OR) while controlling for chest radiation (yes or no), age at cancer diagnosis (continuous variable in years), and sex, using conditional logistic regression techniques. Cumulative anthracycline exposure was categorized as 0, 1 to 100, 101 to 150, 151 to 200, 201 to 250, 251 to 300, and more than 300 mg/m².

CBR genes and cardiomyopathy. SNPs in *CBR1* and *CBR3* genes were examined individually and in combination. For the combined analysis, patients homozygous for both the *CBR1* and *CBR3* G allele (ie, *CBR1*:GG and *CBR3*:GG [associated with increased CBR activity]) served as the group of interest, whereas patients carrying at least one copy of the variant A allele in either of the *CBR* genes (ie, *CBR1*:GA/AA and/or *CBR3*:GA/AA) served as the referent group. Conditional logistic regression method was used with adjustment for cumulative anthracycline exposure in addition to sex, age at cancer diagnosis, and chest radiation.

Modifying effect of CBR genes on dose-dependent risk of anthracycline-related cardiomyopathy. The interactive effect of genes and anthracycline exposure was examined by including genotypes (*CBR1* and *CBR3* individually and in combination), anthracycline exposure, and the product of *CBR* genotypes and anthracycline exposure in the conditional logistic regression model. The risk of cardiomyopathy was examined at varying levels of anthracycline exposure (no exposure: 0 mg/m²; low to moderate dose: 1 to 250 mg/m²; and high dose: > 250 mg/m²) for the two risk categories of the *CBR* genotypes (homozygosis for GG v GA/AA), using *CBR* GA/AA genotype and no exposure to anthracyclines as the referent group. The modifying effect of the *CBR* genotypes was examined by stratifying on the dose categories of anthracyclines.

Data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC). All statistical tests were two-sided; $P < .05$ was considered statistically significant. Bonferroni adjustment was used for multiple comparisons ($0.05/2 = 0.025$) when analyzing the association with the two SNPs.

RESULTS

Demographic and Clinical Characteristics and Risk of Cardiomyopathy

Demographics and clinical characteristics of the 170 patient cases and 317 matched controls are listed in Table 1. Median EF and SF for the patient cases was 42% (range, 10% to 68%) and 24% (range, 5% to 33%), respectively. One hundred sixty-six patient cases (98%) met the echocardiographic cutoffs for cardiomyopathy (EF $\leq 40\%$ and/or SF $\leq 28\%$); the remaining four patient cases presented with signs or symptoms of cardiac compromise per American Heart Association

Table 1. Demographic and Clinical Characteristics of the Study Population

Demographic or Clinical Characteristic	Patient Cases (n = 170)		Controls (n = 317)		P
	No.	%	No.	%	
Age at primary cancer diagnosis, years					.67
Mean	8.3		8.2		
SD	6		6		
Median	7.3		7.6		
Range	0-20.7		0-21.1		
Age at study participation, years					< .001
Mean	17.6		20.6		
SD	9		10		
Median	16.6		18.5		
Range	0.4-41		2.0-49		
Female	94	55	155	49	.15
Race/ethnicity*					—
Non-Hispanic whites	124	73	252	79	
Hispanics	16	9	29	9	
Blacks	12	7	14	5	
Other	18	11	22	7	
Primary diagnosis*					—
Hodgkin's lymphoma	19	11	36	11	
Non-Hodgkin's lymphoma	21	12	34	11	
Bone tumors	35	21	44	14	
Soft tissue sarcoma	20	12	21	7	
Acute lymphoblastic leukemia	23	14	93	29	
Acute myeloid leukemia	19	11	29	9	
Other	33	19	60	19	
Year of primary cancer diagnosis*					—
1966-1980	20	12	25	8	
1981-1990	41	24	77	24	
1991-2000	68	40	147	46	
2001-2008	41	24	68	22	
Length of follow-up, years*					< .001
Mean	9.2		12.3		
SD	9		9		
Median	7.0		11.2		
Range	0.1-35.1		0.4-40.3		
Cumulative anthracycline exposure, mg/m ²					< .001
Mean	291		168		
SD	142		172		
Median	300		140		
Range	0-575		0-1,050		
Categories of anthracycline exposure					< .001
0	15	9	93	29	
1-100	7	4	48	15	
101-150	7	4	38	12	
151-200	9	5	25	8	
201-250	18	11	29	9	
251-300	33	19	20	6	
≥ 301	81	48	64	20	
Chest radiation†	42	25	43	14	< .001
Genotype status‡					
CBR1 1096G>A					
AA	2	1	5	2	.94§
GA	36	21	61	19	
GG	132	78	246	79	
CBR3 V244M					
AA	21	13	49	16	.13
GA	70	41	142	45	
GG	78	46	121	39	

(continued in next column)

Table 1. Demographic and Clinical Characteristics of the Study Population (continued)

Demographic or Clinical Characteristic	Patient Cases (n = 170)		Controls (n = 317)		P
	No.	%	No.	%	
Combination of CBR1 and CBR3					
Both AA/GA	24	14	42	14	.27
CBR1 GG and CBR3 AA/GA	67	40	146	47	
CBR1 AA/GA and CBR3 GG	14	8	23	7	
Both GG	64	38	98	32	
Either AA/GA	105	62	211	68	.09
Both GG	64	38	98	32	

Abbreviation: SD, standard deviation.

*Matching variables. Because of variation in the number of controls per patient case, the percentage of controls and patient cases in each category of a specific matching variable may not be identical.

†Five patients with unknown chest radiation were excluded.

‡Nine patients with noninformative genotypes were excluded.

§Exact P value.

||Summation of rows Both AA/GA; CBR1 GG and CBR3 AA/GA; CBR1 AA/GA and CBR3 GG; and Both GG.

guidelines, despite echocardiographic values exceeding the cutoffs. Echocardiographic and clinical details of the patient cases are summarized in the Data Supplement. Patient cases had been treated for bone tumor/soft tissue sarcoma (33%), acute leukemia (25%), lymphoma (23%), and other diagnoses (19%). Patient cases, compared with controls, were more likely to have received chest radiation (25% v 14%, respectively; $P < .001$) and anthracyclines (91% v 71%, respectively; $P < .001$), as well as higher cumulative doses of anthracyclines (mean, 291 v 168 mg/m², respectively; $P < .001$). The association between demographics and clinical characteristics and risk of cardiomyopathy is detailed in Table 2.

Anthracycline dose. After adjusting for age at diagnosis of cancer, sex, and chest radiation, there was a clear dose-dependent association

Table 2. Demographic/Therapeutic Characteristics and Risk of Cardiomyopathy

Characteristic	Odds Ratio*	95% CI	P
Sex			
Male	1.0		
Female	1.47	0.9 to 2.4	.13
Age at primary cancer diagnosis, per year increase	0.99	0.93 to 1.04	.59
Chest radiation			
No	1.0		
Yes	4.29	1.9 to 9.6	< .001
Cumulative anthracycline exposure, mg/m ²			< .001†
0	1.0		
1-100	1.65‡	0.5 to 5.6	
101-150	3.85	1.1 to 13.9	
151-200	3.69	1.0 to 13.6	
201-250	7.23	2.3 to 22.5	
251-300	23.47	7.4 to 74.2	
≥ 301	27.59	9.3 to 82.1	

*Odds ratios were obtained using multivariate conditional logistic regression.

†P for trend.

‡Association between anthracycline exposure at 1 to 100 mg/m² and cardiomyopathy is not statistically significant.

Fig 1. Dose-response relationship between cumulative anthracycline exposure and risk of cardiomyopathy. Patients with no exposure to anthracyclines served as the referent group. Magnitude of risk is expressed as odds ratio, which was obtained using conditional logistic regression adjusting for age at diagnosis, sex, and chest radiation.

between anthracycline exposure and cardiomyopathy risk. Compared with patients without anthracycline exposure, the risk of cardiomyopathy increased with each dose category (1 to 100 mg/m²: OR, 1.65; 101 to 150 mg/m²: OR, 3.85; 151 to 200 mg/m²: OR, 3.69; 201 to 250 mg/m²: OR, 7.23; 251 to 300 mg/m²: OR, 23.47; and ≥ 301 mg/m²: OR, 27.59; *P* for trend < .001; Fig 1).

Other risk factors. Chest radiation was associated with an increase in risk of cardiomyopathy (OR, 4.29; 95% CI, 1.9 to 9.6; *P* < .001). However, female sex (OR, 1.47; 95% CI, 0.9 to 2.4; *P* = .13) and older age at cancer diagnosis (OR, 0.99/yr; 95% CI, 0.93 to 1.04; *P* = .59) were not associated with cardiomyopathy risk.

Polymorphisms in CBR Genes and Cardiomyopathy

CBR1 1096G>A and *CBR3* V244M genotype distributions were consistent with those predicted under conditions of Hardy-Weinberg equilibrium (*CBR1* 1096G>A: *P* = .79; *CBR3* V244M: *P* = .27) and were similar to those previously reported (*P* > .05)^{13,14} (Table 1). As shown in Table 3, there was no association between *CBR1* genotype

status and cardiomyopathy risk. However, the risk of cardiomyopathy was higher for individuals with homozygous *CBR3* GG genotype compared with those carrying at least one copy of the variant *CBR3* A allele (OR, 1.79; *P* = .02; Table 3). Combined analysis of *CBR1* and *CBR3* genotype status revealed a nonsignificant increase in cardiomyopathy risk for individuals with *CBR1*:GG and *CBR3*:GG genotypes (OR, 1.53; *P* = .09; Table 3).

Modifying Effect of Polymorphisms in CBR Genes and Dose-Dependent Risk of Anthracycline-Related Cardiomyopathy

The modifying effect of *CBR* genotype status on the dose-dependent association between anthracyclines and risk of cardiomyopathy was analyzed for *CBR1*, *CBR3*, and the *CBR1*-*CBR3* combination.

***CBR1* 1096G>A.** Results are summarized in the Data Supplement. For individuals with *CBR1*:GA/AA genotypes exposed to low- to moderate-dose anthracyclines (1 to 250 mg/m²), the risk of cardiomyopathy was not statistically significantly different from individuals with *CBR1*:GA/AA not exposed to anthracyclines (OR, 0.68; *P* = .65). Individuals with *CBR1*:GG genotype exposed to low to moderate doses of anthracycline showed a nonsignificant increase in the risk for cardiomyopathy when compared with individuals with *CBR1*:GA/AA genotypes not exposed to anthracyclines (OR, 2.25; *P* = .22). Within the low- to moderate-dose category, the *CBR1*:GG genotype was associated with an increased risk of cardiomyopathy when compared with the *CBR1*:GA/AA genotypes (OR, 3.29; *P* = .05). Further adjustment for *CBR3* genotype attenuated the association (OR, 2.63; *P* = .11). *CBR1*:GG genotype was not associated with increased risk of cardiomyopathy within the high-dose category.

***CBR3* V244M.** Results are listed in Table 4. For individuals with *CBR3*:GA/AA genotype exposed to low- to moderate-dose anthracyclines (1 to 250 mg/m²), the risk of cardiomyopathy was not statistically significantly different from individuals with *CBR3*:GA/AA genotype with no anthracycline exposure (OR, 1.66; *P* = .42). However, individuals with *CBR3*:GG genotype exposed to low- to moderate-dose anthracyclines were at increased risk of cardiomyopathy when compared with patients with *CBR3*:GA/AA genotypes not exposed to anthracycline (OR, 5.48; *P* = .003). Within the low- to moderate-dose category, *CBR3*:GG genotype status was associated with an increased risk of cardiomyopathy when compared with the *CBR3*:GA/AA genotypes (OR, 3.30; *P* = .006). Further adjustment for *CBR1* genotype did not change the association (OR, 3.48; *P* = .005). *CBR3*:GG genotype was not associated with cardiomyopathy within the high-dose category.

***CBR1* and/or *CBR3*.** Results are summarized in the Data Supplement. The modifying effect of the combined *CBR1* and *CBR3* genotypes on the dose-dependent association between anthracyclines and cardiomyopathy risk was similar to that observed for the *CBR3* genotype.

We next examined the dose-response relationship between anthracycline exposure and cardiomyopathy, stratified by the patients' *CBR* genotype status (patients carrying at least one copy of the variant A allele v patients homozygous for the G allele; Data Supplement). These results indicate that homozygosity for the G allele in *CBR3* gene was primarily responsible for modifying the risk for cardiomyopathy. As shown in Figure 2, among patients with at least one copy of the A allele in *CBR3*, low- to moderate-dose anthracycline exposure was not associated with cardiomyopathy when compared with patients not

Table 3. *CBR1* and *CBR3* Genotypes and Risk of Cardiomyopathy

Genotype	No. of Patient Cases	No. of Controls	Odds Ratio*	95% CI	<i>P</i>
<i>CBR1</i> 1096G>A					
<i>CBR1</i> :AA or GA	38	66	1.0		.49
<i>CBR1</i> :GG	132	246	0.81	0.45 to 1.47	
<i>CBR3</i> V244M					
<i>CBR3</i> :AA or GA	91	191	1.0		.02
<i>CBR3</i> :GG	78	121	1.79	1.08 to 2.96	
Combination of <i>CBR1</i> and <i>CBR3</i>					
<i>CBR1</i> :AA or GA and/or <i>CBR3</i> :AA or GA	105	211	1.0		.09
<i>CBR1</i> :GG- <i>CBR3</i> :GG	64	98	1.53	0.93 to 2.51	

*Odds ratios were obtained from conditional logistic regression adjusting for age at diagnosis, sex, chest radiation, and cumulative anthracycline exposure (0, 1 to 100, 101 to 150, 151 to 200, 201 to 250, 251 to 300, ≥ 301 mg/m²).

Table 4. Modifying Effect of *CBR3* Genotypes on Dose-Dependent Risk of Anthracycline-Related Cardiomyopathy

<i>CBR3</i> Genotype by Anthracycline Exposure	No. of Patient Cases	No. of Controls	Risk of Cardiomyopathy for All Patients*			Risk of Cardiomyopathy Stratified by Anthracycline Exposure (1-250; > 250 mg/m ²)†		
			Odds Ratio‡	95% CI	P	Odds Ratio‡	95% CI	P
No exposure								
<i>CBR3</i> :GA/AA	9	56	1.0					
<i>CBR3</i> :GG	6	36	0.86	0.22 to 3.39	.83			
1-250 mg/m ²								
<i>CBR3</i> :GA/AA	15	84	1.66	0.49 to 5.69	.42	1.0		
<i>CBR3</i> :GG	26	54	5.48	1.81 to 16.63	.003	3.30	1.41 to 7.73	.006
> 251 mg/m ²								
<i>CBR3</i> :GA/AA	67	51	18.92	6.13 to 58.45	< .001	1.0		
<i>CBR3</i> :GG	46	31	25.91	7.67 to 87.57	< .001	1.37	0.66 to 2.84	.40

*Reference group: no anthracycline exposure, *CBR3*:GA/AA genotype.

†Reference group: *CBR3*:GA/AA genotype for the corresponding anthracycline exposure level.

‡Odds ratios were obtained from conditional logistic regression adjusting for age at diagnosis, sex, and chest radiation.

exposed to anthracyclines. However, among patients homozygous for the G allele in *CBR3*, low-dose anthracycline exposure was associated with an increased risk of cardiomyopathy (101 to 150 mg/m²: OR, 6.15; 95% CI, 1.3 to 28.8; *P* = .02; 151 to 200 mg/m²: OR, 6.37; 95% CI, 1.0 to 39.5; *P* = .05; and 201 to 250 mg/m²: OR, 10.85; 95% CI, 2.7 to 43.2; *P* < .001) when compared with patients with at least one copy of the variant A allele in *CBR3* and no exposure to anthracyclines. In contrast, doses exceeding 250 mg/m² were associated with an increased risk of cardiomyopathy, irrespective of patients' *CBR3* genotype status.

DISCUSSION

Anthracyclines play an undisputed role in the treatment of childhood cancer¹⁶⁻¹⁸; unfortunately, development of dose-dependent cardiomyopathy limits their therapeutic potential.¹⁹ An elevated risk of clinical heart failure was reported by ≥ 5-year survivors of childhood

cancer exposed to anthracycline doses exceeding 250 mg/m².⁵ The current study demonstrates elevated risk of clinically validated cardiomyopathy at lower doses, such that patients exposed to a cumulative dose of 101 to 150 mg/m² are at increased risk compared with the unexposed (OR, 3.9). The current study also demonstrates that at low to moderate doses of anthracycline exposure, individual differences in anthracycline pharmacodynamics modify the risk of cardiomyopathy.

Myocardial accumulation of anthracycline alcohol metabolites influences the course of cardiomyopathy.^{9,10} These metabolites form a reservoir in the cardiomyocytes and impair contractility through inhibition of Ca²⁺ and Na⁺/K⁺ pump activity.^{9,20,21} In the human heart, CBRs are considered major anthracycline-metabolizing enzymes.^{7,22} Functional polymorphisms in *CBR1* and *CBR3* modulate the synthesis of anthracycline alcohol metabolites. The rate of doxorubicinol synthesis is 1.5-fold higher in liver samples with *CBR1* 1096G>A homozygous G genotype compared with samples with heterozygous G/A genotype.¹³ *CBR3* V244M polymorphism results in *CBR3* protein isoforms (*CBR3* V244 and *CBR3* M244) with distinct catalytic properties^{12,14}; *CBR3* V244 (G allele) catalyzes the synthesis of doxorubicinol 2.6 times faster than *CBR3* M244 (A allele).²³

The current study examined the modifying effect of *CBR1* 1066G>A and *CBR3* V244M genotype status on the dose-dependent risk of anthracycline-related cardiomyopathy. Among individuals carrying at least one copy of the variant A allele in *CBR1* and/or *CBR3*, low- to moderate-dose anthracycline exposure (1 to 250 mg/m²) was not associated with cardiomyopathy. The risk of cardiomyopathy, however, was significantly increased among individuals with *CBR3*:GG genotype exposed to low- to moderate-dose anthracyclines when compared with unexposed individuals carrying at least one copy of the A allele. However, the risk of cardiomyopathy was modest and statistically nonsignificant for individuals with *CBR1*:GG genotype exposed to low- to moderate-dose anthracyclines. *CBR1* or *CBR3* genotype status did not impact cardiomyopathy risk among patients exposed to high-dose anthracyclines.

Homozygosis for the G allele in *CBR3* gene seems to drive the increase in risk for cardiomyopathy associated with low- to moderate-dose anthracyclines. The differential impact of *CBR1* and *CBR3* genotype status on cardiotoxicity may be explained, in part, by recent findings on the bases that control the transcription of both genes.²⁴⁻²⁶

Fig 2. Dose-response relationship between cumulative anthracycline exposure and risk of cardiomyopathy stratified by patients' *CBR3* genotype status (*CBR3*:GG and *CBR3*:GA/AA). Patients with no exposure to anthracyclines and carrying *CBR3*:GA/AA genotype served as the referent group. Magnitude of risk is expressed as odds ratio, which was obtained using conditional logistic regression adjusting for age at diagnosis, sex, and chest radiation.

CBR3 expression is modulated by the master transcription factor Nrf2 (nuclear factor [erythroid-derived 2]-like 2 [NFE2L2]), whereas expression of *CBR1* seems to be predominantly regulated through the aryl hydrocarbon receptor pathway.^{24,26} Nrf2 coordinates the induction of a battery of genes involved in protection against oxidative stress. We propose that the Nrf2 pathway provides a link between the acute cardiotoxicity mediated mostly by reactive oxidative species (ROS) and chronic cardiotoxicity induced by anthracycline C-13 alcohol metabolites. Parent anthracyclines induce ROS production and Nrf2 translocation into the nucleus. In turn, nuclear Nrf2 upregulates *CBR3* expression with consequent increase in synthesis of cardiotoxic anthracycline alcohol metabolites. This upregulation of *CBR3* activity in a chronic setting may help explain why the association with cardiomyopathy seems to be stronger for polymorphic *CBR3* than for polymorphic *CBR1*.

Restriction of the modifying effect of *CBR* genotype status only to low- to moderate-dose anthracyclines is intriguing. In humans, approximately 50% of anthracyclines remain unmetabolized²⁷ and induce cardiotoxicity through mechanisms that invoke ROS production.^{8,28} Because *CBR* activity contributes to the pathogenesis of cardiomyopathy by generation of cardiotoxic anthracycline alcohol metabolites, three possible scenarios could be envisaged, depending on the dose of anthracyclines. First, in the absence of anthracycline exposure, *CBR* activity does not play a role in the development of cardiomyopathy as a result of lack of substrate. Second, exposure to low- to moderate-dose anthracyclines provides the necessary substrate to synthesize cardiotoxic C-13 alcohol metabolites by the high-risk/high-activity *CBR* variants, resulting in an increased risk of cardiomyopathy. Finally, in the presence of high-dose anthracyclines, cardiotoxicity is mostly mediated by the oxidative stress generated by the excess of unmetabolized anthracyclines, such that anthracycline alcohol metabolites play a relatively minor role. However, it is also possible that cardiomyopathy in individuals exposed to high-dose anthracyclines and carrying the high-risk variants (*CBR1*:GG and *CBR3*:GG) is associated with high lethality, thus removing these individuals from the pool of eligible patient cases and reducing the association in this group to null.

Prevalent case-control studies, by the nature of their design, exclude fatal end points from the patient case set. Presence of survival bias risks underascertainment of genotypes associated with high lethality, with consequent underestimation of disease risk effect size for those genotypes associated with both increased disease risk and disease-associated lethality.²⁹ Using the methodology developed by Anderson et al³⁰ to estimate effect size erosion, we calculated the maximum possible degree of erosion of the true estimate as a result of survival bias in our case-control study to be 12%, assuming that the high-risk genotype was associated with a three-fold increased risk of death (Data Supplement). Furthermore, for early cardiac death to have eliminated the association from an anticipated genotype-outcome risk of 3.0 to 1.0 (ie, associated with a 67%

erosion) as a result of an overrepresentation of the GG genotype among patients who had received high-dose anthracyclines and died before study participation, the high-risk genotype would need to be associated with a four-fold increased risk of death when compared with the low-risk genotype. There are no data to support such a high level of lethality. However, because it is logistically impossible to prove this within the context of a large multi-institutional study, we do acknowledge the possibility that among recipients of high-dose anthracyclines, patients with *CBR1*:GG and *CBR3*:GG genotype could have been more likely to have developed cardiomyopathy and died, thus becoming lost from the sampling frame and eroding the true association between high-risk variants and cardiomyopathy.

We recognize that interindividual variability in alternate metabolic pathways, such as reduction to semiquinone radicals by a number of oxidoreductases or two-electron reduction by aldo-keto reductases, and in pharmacodynamic targets (eg, calsequestrin, iron regulatory proteins) could play a role in modulating cardiomyopathy risk.²⁷ However, functional, biochemical and genetic studies support *CBR*-mediated reduction of anthracyclines to cardiotoxic alcohol metabolites as a major metabolic route implicated in the pathogenesis of cardiotoxicity.^{31,32} Furthermore, findings in this study confirmed the association between *CBR3* V244M genotype and cardiomyopathy reported in a small previously published study.²³

The current study demonstrates that patients with *CBR3* V244M homozygous G genotype exposed to low- to moderate-dose anthracyclines are at an increased risk of cardiomyopathy, even at cumulative exposures between 101 and 150 mg/m². These results could allow for enhanced surveillance and/or prevention strategies among survivors of childhood cancer at increased risk of cardiomyopathy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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