BclI Glucocorticoid Receptor Polymorphism in Relation to Arterial Stiffening and Cardiac Structure and Function

ORIGINAL ARTICLE

*Bcl*I Glucocorticoid Receptor Polymorphism in Relation to Arterial Stiffening and Cardiac Structure and Function: The Hoorn and CODAM Studies

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BACKGROUND
Chronic glucocorticoid excess is associated with arterial stiffening and cardiac dysfunction. The *Bcl*I glucocorticoid receptor (GR) polymorphism increases GR sensitivity and is associated with a higher body mass index (BMI) and some proxies for cardiovascular disease (CVD). Whether *Bcl*I influences arterial stiffening and cardiac dysfunction is currently unknown. Therefore, the aim of the present study was to investigate the association of the *Bcl*I polymorphism with arterial stiffening and cardiac structure and function.

METHODS
We conducted an observational cohort study, combining 2 cohort studies designed to investigate genetic and metabolic determinants of CVD. We genotyped 1,124 individuals (age: 64.7 ± 8.5 years) from the Hoorn study and Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study for *Bcl*I. Several arterial stiffening indices of the carotid (Hoorn and CODAM study), brachial and femoral artery and the carotid-femoral pulse wave velocity (Hoorn study only) were determined. In addition, in the Hoorn study, we determined several variables of cardiac structure and function.

RESULTS
We identified 155 homozygous carriers (GG), 498 heterozygous carriers (CG), and 471 noncarriers (CC) of the *Bcl*I polymorphism. *Bcl*I genotypes did not display significant differences in variables of arterial stiffening (e.g., carotid distensibility coefficient (DC): 12.41 ± 5.37 *vs.* 12.87 ± 5.55 10^{-3}/kPa [mean ± SD]; $P = 0.38$; homozygous *vs.* noncarriers). In addition, no clear differences in estimates of cardiac structure and function were found.

CONCLUSIONS
Even though *Bcl*I is associated with a higher BMI and some proxies of CVD, our results do not support the concept that *Bcl*I carrier status is associated with greater arterial stiffening or cardiac dysfunction.

Keywords: arterial stiffness; arterial stiffening; *Bcl*I; blood pressure; cardiac function; cardiac structure; glucocorticoids; glucocorticoid receptor; glucocorticoid receptor polymorphism; hypertension; left ventricular function; rs41423247.

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Chronically increased glucocorticoid action has been associated with several metabolic and cardiovascular abnormalities, such as obesity, insulin resistance, hypertension, atherosclerosis, and consequently cardiovascular disease (CVD).[1,2] This is true both for endogenous and exogenous excess of glucocorticoids.[3,4] There is, however, considerable variation in the interindividual response to glucocorticoids,[5] which appears to be partially caused by genetic variations in the glucocorticoid receptor (GR) gene, such as single nucleotide polymorphisms.[6] One of the most common single nucleotide polymorphisms is the *Bcl*I polymorphism, caused by a C to G substitution in intron 2, which is associated with an increased sensitivity to glucocorticoids for G-allele carriers.[7] Interestingly, previous studies have shown that homozygous carriers of the *Bcl*I polymorphism exhibit unfavorable metabolic traits, such as greater abdominal obesity and insulin resistance.[8,9] We previously demonstrated a higher mean arterial pressure (MAP) and a lower ankle-brachial index for homozygous carriers, without significant effects on systolic blood pressure, intima-media thickness, and prevalent CVD.[10] In this study, we further explored the association of the *Bcl*I polymorphism with estimates of arterial stiffening and cardiac function.

In recent years, the role of arterial stiffening has been increasingly recognized in the development of cardiovascular morbidity and mortality, independent of classical CVD risk factors.[11–13] Importantly, variation in arterial wall properties across the arterial tree makes it eminent to measure arterial stiffening at different sites,[14] since these local stiffening estimates may be differentially associated with cardiovascular morbidity and mortality.[13,15] Stiffening of the arterial wall decreases coronary circulation and raises systolic blood pressure, thereby increasing the pulsatile load on the microcirculation.[16] In addition, increased arterial stiffening could impair cardiac function by increasing arterial wave reflections, resulting in increased cardiac afterload, increased myocardial oxygen demand, and decreased diastolic coronary perfusion pressure.[17]

Although conditions with chronic glucocorticoid excess have been linked to both arterial stiffening and heart failure,[18–21] potential associations with the *Bcl*I polymorphism have not been evaluated to date. *Bcl*I could increase arterial stiffening and cardiac dysfunction *via* direct cardiovascular effects, or indirectly, *via* the unfavorable metabolic effects that are associated with *Bcl*I, such as obesity and insulin resistance. Therefore, we investigated whether the *Bcl*I GR polymorphism was associated with arterial stiffening and cardiac structure and function. The study was conducted in a sample of two well-defined Dutch cohort studies, enriched with participants with disturbed glucose metabolism and an increased risk of CVD. We hypothesized *Bcl*I carriers to display greater arterial stiffening and unfavorable cardiac structure and function.

METHODS

Study population

In this cross-sectional study, we combined individuals from the baseline examination of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study[22] and the Hoorn study follow-up examination.[23] Research protocols and data collection procedures, including the measurements of arterial stiffening, were similar for both studies and they have been used as a combined cohort before, as was described earlier.[10,24] Briefly, the CODAM study is an ongoing prospective cohort study designed to study the effects of lifestyle, obesity, metabolic disease, and genetics on cardiovascular outcomes. It consists of 574 included and extensively characterized individuals, selected on the basis of an elevated risk of type 2 diabetes mellitus and CVD, as described elsewhere.[22] The Hoorn study started in 1989 as a population-based cohort study, investigating glucose metabolism and complications of cardiovascular risk factors (n = 2484). In 2000–2001, 648 surviving individuals of the original study and 174 individuals with type 2 diabetes mellitus from the Hoorn Screening study[23] were combined in the Hoorn study follow-up examination, resulting in 822 participants.

After combining the individuals from the 2 cohort studies and excluding individuals with missing data on *Bcl*I genotype and all outcome variables, the present study was performed in 1,124 individuals (464 from CODAM and 660 from the Hoorn Study). Both cohorts were approved by the local medical ethics committees and all individuals gave informed consent.

Measures of arterial stiffening

Arterial diameter (D) and distention (ΔD) of the carotid (Hoorn and CODAM study), femoral and brachial arteries (Hoorn study only) were measured according to international guidelines[25] by ultrasound imaging techniques (Pie 350 Series; Pie Medical BV, Maastricht, The Netherlands in the Hoorn study and Ultramark 4þ, Advance Technology Laboratories, Bothel, WA, in the CODAM study). Together with the carotid intima-media thickness and the brachial pulse pressure (PP; systolic blood pressure – diastolic blood pressure), these measurements were used to calculate arterial stiffening of the carotid, femoral, and brachial arteries:

- Distensibility coefficient (DC, a measure of arterial stiffening): $(2\Delta D \times D + \Delta D^2)/(PP \times D^2)$
- Compliance coefficient (CC, a measure of arterial buffering capacity): $\pi \times (2D \times \Delta D + \Delta D^2)/4PP$
- Young's elastic modulus (the intrinsic stiffening of the arterial wall at operating pressure, measured only in carotid artery[26,27]): D/(intima-media thickness × DC)[23,25]

In addition, in a subgroup of the Hoorn study only, carotid-femoral pulse wave velocity (cfPWV) was calculated by dividing estimated travel distance (based on body height[28]) by estimated transit time (based on continuous measurement of the distension curves of the carotid and femoral arteries[26]).

Cardiac structure and function

Echocardiography was only performed in the Hoorn Study population. All echocardiograms were obtained by a single ultrasound technician—and subsequently reviewed by a senior cardiologist—with the HP SONOS 5500 scanner (2–4 MHz transducer, Andover, MA) according to a standardized protocol, with 2-dimensional M-mode recordings, both in parasternal- and apical views. The following structural variables were determined: left ventricular (LV) mass, LV mass index (LV mass divided by body surface[29]), LV end-diastolic diameter, left atrial (LA) volume index (LA volume

divided by body surface), and the product of LA volume and LV mass index (LAV × LVMI).

As an estimate of left ventricular systolic function, left ventricle ejection fraction was determined, calculated by dividing the difference between end-systolic and end-diastolic LV volume by the end-diastolic volume. Diastolic dysfunction can indirectly be estimated based on measures of cardiac structure, as a higher LV mass index,[27] LA volume index,[30] and LAV × LVMI[31] represent a decreased diastolic function.

Single nucleotide polymorphism analysis

*Bcl*I (rs41423247) is a C/G restriction fragment length polymorphism located in intron 2 of the GR gene (*NR3C1*), 646 nucleotides downstream from exon 2.[7] Determination was performed by allelic discrimination with the TaqMan Genotyping Master Mix (Applied Biosystems) using probes as previously described.[7]

Study power and statistical analyses

A power analysis was performed based on previous data, anticipating higher arterial stiffening, and decreased cardiac function in the homozygous carriers of the *Bcl*I G-allele (GG). In a previous study in the same population we identified 169 homozygous carriers and 519 noncarriers of the *Bcl*I polymorphism. Assuming a normal distribution, this population-size would result in a power of 80.6% to demonstrate a significant effect of 0.25SD (i.e., DC: 1.4 10^{-3}/kPa, CC: 0.06 mm^2/kPa, Young's elastic modulus: 0.12 10^3 × kPa) in the analyzed outcome variables. Since variables of brachial and femoral arterial stiffening and cardiac structure and function were only available in the Hoorn study population, we expected to have sufficient power to detect a difference of 0.34 SD in these variables.

The Hardy–Weinberg equilibrium was determined using a χ^2 test. Variables with a skewed distribution were natural log-transformed before further analyses. Multivariate linear regression was used to analyze differences in variables of arterial stiffening and cardiac structure and function across the 3 *Bcl*I genotypes (CC, CG, and GG). These analyses were performed by creating dummy variables, using alternately CG and CC as a reference as described before.[8,10] The analyses were performed crude (model 1), adjusted for sex, age, cohort, glucose metabolism status, and MAP (model 2), additional adjustment for estimated glomerular filtration rate,[32] antihypertensive medication, and angiotensin-converting enzyme inhibitors (use of angiotensin receptor blockers or mineralocorticoid receptor antagonists was uncommon in our population) (model 3) and for prior CVD (model 4). Since we previously demonstrated higher body mass index (BMI) in homozygous *Bcl*I carriers, we additionally performed mediation analyses to investigate whether a possible association with arterial stiffening or cardiac structure and function was mediated by BMI (model 5). A 2-sided P value <0.05 was considered statistically significant. Data throughout the manuscript are presented as mean ± SD unless otherwise indicated. Statistical analyses were performed with the IBM Statistical Package for Social Sciences for MAC, version 21 (SPSS).

Additional analyses

Since almost 40% of the study cohort was treated for hypertension, sensitivity analyses were performed on the arterial stiffening indices and cardiac structure and function variables excluding participants using antihypertensive medication. In addition, we analyzed the association of the *Bcl*I polymorphism with carotid arterial lumen, diameter, distention, and brachial PP separately, to detect the possibility of arterial remodeling.

RESULTS

Participants of the Hoorn and CODAM studies were combined and genotyped for the *Bcl*I GR polymorphism. The Hoorn study population was older than the CODAM study population, consisted of more women, more individuals with type 2 diabetes mellitus, and more individuals with prior CVD (Table 1). In the analyses, all genotyped individuals with available data on arterial stiffening or cardiac structure and function were included, resulting in a total study population of 1,124 participants, comprising 155 homozygous carriers (GG), 498 heterozygous carriers (CG), and 471 noncarriers of the *Bcl*I polymorphism. These genotypes were in Hardy–Weinberg equilibrium (used to measure whether the observed genotype frequencies in a population differ significantly from the frequencies predicted by the equation based on the allele frequency) ($P > 0.05$). Furthermore, the frequencies of the *Bcl*I genotypes did not differ between the cohorts (Table 1). Mean values and SDs of the study variables across the *Bcl*I GR polymorphism genotypes are displayed in Table 2.

*Bcl*I GR polymorphism and arterial stiffening variables

Table 3 shows the unstandardized regression coefficients for the association of the *Bcl*I genotypes with the DC, CC, and Young's elastic modulus, measured in the common carotid artery. *Bcl*I genotypes were not associated with differences in any of these carotid arterial stiffening measurements, in any of the statistical models.

Additionally, we investigated associations of *Bcl*I with the DC and CC in the brachial and femoral arteries, and by estimating the cfPWV. These measurements were performed in a subpopulation, since they were available in the Hoorn Study only. Due to technical reasons (i.e., no qualitatively acceptable distension curve available for both the carotid and femoral artery), as well as due to later addition of the automatic calculation of carotid-femoral transit time to the vascular ultrasound protocol, cfPWV was only available in 257 participants. Just as for the carotid stiffening estimates, the DC and CC of the brachial and femoral arteries, as well as the cfPWV, were not significantly different between genotypes of the *Bcl*I GR polymorphism (Supplementary Table 1).

*Bcl*I polymorphism and variables of cardiac structure and function

Next, we investigated whether *Bcl*I genotypes were associated with differences in variables of cardiac

Table 1. General characteristics of the CODAM and Hoorn study population

	CODAM (*n* = 464)	Hoorn (*n* = 660)	Total (*n* = 1124)
Women (%)	38.8	50.2	45.5
CC/CG/GG (%)	41/45/14	43/43/14	42/44/14
NGM/IGM/T2DM (%)	55/21/24	38/23/39	45/22/33
Age (years)	59.2 ± 7.1	68.7 ± 7.1	64.7 ± 8.5
BMI (kg/m^2)	28.1 ± 3.9	27.5 ± 3.8	27.8 ± 3.9
Obesity (%)	27.2	23.5	25.0
Current smoking (%)	19.4	15.7	17.2
Antihyp Med (%)	37.3	38.8	38.2
Prior CVD (%)	25.9	54.9	42.6
Systolic BP (mm Hg)	140 ± 20	143 ± 20	141 ± 20
Diastolic BP (mm Hg)	82 ± 9	83 ± 11	83 ± 10
Mean arterial pressure (mm Hg)	101 ± 12	103 ± 12	102 ± 12
Carotid-femoral PWV (m/s)	–	10.6 ± 5.6	–
Carotid artery			
Distensibility coefficient (10^{-3}/kPa)	15.23 ± 6.01	10.73 ± 4.09	12.65 ± 5.47
Compliance coefficient (mm^2/kPa)	0.703 ± 0.271	0.526 ± 0.216	0.601 ± 0.256
Young's elastic modulus (10^3 × kPa)	0.788 ± 0.372	1.036 ± 0.532	0.932 ± 0.487
Brachial artery			
Distensibility coefficient (10^{-3}/kPa)	–	7.53 ± 4.04	–
Compliance coefficient (mm^2/kPa)	–	0.127 ± 0.071	–
Femoral artery			
Distensibility coefficient (10^{-3}/kPa)	–	5.06 ± 2.31	–
Compliance coefficient (mm^2/kPa)	–	0.395 ± 0.202	–
Cardiac structure and function			
LV mass (g)	–	177 ± 57	–
LV mass index (g/m^2)	–	93.3 ± 27.7	–
LV end-diastolic diameter (mm)	–	50.8 ± 6.0	–
LA volume index (ml/m^2)	–	24.7 ± 9.9	–
LAV × LV mass index (ml × g/m^2)	–	4,571 ± 3,359	–
Ejection fraction (%)	–	61.2 ± 8.3	–

Data are shown as mean ± SD. Obesity is defined as BMI ≥ 30 kg/m^2. Abbreviations: Antihyp Med, antihypertensive medication; BMI, body mass index; BP, blood pressure; CC, noncarriers; CG, heterozygous carriers; CVD, cardiovascular disease; GG, homozygous carriers; IGM, impaired glucose metabolism; LA, left atrial; LAV, left atrial volume; LV, left ventricular; LVMI, left ventricular mass index; NGM, normal glucose metabolism; PWV, pulse wave velocity; T2DM, type 2 diabetes mellitus.

structure and function, which were only available in the Hoorn study population. In general, we observed no consistently significant differences in the cardiac structure variables LV mass, LV mass index, LV end-diastolic diameter, and LAV × LVMI across *Bcl*I genotypes (Tables 2 and 4). LA volume index was slightly lower for heterozygous carriers, but only when compared with noncarriers of the *Bcl*I polymorphism. This association remained significant after adjustment for all covariates and after additional adjustment for the effect mediator BMI (Table 4). In addition, left ventricular function measured by left ventricle ejection fraction was not consistently different between genotypes.

Additional analyses

Excluding participants using antihypertensive medication did not result in significant associations of the *Bcl*I polymorphism with variables of arterial stiffening or cardiac structure and function (Supplementary Tables 2–4). Additional analyses investigating the arterial wall properties showed that in GG-carriers, carotid artery diameter was statistically significantly increased as compared with CC-carriers, without concomitant alterations in arterial stiffening. PP, carotid artery distensibility, and carotid artery lumen were not significantly altered in GG-carriers as compared with CC-carriers (Supplementary Table 5).

Table 2. Distribution of outcome variables across *Bcl*I genotypes

	CC	CG	GG
Hoorn and CODAM study			
N	471	498	155
Age (years)	64.6 ± 8.7	64.8 ± 8.4	65.1 ± 8.5
Systolic BP (mm Hg)	141 ± 20	142 ± 20	144 ± 21
Diastolic BP (mm Hg)	82 ± 11	83 ± 10	84 ± 10
Mean arterial pressure (mm Hg)	102 ± 12	102 ± 12	104 ± 13
Carotid artery			
Distensibility coefficient (10^{-3}/kPa)	12.87 ± 5.55	12.51 ± 5.43	12.41 ± 5.37
Compliance coefficient (mm^2/kPa)	0.610 ± 0.272	0.590 ± 0.239	0.611 ± 0.260
Young's elastic modulus (10^3 × kPa)	0.914 ± 0.499	0.941 ± 0.459	0.959 ± 0.535
Hoorn study only			
N	283	287	90
Carotid-femoral PWV (m/s)	10.1 ± 4.4	10.9 ± 6.7	11.1 ± 4.7
Brachial artery			
Distensibility coefficient (10^{-3}/kPa)	7.63 ± 3.95	7.43 ± 4.07	7.55 ± 4.26
Compliance coefficient (mm^2/kPa)	0.126 ± 0.066	0.126 ± 0.069	0.138 ± 0.088
Femoral artery			
Distensibility coefficient (10^{-3}/kPa)	5.11 ± 2.43	5.09 ± 2.30	4.74 ± 1.96
Compliance coefficient (mm^2/kPa)	0.400 ± 0.206	0.394 ± 0.201	0.383 ± 0.191
Cardiac structure and function			
LV mass (g)	176 ± 56	178 ± 59	181 ± 52
LV mass index (g/m^2)	93.3 ± 28.8	92.7 ± 27.4	94.8 ± 24.8
LV end-diastolic diameter (mm)	50.8 ± 5.9	50.6 ± 6.1	51.1 ± 5.6
LA volume index (ml/m^2)	25.3 ± 9.9	24.1 ± 10.6	24.9 ± 7.1
LAV × LVMI (ml × g/m^2)	4,573 ± 3,165	4,520 ± 3,722	4,724 ± 2,716
Ejection fraction (%)	60.4 ± 8.8	61.9 ± 8.0	61.8 ± 7.7

Data are shown as mean ± SD. Abbreviations: BP, blood pressure; CC, noncarriers; CG, heterozygous carriers; GG, homozygous carriers; LA, left atrial, LAV; left atrial volume; LV, left ventricular; LVMI, left ventricular mass index; PWV, pulse wave velocity.

DISCUSSION

In this study, we combined data from 2 well-defined cohort studies based in the Netherlands to evaluate arterial stiffening and cardiac structure and function across genotypes of the *Bcl*I GR polymorphism. No differences in carotid, brachial, and femoral measures of arterial stiffening, nor in the cfPWV, were found. In addition, our study revealed no clear differences in estimates of cardiac structure and function. Thus, even though homozygous carriers of the *Bcl*I polymorphism display higher BMI, higher MAP, and lower ankle-brachial index, as demonstrated in earlier studies in these cohorts,[8,10] no clinically significant differences in other proxies for CVD were observed.

Arterial stiffening is increased in conditions of prolonged endogenous and exogenous glucocorticoid excess,[19,21] contributing to a rise is systolic blood pressure and increased cardiac afterload.[17] This effect could be mediated by direct vascular effects of the glucocorticoid or mineralocorticoid receptor,[2] or indirectly *via* well-known deleterious effects of glucocorticoids on metabolism.[33] In our current study, however, we did not observe any relevant effect of the *Bcl*I GR polymorphism on arterial stiffening, in line with our earlier observation that systolic blood pressure did not differ across *Bcl*I genotypes in this population.[10] Possibly, the effect of exposure to high doses of glucocorticoids is larger than the effects that we can expect of an increased GR sensitivity by *Bcl*I. Alternatively, since we did demonstrate differences in MAP but not in arterial stiffening, the current null findings could be explained by arterial remodeling in *Bcl*I carriers, neutralizing the effects of increased pressure on stiffening indices. In this respect, our additional analyses displayed increased arterial diameter without altering PP, arterial distensibility, and arterial lumen. The results of these analyses suggest that indeed mechanisms are operative to maintain the hemodynamic integrity of the arterial wall (i.e., keeping circumferential wall stress constant).[34] Taken together, although homozygous carriage of the G-allele has been associated with an unfavorable metabolic profile and possibly with peripheral atherosclerosis,[8,10] the results of our

Table 3. Associations of *Bcl*I polymorphism with carotid arterial stiffening

		GG vs. CG		GG vs. CC		CG vs. CC	
	Model	β	95% CI	β	95% CI	β	95% CI
Distensibility coefficient	1	−0.193	−1.222; 0.836	−0.461	−1.494; 0.572	−0.268	−0.987; 0.451
	2	0.072	−0.657; 0.801	−0.074	−0.806; 0.659	−0.146	−0.656; 0.364
	3	0.069	−0.659; 0.796	−0.071	−0.802; 0.661	−0.139	−0.648; 0.370
	4	0.089	−0.637; 0.814	−0.076	−0.805; 0.653	−0.165	−0.673; 0.343
	5	0.160	−0.561; 0.880	−0.033	−0.756; 0.691	−0.192	−0.696; 0.312
Compliance coefficient	1	0.019	−0.030; 0.067	0.005	−0.044; 0.053	−0.014	−0.047; 0.020
	2	0.024	−0.015; 0.064	0.018	−0.022; 0.058	−0.006	−0.034; 0.022
	3	0.024	−0.016; 0.064	0.018	−0.022; 0.058	−0.006	−0.034; 0.022
	4	0.025	−0.015; 0.064	0.018	−0.022; 0.058	−0.007	−0.035; 0.021
	5	0.025	−0.015; 0.065	0.018	−0.022; 0.058	−0.007	−0.035; 0.021
Young's elastic modulus	1	0.022	−0.071; 0.116	0.046	−0.048; 0.140	0.023	−0.042; 0.088
	2	0.008	−0.073; 0.089	0.022	−0.059; 0.104	0.015	−0.041; 0.071
	3	0.008	−0.073; 0.089	0.022	−0.059; 0.103	0.014	−0.042; 0.070
	4	0.006	−0.075; 0.086	0.024	−0.057; 0.105	0.018	−0.038; 0.074
	5	0.003	−0.077; 0.084	0.022	−0.059; 0.103	0.019	−0.037; 0.075

Comparison across genotypes. Model 1; crude analysis. Model 2; adjusted for sex, age, cohort, glucose metabolism status, and mean arterial pressure. Model 3; model 2 + antihypertensive medication, ACE-inhibitors, and estimated GFR. Model 4; model 3 + prior CVD. Mediation analysis: model 5; model 4 + BMI. *$P < 0.05$. n = 1,040 for DC and CC, n = 1016 for YEM. Abbreviations: ACE, angiotensin-converting enzyme; β, unstandardized regression coefficient: indicates the difference in dependent variable (in its units) between groups being compared; BMI, body mass index; CC, noncarriers; CG, heterozygous carriers; CI, confidence interval; CVD, cardiovascular disease; GFR, glomerular filtration rate; GG, homozygous carriers; YEM, Young's elastic modulus.

study do not support the concept that *Bcl*I increases cardiovascular risk through increased arterial stiffening.

In addition, none of the cardiac structure and function variables were in our opinion clearly associated with the *Bcl*I polymorphism. In the current analyses, heterozygous carriers of the *Bcl*I polymorphism displayed a slightly lower LA volume index in all statistical models and a slightly higher ejection fraction in partly adjusted statistical models when compared to noncarriers. In fact, homozygous carriers did not display differences in these variables compared with the other genotypes, while in previous studies homozygous carriers consistently displayed disadvantageous metabolic and cardiovascular characteristics. Potentially, for the *Bcl*I variant there is no allele-dosage effect with respect to a relation with cardiac structure and function variables or the effects may be tissue-specific as suggested previously.[7] However, since multiple statistical tests were performed in this study, these significant results might well be spurious.

Since both obesity and insulin resistance are associated with arterial stiffening and cardiac dysfunction,[23,35–37] and earlier studies demonstrated greater obesity and insulin resistance for carriers of the *Bcl*I polymorphism,[8] one might expect these factors to have effect on the current cardiovascular outcomes as well. Our data, however, suggest that the metabolic traits associated with *Bcl*I have only minor cardiovascular effects. Possibly, actions of *Bcl*I are pleiotropic; exerting cardioprotective effects next to the disruptive effects on metabolism. Another explanation could be that the metabolic effects of *Bcl*I are only manifested at a later age, thereby not yet having impacted the cardiovascular system at the age of our study population.

A limitation of the study is that the femoral and brachial measurements, the cfPWV, and the cardiac measurements were performed in a subset of participants, therefore limiting the power of these analyses. A strength of our study is its well-characterized study population, enriched with participants at risk for CVD, increasing the power to investigate cardiovascular proxies and outcomes. In addition, many of the earlier studies investigating the effects of increased glucocorticoid action on cardiovascular outcomes, were performed in patient populations with active diseases or treatment,[18,19,21,38] thereby generating many possible confounding factors.[39] The current study was performed in an extensively characterized cohort while controlling for many known CVD risk factors, making it possible to reliably investigate the influence of a genetically increased sensitivity of the GR to glucocorticoids on several proxies for CVD.

In conclusion, in a combination of 2 Dutch cohorts enriched with participants with type 2 diabetes mellitus and with a higher CVD risk, the *Bcl*I GR polymorphism was not associated with several measures of arterial stiffening or differences in cardiac structure or function. Therefore, even though carriers of the *Bcl*I polymorphism may display several disadvantageous metabolic traits, as well as higher MAP and possibly peripheral atherosclerosis, this did not translate into greater arterial stiffening or

Table 4. Associations of *BclI* polymorphism with cardiac structure and function (Hoorn study only)

		GG vs. CG		GG vs. CC		CG vs. CC	
	Model	β	95% CI	β	95% CI	β	95% CI
Log LV mass	1	0.020	−0.055; 0.094	0.029	−0.045; 0.103	0.010	−0.042; 0.061
	2	0.022	−0.046; 0.090	0.030	−0.038; 0.098	0.008	−0.039; 0.055
	3	0.023	−0.043; 0.089	0.031	−0.035; 0.097	0.008	−0.038; 0.054
	4	0.020	−0.045; 0.086	0.032	−0.034; 0.098	0.012	−0.034; 0.057
	5	0.018	−0.046; 0.082	0.026	−0.038; 0.090	0.008	−0.036; 0.052
Log LV mass index	1	0.028	−0.040; 0.096	0.022	−0.046; 0.090	−0.007	−0.054; 0.041
	2	0.021	−0.044; 0.087	0.016	−0.049; 0.081	−0.005	−0.051; 0.040
	3	0.022	−0.041; 0.086	0.017	−0.047; 0.080	−0.005	−0.049; 0.038
	4	0.019	−0.044; 0.082	0.018	−0.045; 0.081	−0.001	−0.044; 0.043
	5	0.018	−0.045; 0.081	0.017	−0.046; 0.080	−0.002	−0.045; 0.042
LV end-diastolic diameter	1	0.352	−1.109; 1.814	0.190	−1.268; 1.648	−0.162	−1.174; 0.849
	2	0.681	−0.696; 2.059	0.468	−0.905; 1.842	−0.213	−1.162; 0.736
	3	0.652	−0.718; 2.023	0.441	−0.926; 1.807	−0.211	−1.155; 0.732
	4	0.651	−0.722; 2.023	0.441	−0.926; 1.809	−0.209	−1.156; 0.737
	5	0.625	−0.742; 1.991	0.386	−0.977; 1.748	−0.239	−1.181; 0.704
Log LA volume index	1	0.062	−0.017; 0.141	0.000	−0.079; 0.079	**−0.062***	**−0.117; −0.006**
	2	0.059	−0.018; 0.136	0.003	−0.074; 0.080	**−0.056***	**−0.110; −0.003**
	3	0.062	−0.013; 0.137	0.004	−0.070; 0.079	**−0.057***	**−0.109; −0.005**
	4	0.061	−0.014; 0.136	0.005	−0.070; 0.080	**−0.056***	**−0.108; −0.004**
	5	0.061	−0.014; 0.136	0.005	−0.070; 0.080	**−0.056***	**−0.108; −0.004**
Log LAV × LVMI	1	0.079	−0.046; 0.203	0.037	−0.087; 0.161	−0.042	−0.128; 0.045
	2	0.074	−0.043; 0.190	0.036	−0.080; 0.152	−0.038	−0.119; 0.043
	3	0.078	−0.034; 0.189	0.040	−0.072; 0.151	−0.038	−0.115; 0.039
	4	0.075	−0.037; 0.186	0.042	−0.069; 0.153	−0.033	−0.110; 0.004
	5	0.071	−0.040; 0.181	0.034	−0.076; 0.144	−0.037	−0.113; 0.040
Ejection fraction	1	0.0	−2.1; 2.1	1.4	−0.7; 3.5	1.4	−0.1; 2.8
	2	−0.4	−2.4; 1.7	1.1	−0.9; 3.1	**1.4***	**0.0; 2.8**
	3	−0.4	−2.4; 1.6	1.0	−1.0; 3.0	**1.5***	**0.1; 2.8**
	4	−0.4	−2.4; 1.6	1.0	−1.0; 3.0	1.4	0.0; 2.8
	5	−0.4	−2.4; 1.6	1.0	−1.0; 3.0	1.4	0.0; 2.8

Comparison across genotypes. Model 1; crude analysis. Model 2; adjusted for sex, age, glucose metabolism status, and mean arterial pressure. Model 3; model 2 + antihypertensive medication, ACE-inhibitors, and estimated GFR. Model 4; model 3 + prior CVD. Mediation analysis: model 5; model 4 + BMI. A lower LV ejection fraction indicates a worse LV systolic function, unlike all other estimates of which higher values indicate a worse LV systolic and/or diastolic function. Bold formatted text is used to underscore that these associations were statistically significant. *$P < 0.05$. $n = 623$ for log LV mass, log LV mass index and LV end-diastolic diameter, $n = 602$ for log LA volume index, $n = 600$ for log LAV × LVMI, and $n = 580$ for ejection fraction. Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; β, unstandardized regression coefficient: indicates the difference in dependent variable (in its units) between groups being compared; CC, noncarriers; CG, heterozygous carriers; CI, confidence interval; CVD, cardiovascular disease; GFR, glomerular filtration rate; GG, homozygous carriers; LA, left atrial, LAV; left atrial volume; LV, left ventricular; LVMI, left ventricular mass index.

cardiac dysfunction in this population. These findings suggest that genetic variants of the GR exert pleiotropic effects on the cardiovascular system.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *American Journal of Hypertension* online.

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DISCLOSURE

The authors declared no conflict of interest.

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