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Risk factors for anthracycline-associated cardiotoxicity

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Abstract

Purpose—Carbonyl reductase (CBR) catalyzes anthracycline metabolism, and single nucleotide polymorphisms (SNPs) in *CBR* impact metabolic efficiency. In pediatric patients, homo-zygosity for the major allele (G) in the *CBR3* gene was associated with increased risk of anthracycline cardiotoxicity. We hypothesized that CBR SNPs contribute to cardiotoxicity in adults

Methods—We retrospectively identified female breast cancer patients in the Columbus Breast Tissue Bank Registry treated with adriamycin and cytoxan (AC) from 2003 to 2012. We selected patients who developed cardiomyopathy, defined as a drop in ejection fraction to <50 % or >15 % decrease from pre-therapy. Univariate and multivariate logistic regressions were performed to identify cardiotoxicity risk factors. SNPs were genotyped, and frequency of the major allele (G)/minor allele (A) of the *CBR3* and *CBR1* genes was calculated.

Results—We identified 52 cases of cardiotoxicity after AC and 110 controls. Multivariate analysis showed that trastuzumab ($p=0.009$), diabetes ($p=0.05$), and consumption of >8 alcoholic drinks/week ($p=0.024$) were associated with higher cardiotoxicity risk. Moderate alcohol consumption (<8 drinks/week) was associated with lower risk ($p=0.009$). No association was identified between CBR SNPs and cardiotoxicity (*CBR1* $p=0.261$; *CBR3* $p=0.556$).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Conclusions—This is the first study to evaluate SNPs in the CBR pathway as predictors of AC cardiotoxicity in adults. We did not observe any significant correlation between cardiotoxicity and SNPs within the CBR pathway. Further investigation into CBR SNPs in a larger adult sample is needed. Additional exploration into genomic predictors of anthracycline cardiotoxicity may allow for the development of preventative and therapeutic strategies for those at risk.

Keywords

Anthracycline; Cardiotoxicity; CBR; Trastuzumab; Single nucleotide polymorphisms

Introduction

Anthracyclines are widely used in both adult and pediatric cancer treatment regimens. Due to their association with improved rates of disease-free and overall survival, anthracyclines remain an integral part of today's neoadjuvant, adjuvant, and metastatic breast cancer treatment regimens. Despite their efficacy, the decision to treat patients with anthracyclines must be weighed against their associated cardiotoxicity risk, which may range from subclinical decrements of left ventricular ejection fraction (LVEF) to overt cardiomyopathy [1, 2].

Anthracycline-related cardiac toxicity is broadly classified as acute onset (within the first week of treatment), early onset, and late onset (>1 year after treatment). Acute-onset toxicity, which occurs in less than 1 % of patients, is a dose-independent, pericarditis-myocarditis syndrome. More common is late-onset toxicity, which manifests in a dose-dependent fashion and results in progressive endomyocardial damage causing dilated cardiomyopathy that can occur decades from first exposure [3]. Cumulative doses above 500 mg/m² in adults and >300 mg/m² in pediatric patients are associated with a higher risk of therapy-related cardiotoxicity [4, 5]. Retrospective studies show that the incidence of cardiomyopathy is 4–5 % for cumulative doses up to 500 mg/m² and 11–31 % for cumulative doses greater than 500 mg/m² [4, 5]. These retrospective models may even underestimate the frequency of cardiotoxicity in patients receiving lower cumulative anthracycline doses. Prospective monitoring of LVEF in breast cancer patients receiving adjuvant doxorubicin doses of 240 mg/m² showed that 25 % of women had grade 1–2 cardiac toxicity [6]. Some studies have demonstrated stabilization of LVEF after discontinuation of anthracycline therapy, though toxicity is generally considered to be irreversible [7].

There are a variety of clinical risk factors that have been linked to the development of anthracycline-associated cardiotoxicity. Available studies suggest that conditions like pre-existing cardiac disease, age >65 years, hypertension, hyperlipidemia, and radiation exposure may impact cardiotoxicity risk after anthracycline therapy [3, 8, 9]. The detrimental cardiac effects of anthracyclines may also be enhanced by other treatments, especially by the monoclonal antibody trastuzumab (Trz). This targeted agent is an effective treatment option approved for breast cancer patients whose disease manifests amplification of the *HER2* (*ERBB2*) gene, which occurs in up to 20 % of breast cancers [10]. Trz also has its own associated cardiotoxicity risks; approximately 2.5–10 % of breast cancer patients

treated with Trz develop therapy-mediated cardiomyopathy, although rates up to nearly 25 % have been reported in a retrospective report of patients who also received doxorubicin [11–13].

The exact mechanism for anthracycline cardiotoxicity is not fully understood, though multiple pathways have been implicated. One proposed mechanism is the generation of free radicals and reactive oxygen species (ROS) that are formed in response to injury caused by anthracyclines [14]. The formation of iron-anthracycline complexes, which cause mitochondrial DNA damage and direct damage to myocytes, is further implicated [15]. In addition, toxicity is posited to arise from the drug metabolism process itself. Anthracyclines are primarily metabolized through an alteration of the anthracycline side chain, specifically a reduction of the C-13 carbonyl group. This process forms alcohol metabolites, like doxorubicinol, a potent ATPase inhibitor that acts subcellularly and inhibits cytoplasmic Ca^{2+} uptake by the sarcoplasmic reticulum, leading to impaired relaxation or contractility [16, 17]. Carbonyl reductases (CBRs) are the catalysts for these reactions that form cardiotoxic alcohol metabolites. Chromosome 21 encodes the genes for CBR1 and CBR3, two highly homologous monomeric CBR proteins found in the cytoplasm [18, 19]. While certain CBR variants have been shown to increase the risk of anthracycline-related cardiotoxicity in childhood cancer survivors, this has not been evaluated in adults. The study conducted by the Children's Oncology Group compared 170 cancer survivors with cardiomyopathy to 317 survivors without cardiomyopathy. They found that homozygosity for the G allele in *CBR3* contributes to cardiotoxicity in patients taking low-moderate doses of anthracyclines, whereas high doses increase cardiotoxicity irrespective of *CBR3* status, suggesting there is no safe dosage of anthracyclines for patients homozygous for the G allele in *CBR3* [18].

Aside from prospective clinical monitoring typically in the form of serial echocardiograms, there are no accurate methods of predicting those at greatest risk of therapy-associated cardiotoxicity in the adult breast cancer population. Furthermore, definitive clinical risk factors that correlate with increased rates of toxicity are lacking. With improving patient outcomes resulting in a greater number of survivors, the ability to accurately predict those at greatest risk of treatment complications is paramount. In an effort to better characterize this issue, our study attempted to retrospectively identify clinical and genomic risk factors that contribute to cardiotoxicity genesis in breast cancer patients who received anthracycline therapy.

Methods

Study design

Using the Columbus Breast Cancer Tissue Bank (CBCTB) registry, we conducted a retrospective case-control study to identify clinical and genetic predictors in women treated with anthracyclines who developed cardiac complications vs. those who did not develop complications. The CBCTB is an IRB-approved protocol of the Stefanie Spielman Breast Center that enrolls incoming breast cancer patients in a tissue and clinical registry. Clinical information, stored DNA, and tumor tissue samples were available for each of these patients.

Procedures

The registry included over 700 breast cancer patients from 2003 to 2012. Patient charts were screened for those individuals treated with anthracyclines and then classified by the development of cardiac complications during/after treatment. Patients identified included those who developed cardiomyopathy, which was defined as a drop in ejection fraction to <50 % or >15 % decrease from pre-therapeutic levels, and those who developed a new arrhythmia or myocardial infarction after therapy. Cardiac diagnosis codes were used to screen patients for these complications. Diagnosis codes included cardiomyopathy; cardiomyopathy, treatment related; congestive heart failure; congestive heart failure, treatment related; dysrhythmias, cardiac, treatment related; heart palpitations, treatment related; lower ejection fraction, treatment related; myocardial infarct, treatment related; supraventricular tachycardia, treatment related; and ventricular tachycardia, treatment related. Patients were excluded for the following: no available documentation of LVEF decline by echocardiogram, MRI, or stress test; the identified post-treatment cardiac complication was also present pre-treatment; inability to confirm cardiotoxicity was directly related to therapy; and no prior receipt of anthracycline.

A total of 110 cases and 729 controls were identified from the database using these screening criteria. Ultimately, 52 female patients who developed a cardiac complication and met study criteria were identified; 110 asymptomatic patients were randomly selected to serve as controls. Detailed chart review was performed on these patients and included examination of demographic data; body mass index (BMI); age; cancer staging; estrogen receptor (ER) status; progesterone receptor (PR) status; Her2-neu status; location of tumor; surgery type; axillary dissection status; tumor histology; radiation receipt; treatment history; and cardiac medication receipt (including beta blockers; ACE inhibitors; ARBs; spironolactone; diuretics; statins; antidepressants/anxiolytics). Past medical, social, and family histories were also assessed.

All study patients provided informed consent to allow the use of DNA samples for research purposes. Genotyping for variants in the *CBR1* and *CBR3* (rs9024 and rs1056892) genes was performed using a fluorogenic 5' nuclease allelic discrimination assay (TaqMan® single nucleotide polymorphisms (SNP) genotyping assay, Applied Biosystems, Foster City, CA, USA). Approximately 20 ng of the genomic DNA was used to genotype each sample. Reactions were prepared using TaqMan Universal PCR Mastermix, No AmpErase UNG, and 20× SNP Genotyping Assays (Applied Biosystems) and run according to the manufacturer's instructions. Thermal cycling and analysis were performed using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). A no-template (water) control was included to assess DNA contamination. Genotype assignment was achieved automatically with SDS software (Applied Biosystems) using a proprietary algorithm.

Endpoints

We hypothesized genomic polymorphisms in the CBR pathway would predict therapy-associated cardiotoxicity in female breast cancer patients previously treated with anthracyclines. The primary endpoint was to genotype for SNPs in the *CBR1* and *CBR3*

genes on stored DNA samples of women treated with anthracyclines. Secondary endpoints were to identify both clinical and genetic predictors of therapy-related cardiotoxicity.

Statistics

The demographic information, medical history, treatment, and all potential risk factors were summarized for each group, and the difference between the two groups were compared using *t* test for continuous variables or chi-square test for categorical data. Simple logistic regression and multivariate logistic regression analyses were conducted to estimate the risk of cardiac complication development. The frequencies of the selected alleles of the SNPs, *CBR1* and *CBR3*, were also summarized for these two groups and compared using the chi-square test.

Before the study was conducted, we anticipated identifying 100 cases and 100 controls to provide 80 % power to detect a difference of 0.40 standard deviation between the two groups at a significance level of 0.05. However, only 52 cases and 110 controls were identified. This sample size provides 80 % power to detect a 0.45 standard deviation difference between the two groups at a significance level of 0.05.

Results

Baseline characteristics for both cohorts in this study were relatively similar (Table 1). All included patients were treated with anthracyclines. Approximately 48 % of the toxicity group was treated with Trz compared to only 24 % of the control group ($p=0.0018$); 50–60 % of patients received radiation therapy in both groups ($p=0.344$). Only nine patients from both groups were given bevacizumab. A greater number of patients in the toxicity group had breast cancers that were Her2-neu amplified ($p=0.0037$) and, hence, received Trz. Also, diabetes was significantly ($p=0.039$) more prevalent in those patients who experienced cardiotoxicity. Finally, a significantly greater use of beta blockers, ACE inhibitors, diuretics, and ARBs was demonstrated in the cardiotoxicity group. Multivariate logistic regression analysis revealed that DM (vs. no DM, odds ratio 1.93 and $p=0.05$) and prior Trz receipt (odds ratio 1.69 and $p=0.009$) were associated with a higher rate of cardiotoxicity development. Compared to no consumption of alcohol, moderate alcohol consumption (<8 drinks/week) was associated with a lower rate (odds ratio 0.43, $p=0.009$) of cardiotoxicity development, while greater amounts of alcohol consumption (>8 drinks/week) were associated with a higher risk of cardiotoxicity (odds ratio 3.07, $p=0.024$) (Table 2).

Of the 52 patients with anthracycline therapy-related cardiac complications, 41 patients (79 %) developed systolic dysfunction. Nine of these patients did not show recovery of EF in subsequent echocardiograms. Ten patients (19.2 %) developed an arrhythmia, and two patients (3.8 %) developed myocardial infarctions. Finally, no statistically significant difference in the prevalence of the major allele (G) or minor allele (A) of the *CBR1* ($p=0.480$) and *CBR3* ($p=0.395$) genes was noted between the toxicity and control groups (Table 3). Further, no difference in *CBR1* ($p=0.261$) and *CBR3* ($p=0.556$) variant genotypes was identified.

Discussion

The identification of genetic determinants linked to treatment toxicity represents an important step in efforts to personalize cancer treatments while mitigating side effects. Our study represents the first attempt to evaluate SNPs in the *CBR1* and *CBR3* genes as potential risk factors for cardiotoxicity in an adult population receiving anthracycline chemotherapy. In this study, we did not identify any significant correlation between cardiotoxicity and polymorphisms within the *CBR1* or *CBR3* gene. We suspect that our small sample size may have contributed to the noted equivalency. One other potential reason for our inability to confirm the correlation observed in the pediatric population is the inherent difference between adult and pediatric susceptibilities to anthracyclines [20].

Interestingly, our initial analysis included seven additional toxicity cases that had received Trz alone, in the absence of anthracycline receipt. When these Trz monotherapy cases were included in the study analysis, a non-statistically significant trend toward an increased frequency of the allele G of the *CBR3* gene was observed in patients with cardiotoxicity compared to controls (66 vs. 59 %, $p=0.16$, OR=1.39, 95 % CI 0.88–2.21). Several studies have linked the *HER2* polymorphism Ile644Val as a risk factor for Trz-associated cardiotoxicity [21, 22]. However, there is no published data to date demonstrating a link between Trz-associated cardiotoxicity and polymorphisms in the CBR pathway. Thus, the implications of our observation are unclear; however, this certainly represents an important area of future study.

A variety of other investigations have undertaken to identify candidate-gene variants conceivably linked to anthracycline cardiotoxicity. Polymorphisms in genes encoding NADPH oxidase subunits, the doxorubicin efflux transporter ABCB1/MDR1, as well as genes involved in the doxorubicin metabolism pathway like *ABCC1* have been implicated in cardiotoxicity development [23–25]. In a similar fashion, several gene variants have been found to confer significant protection against anthracycline-induced cardiotoxicity, including variants in the solute *SLC28A3* or *hCNT3* gene and variants in *ABCB1*, *ABCB4*, and *ABCC1* [26]. The potential role of CBR pathway overexpression in anthracycline-induced cardiotoxicity was previously described in a transgenic mouse model [27]. In the investigation completed by the Children's Oncology Group in pediatric patients, patients homozygous for the G allele of the *CBR3* gene (46 % of cases; 39 % of controls) experienced an increased risk of cardiotoxicity irrespective of anthracycline dose [18]. Our investigation found a similar proportion of patients to be homozygous for the G allele of the *CBR3* gene (approximately 40 % of cases and 36 % of controls), although no increased risk of cardiotoxicity was observed.

Our study also investigated potential clinical risk factors associated with the development of therapy-related cardiotoxicity. Data from the Surveillance, Epidemiology, and End Results (SEER) Medicare database reported that female breast cancer patients 65 years old who received an anthracycline-containing chemotherapy regimen had a 10.23 % cumulative incidence of cardiotoxicity compared to 4.97 % of patients who did not receive chemotherapy [8]. Other reported risk factors besides age include pre-existing cardiac dysfunction (such as decreased LVEF), high BMI, heavy alcohol intake, and

antihypertensive therapy [28, 29]. In our analysis, diabetes was shown to be associated with a greater risk of cardiotoxicity, which was similarly demonstrated in a large-scale meta-analysis [28]. We also observed that patients who consumed a moderate amount (<8 drinks/week) of alcohol were less likely to develop cardiotoxicity, while a greater degree of consumption (>8 drinks/week) was associated with an increased risk of toxicity. In the non-cancer setting, patients with an average drinking history of 15 years and reporting consumption of >90 g/day of alcohol (8–21 standard drinks) have been shown to have asymptomatic cardiac dysfunction [30]. However, little is known about the effects of alcohol on chemotherapy-related cardiotoxicity. Further study is required to better understand the potential positive and negative impacts of alcohol on patients undergoing cardiotoxic cancer treatments. Finally, our study observed an increased use of aromatase inhibitors in the cohort without cardiotoxicity. This finding is in contrast to prior reports, which have suggested an increased risk of cardiac issues with aromatase inhibitor use [31]. These disparate results may be attributable to our small sample size. Nonetheless, due to the limited number of studies to date, aromatase inhibitors remain an interesting risk factor to consider in future investigation design.

Trz therapy was additionally identified as a significant clinical risk factor in our study. Unlike anthracyclines, Trz cardiotoxicity is largely reversible after discontinuation of treatment and does not show significant myocyte destruction [15]. However, despite recovery of LVEF, late mitochondrial scarring persists in many patients for up to 6 months as detected through late gadolinium enhancement visualized by cardiac magnetic resonance imaging (CMR). This enhancement is located in the lateral sub-epicardial wall and could imply a typical distribution or location for Trz-related myocarditis [32]. Risk factors for adjuvant Trz-associated cardiotoxicity are not well characterized [33]. However, some studies have suggested potential risk factors such as prior cardiac disease, older age, increased BMI, antihypertensive therapy, previous chemotherapy receipt, and diabetes [15, 34]. In addition, other investigations have demonstrated a synergistic cardiotoxic effect when patients are treated with Trz after adjuvant anthracycline-based chemotherapy, resulting in symptomatic cardiac failure in 0.6–4.1 % of patients. For 5–19 % of patients, cardiomyopathy development results in permanent discontinuation of Trz therapy, which can impact treatment outcomes [3]. This synergistic effect between anthracyclines and Trz may be a result of anthracycline-induced dilation of T-tubules, which allows for greater access of Trz to epidermal growth factor receptor 2 (*ErbB2*), a tyrosine kinase receptor that promotes the proliferation and survival of cells through a downstream signaling pathway. Trz binds to the extracellular domain of ErbB2, inhibiting uncontrolled downstream signaling in patients who overexpress the receptor [35, 36]. However, ERbB2 is also responsible for binding to neuregulin-1 (NRG-1), which activates a downstream pathway involved in the proliferation, differentiation, and survival in many tissues, including cardiomyocytes [14, 36]. Indirect inhibition of NRG-1 by Trz can enhance anthracycline toxicity by increasing myofibrillar disarray, leaving cardiomyocytes more susceptible to anthracycline-induced oxidative stress [37].

Our study was limited by the retrospective nature of the data, although a detailed review of patient medical records was performed. Patients lacking all required data or those who had a prior history of cardiac disease were excluded, a practice that likely contributed to our

study's small sample size. Additionally, the ability to evaluate for long-term toxicities, as often seen with anthracycline-related cardiac impairment, was beyond the scope of this investigation, which may have further limited our data set. Ideally, future research would include observations of a large patient cohort undergoing anthracycline treatment with long-term, prospective cardiac monitoring.

Conclusion

This study represents the first attempt to characterize SNPs in the *CBR1* and *CBR3* genes as predictors of anthracycline-associated cardiotoxicity in the adult cancer population. Unlike our pediatric counterparts, we did not observe any significant correlation between cardiotoxicity and polymorphisms within the CBR pathway. As with many studies that endeavor to evaluate risk factors for anthracycline-associated toxicity, our study was limited by small sample size. Thus, selection of a larger patient cohort via multi-institutional collaboration and possible meta-analysis will be critical to future scientific inquiries on this topic. Further research is required to identify potentially predictive genetic abnormalities of therapy-associated toxicities. Ultimately, prospective identification of patients at greatest risk of toxicity will allow for earlier therapeutic interventions and perhaps even toxicity avoidance.

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Table 1

Baseline patient characteristics

	Cardiotoxicity cohort (cases; n=52)	Control cohort (controls; n=110)	p values
Age (mean, standard deviation)	51.9±11.9	50.1±9.3	0.324
Body mass index	29.1±7.8	29.3±7.4	0.861
Menopause at diagnosis	35 (67.3 %)	65 (59.6 %)	0.348
Cancer stage at diagnosis			
1	13 (25.5 %)	31 (28.7 %)	0.548
2	21 (41.2 %)	50 (46.3 %)	
3	17 (33.3 %)	17 (25.0 %)	
Location			
Right	23 (44.2 %)	47 (42.7 %)	0.92
Left	25 (48.1 %)	56 (50.9 %)	
Bilateral	4 (7.7 %)	7 (6.4 %)	
Surgery type			
None	1 (1.9 %)	0 (0 %)	0.248
Lumpectomy	13 (25.0 %)	35 (31.8 %)	
Mastectomy/quadrantectomy	38 (73.1 %)	75 (68.2 %)	
Axillary dissection	35 (67.3 %)	73 (67.6 %)	0.971
Histology			
Invasive ductal	44 (86.3 %)	93 (85.3 %)	0.33
Invasive lobular	5 (9.8 %)	6 (5.5 %)	
Other	2 (3.9 %)	10 (9.3 %)	
ER+	33 (63.5 %)	76 (69.1 %)	0.476
PR+	26 (50 %)	70 (63.6 %)	0.099
Her2+	23 (44.2 %)	22 (20 %)	0.0037
Radiation	32 (61.5 %)	59 (53.6 %)	0.344
Treatment			
Trastuzumab	25 (48.1 %)	26 (23.6 %)	0.0018
Aromatase inhibitor	20 (38.5 %)	63 (57.8 %)	0.022
Bevacizumab	5 (9.6 %)	4 (3.6 %)	0.121
Past medical history			
Hypertension	15 (30.6 %)	20 (18.4 %)	0.086
Hyperlipidemia	5 (10.2 %)	10 (9.2 %)	0.838
Asthma	8 (15.7 %)	11 (10 %)	0.298
Diabetes	7 (13.7 %)	5 (4.6 %)	0.039
Hypothyroidism	9 (18.0 %)	14 (12.7 %)	0.467
Social history			
Smoking status			
Never	37 (71.2 %)	83 (76.2)	0.785
Current	6 (11.5 %)	11 (10.1 %)	
Former	9 (17.3 %)	15 (13.8 %)	

	Cardiotoxicity cohort (cases; n=52)	Control cohort (controls; n=110)	p values
Alcohol use			
None	27 (51.9)	43 (39.5)	0.022
<8 drinks a week	19 (36.5 %)	62 (56.9 %)	
>8 drinks a week	6 (11.5 %)	4 (3.7 %)	
Family history of heart disease	16 (32.0 %)	27 (24.6 %)	0.324
Medications			
Beta blocker use	37 (32.6 %)	22 (20.6 %)	<0.001
Ace inhibitor use	28 (54.9 %)	21 (19.6 %)	<0.001
Diuretic use	19 (38 %)	24 (22.4 %)	0.042
Statin use	14 (28.0 %)	23 (21.5 %)	0.371
Angiotensin receptor blocker use	8 (16.0 %)	5 (4.7 %)	0.016
Spironolactone use	6 (12 %)	5 (4.7 %)	0.094
Anxiolytic/anti-depressant use	32 (64.0 %)	65 (60.8 %)	0.696

Table 2

Analysis of clinical risk factors for cardiotoxicity

Risk factor	Univariate logistics regression			Multivariate logistic regression		
	Odds ratio	95 % confidence interval	p value	Odds ratio	95 % confidence interval	p value
Aromatase inhibitor	0.46	0.23, 0.90	0.023	0.74	0.5, 1.09	0.129
Trastuzumab	2.99	1.49, 6.02	0.002	1.69	1.14, 2.52	0.009
Alcohol (<8 drinks/week) vs. none	0.49	0.24, 0.99	0.008	0.43	0.23, 0.81	0.009
Alcohol 2 (>8 drinks/week) vs. none	2.39	0.62, 9.25	0.067	3.07	1.16, 8.12	0.024
Family history of heart disease	1.45	0.69, 3.02	0.325	1.23	0.8, 1.89	0.352
Hypertension	1.96	0.90, 4.27	0.089	1.17	0.74, 1.84	0.494
Hyperlipidemia	1.13	0.36, 3.49	0.838	1.24	0.67, 2.29	0.5
Diabetes	3.34	1.01, 11.1	0.049	1.93	1.00, 3.73	0.05

Table 3

Polymorphisms in the CBR pathway

	Cases (n=52)	Controls (n=110)	General population	p value
CBR1 1096G>A (rs9024)				
Homogeneous A/A	2 (3.8 %)	2 (1.8 %)	–	0.261
Heterogeneous A/G	7 (13.5 %)	26 (23.6 %)	–	
Homogeneous G/G	43 (82.7 %)	82 (74.6 %)	–	
Major allele frequency (G)	0.894	0.864	0.89	
Minor allele frequency (A)	0.106	0.136	0.11	
CBR 3 (rs6892)				
Homogeneous A/A	6 (11.5 %)	20 (18.2 %)	–	0.556
Heterogeneous A/G	25 (48.1 %)	50 (45.5 %)	–	
Homogeneous G/G	21 (40.4 %)	40 (36.4 %)	–	
Major allele frequency (G)	0.644	0.591	0.60	
Minor allele frequency (A)	0.356	0.409	0.40	