Association of Coronary Artery Disease With Glucocorticoid Receptor N363S Variant

Ruby C.Y. Lin, Xing Li Wang, Brian J. Morris

Abstract—Overweight is associated with the N363S variant in the glucocorticoid receptor (encoded by nuclear receptor subfamily 3, group C, member 1 gene: NR3C1). The present study examined whether the N363S polymorphism might also be associated with coronary artery disease (CAD). This involved 556 patients with CAD, of which 437 were analyzed, and 302 control subjects, all being of Anglo-Celtic descent residing in Sydney. An extensive range of phenotypic parameters was collected from the patients, and leukocyte DNA from all subjects was genotyped by polymerase chain reaction–restriction fragment length polymorphism analysis for the A1218G (N363S) variant. Frequency of the S363 allele was 0.04 in healthy normal-weight control subjects but was 0.15 in patients with CAD ($P=2.0 \times 10^{-5}$) and was also elevated in subjects with CAD who were not overweight (0.14) ($P=2.6 \times 10^{-5}$), supporting a primary association with CAD. Frequency of S363 allele carriers in subjects with CAD who had angina was particularly high: unstable angina (0.45), stable angina (0.29), and no angina (0.26) ($P$ for trend = 0.016). Elevated cholesterol ($P=0.027$), triglycerides ($P=0.005$), and total cholesterol/HDL ratio ($P=0.011$), after Bonferroni, tracked with the S363 allele, consistent with accentuation of mechanisms that predispose to atheroma formation in coronary vessels. The data suggest a role for glucocorticoid receptor variation in the underlying cause of CAD. (Hypertension. 2003;41:1111–1116.)

Key Words: glucocorticoids ■ genetics ■ coronary artery disease ■ population

Coronary artery disease (CAD) and overweight are 2 of the conditions that constitute the “metabolic syndrome.”1,2 One of the most important regulators of both cardiovascular function and metabolism is the glucocorticoid class of hormones, whose effects are mediated by the glucocorticoid receptor (GR). Genetic variation in the GR gene (NR3C1)3–12 has the potential to predispose to each of these conditions. Rare point mutations or deletions can lead to reduced intracellular concentration or biological activity of GR in glucocorticoid target tissues.3,5,13 Patients with such NR3C1 defects can exhibit one or more of a plethora of clinical manifestations, such as Cushingoid symptoms and signs, hypertension, hypokalemic alkalosis, and/or familial glucocorticoid resistance. In addition, tissue sensitivity to glucocorticoids is associated with insulin resistance, glucose intolerance, and hypertriglyceridemia.14 In contrast, for common variants, promoter activity and ligand binding of GR are similar.7,15 For one, an intronic BclI restriction fragment length polymorphism (RFLP) that was the subject of early studies, an association with obesity, abdominal visceral fat and hyperinsulinemia was reported.4,16–18 More recently, association was seen between an N363S variant19 and elevated body mass index (BMI) in a Dutch cohort and overweight in 2 different groups of Anglo-Celtic whites20 but not in Danish subjects21 or Swedish men.11 Other variants in or near NR3C1 showed no association with overweight.22 The N363S variant has thus become a topic of great interest. Although it has shown no association with essential hypertension,22 it was associated with overweight/obesity in hypertensive patients.20 N363S is 101 residues from the transactivation ($\tau_1$) domain (amino acids 77 to 262), which interacts with coactivator and corepressor proteins during transcription complex formation.23–25 In mouse, ligand binding leads to phosphorylation of 6 amino acids in $\tau_1$ as well as S315, which has no homologue in the human GR.26 Phosphorylation of serine and threonine residues is important for DNA binding by GR dimers.24,26 Thus the extra serine in the N363S GR variant has the potential to offer an additional phosphorylation site, with possible biological sequelae. Moreover, deletion of amino acids 328 to 382 from the human mineralocorticoid receptor markedly decreases transcriptional activation,27 so one wonders if this region of the GR, which includes the N363S site, might also affect GR activity.

In view of the effects of glucocorticoids on the heart, vasculature, and plasma lipids, we considered it important to...
test whether the GR N363S variant might be associated with CAD.

**Methods**

**Study Cohorts**

Subjects were all of Anglo-Celtic Caucasian extraction and resided in the same geographic locality in or near Sydney. Height and weight were recorded, and a 10-mL blood sample was collected for DNA extraction and plasma biochemistry. The study had ethical approval, and all subjects gave informed consent. Specific details of each group follow.

**Control Subjects**

These consisted of 302 volunteers from the Sydney Red Cross Blood Bank. They were nong diabetic, had no heart or kidney disease, nor hypertension (blood pressure <140/90 mm Hg), and reported similar absence in their parents. In all of our many previous genetic studies over more than a decade, this cohort has exhibited allele frequencies for all polymorphisms examined that were similar to values reported for other healthy white populations elsewhere.

**Patients With CAD**

There were 556 patients with CAD, all <65 years of age, who had been consecutively referred to the Eastern Heart Clinic at Prince of Wales Hospital, Sydney, for coronary angiography between 1996 and 1998. Each angiogram was classified as revealing either normal coronary arteries or having a coronary lesion with <50% luminal stenosis or as having 1, 2, or 3 major epicardial coronary arteries with >50% luminal obstruction. The medical history of each patient was obtained by a physician using a questionnaire with standardized choices of answers to be ticked during the interview, and DNA samples were collected for each patient. Three hundred twenty-seven (59.3%) had a history of myocardial infarction, 206 (37.5%) had unstable angina, 200 (36.4%) had stable angina, 62 (11.3%) had type 2 diabetes, 246 (45.1%) were being or had been treated for hypertension, 255 (46.5%) were ex-smokers, 117 (21.4%) were current smokers, and 437 (79%) had angiographically confirmed CAD, the severity of which was categorized by percent luminal obstruction (>50%) on 3 major epicardial coronary arteries, as observed on coronary angiograms. This has often been regarded as severity of CAD. All vascular events were documented during hospitalization. The remaining 119 (21%) patients had no angiographically demonstrable coronary lesions and were therefore excluded.

**Genotyping**

DNA was isolated from whole blood by use of a DNA extraction kit (Qiagen). Genotypes for the A1218G (N363S) NR3C1 variant were determined by polymerase chain reaction (PCR)-RFLP analysis, as described previously, except that PCR steps were modified to 1 minute each. PCR products were digested at 65°C for 1 hour with 1 U of Tsp509I (New England BioLabs). Random samples were purified and cloned (pGEM-T Easy Vector System; Promega) and sequenced (Australian Genome Research Facility) to confirm accuracy of genotyping. As well, 106 were subjected to melt-curve genotyping analysis with real-time PCR (Rotorgene, Corbett Research) with FAM and Cy5 labeled probes, and primers: forward, 5′-TGTTCGACCAGGGAAGTTCAGAG-3′; reverse, 5′-TGTTGCACCGGGAAGTTCAGG-3′. Genotypes were assigned according to appearance of peaks in the melt curves.

**Statistical Analysis**

χ² and 1-way ANOVA was by StatView (Abacus Concepts) and SPSS v 9.0 for Windows. In some subgroup analyses, S363 carriers were grouped together because of low SS prevalence. Power (at 0.005 end point) indicated adequate sample size; healthy control subjects, 99%; CAD, 99%. Odds ratio (OR) was calculated as described.

**Results**

**Group Characteristics**

Table 1 shows group characteristics. The Hardy-Weinberg equilibrium applied for the control (χ²=6.0; P=0.031) but not the CAD cohort (χ²=12; P=0.003) because of excess NS and SS. Although higher S363 could theoretically be contributed by underdigestion during PCR-RFLP analysis, this was ruled out by direct sequencing. The excess could thus reflect disease-driven self-selection of S363 into the CAD patient group. The frequency (0.04) noted for S363 in normal-weight control subjects (Table 2) was similar to values reported by others of 0.03, 0.04, and 0.05 for healthy white Anglo-Celtic or Northern European subjects.

**Association Analyses**

CAD was strongly associated with the S363 allele (Table 2). OR for CAD to carry S363 was 1.6 (95% CI 1.1 to 2.3). Association of CAD with S363 was similar in male (0.16) and female subjects (0.18). S363 frequency in patients with a BMI ≥30 kg/m² was 0.19 (Table 3). Since we also saw a highly significant association when normal-weight patients with CAD were compared with normal-weight control subjects (Table 3), the data suggest a primary association with CAD. No association was, however, seen with severity of CAD, based on number of diseased vessels determined by angiography, nor with angina, diabetes, or hypertension within the patients with CAD. Frequency of S363 carriers was higher in patients with a history of myocardial infarction (0.30) than those without (0.24), but not significantly so (χ²=2.8, P=0.095). Of the 179 patients who had different stages of angina, those with unstable angina had higher S363 carrier frequency (0.45) than those with stable angina (0.29) or no angina (0.26) (P for trend=0.016).

In patients with CAD, BMI was elevated in S363 allele carriers (29±5 SD in SS+SN versus 28±4 SD kg/m² for NN; P=0.01), as were total cholesterol (P=0.04), plasma triglycerides (P=0.004), and total cholesterol/HDL ratio (P=0.006) after Bonferroni correction (Table 4). The associations were independent of gender, BMI, waist-to-hip ratio, and cigarette smoking in a factorial design ANOVA. Analysis

**TABLE 1. Characteristics of Subjects in Each Group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=302)</th>
<th>CAD (n=437)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female</td>
<td>173:129</td>
<td>353:84</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>47±11</td>
<td>56±7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26±4</td>
<td>28±4*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>120±11</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>73±8</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.0±0.1</td>
<td>5.4±0.05*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.3±0.05</td>
<td>2.0±0.05*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.3±0.03</td>
<td>1.0±0.01*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.7±0.1</td>
<td>3.5±0.05*</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Values are mean±SD or, for lipid measurements, mean±SE. HDL indicates high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; and ND, not determined.

*Bonferroni-corrected P values following t test.
of genotype data for subjects higher versus lower than median age of 57 years excluded survivorship bias.

### Discussion

Our study found a 4-fold elevated frequency of the S363 allele of GR in CAD. Lean patients with CAD showed 3-fold higher frequency. In obese patients with CAD, S363 frequency was elevated 5-fold, which adds to previous data for an association of S363 with overweight. Such elevations are striking for an association study, and the statistical significance observed were consequently very high. No sex difference was apparent. Increased waist-to-hip ratio for the S363 variant in male but not female subjects has been noted.

Numerous studies have confirmed that obesity is a strong indicator for CAD. The higher prevalence of the S363 allele in CAD appeared not, however, to be secondary to obesity in the patients with CAD, since the strong association was also apparent in a subgroup of patients with CAD of normal weight. Nevertheless, we cannot exclude the possibility that some of the patients with CAD who were lean had previously been obese but had lost weight in view of earlier clinical indications such as CAD or CAD risk.

The mechanism responsible for the association with CAD might be reflected in the influence of genotype we found on plasma lipid variables in patients with CAD, that is, higher total cholesterol, triglyceride, and total cholesterol/HDL ratio being strongly associated with the S363 allele. This could contribute to atheroma formation in coronary vessels and, of interest, particularly high S363 carrier frequency (0.45) was observed in patients with CAD with unstable angina, the value being 6 times higher than in healthy, normal-weight control subjects (0.07). Perhaps adipocytes having the S363 variant of GR could be more sensitive to glucocorticoids, leading to increased lipid mobilization. Whether the S363 allele plays a central role in overweight CAD requires further investigation.

In conclusion, the present study has demonstrated that CAD is associated with the S363 allele of the GR N363S variant. Early testing for the S363 allele could have merit in predicting risk of CAD later in life.

### Table 2. Association Analyses of NR3C1 N363S Variant in CAD

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes (Frequency)</th>
<th>Total Alleles on All Chromosomes (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NN NS SS χ² P</td>
<td>N S χ² P</td>
</tr>
<tr>
<td>Control</td>
<td>263 31 8 (0.87) (0.10) (0.03) 18 0.0003</td>
<td>557 47 (0.92) (0.08) 20 0.00002</td>
</tr>
<tr>
<td>CAD</td>
<td>330 84 23 (0.76) (0.19) (0.05)</td>
<td>744 130 (0.85) (0.15)</td>
</tr>
</tbody>
</table>

### Table 3. N363S Frequencies in CAD Patients Differing in BMI Status

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes (Frequency)</th>
<th>Total Alleles on All Chromosomes (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NN NS SS χ² P</td>
<td>N S χ² P</td>
</tr>
<tr>
<td>CAD with BMI&lt;25 (23±2 kg/m²)</td>
<td>79 16 6 (0.78) (0.16) (0.06)</td>
<td>174 28 (0.86) (0.14)</td>
</tr>
<tr>
<td>CAD with BMI 25–29.9 (27±1 kg/m²)</td>
<td>157 37 8 (0.78) (0.18) (0.04)</td>
<td>351 53 (0.87) (0.13)</td>
</tr>
<tr>
<td>CAD with BMI≥30 (33±3 kg/m²)</td>
<td>80 29 7 (0.69) (0.25) (0.06)</td>
<td>189 43 (0.81) (0.19)</td>
</tr>
<tr>
<td>Control with BMI&lt;25 (23±1 kg/m²)</td>
<td>141 9 1 (0.93) (0.06) (0.01)</td>
<td>291 11 (0.96) (0.04)</td>
</tr>
</tbody>
</table>

*All comparisons are with values for the CAD with BMI<25 kg/m² group, except where otherwise indicated.
†CAD with BMI≥30 kg/m² vs CAD with BMI 25–29.9 kg/m².
TABLE 4. Plasma Lipids in CAD Patients in the Presence and Absence of the N363 Allele

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S363/−</th>
<th>N363/N363</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (TC), mmol/L</td>
<td>5.6±0.1</td>
<td>5.3±0.05</td>
<td>0.027</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.3±0.1</td>
<td>1.9±0.05</td>
<td>0.005</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>6.0±0.2</td>
<td>5.5±0.09</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*P values obtained after Bonferroni correction.

Perspectives

The now dual associations found for the N363S variant of GR might suggest a general role in the metabolic syndrome. However, a lack of association with hypertension is consistent with an effect on lipid metabolism being the underlying defect, and the mechanism should now be explored. It will also be of interest to perform association studies of the N363S variant in type 2 diabetes and obesity as opposed to overweight subjects. Because of the key role of GR effects in many metabolic processes, it would also be of interest to see whether the N363S variant represents a genetic component in the fetal undernutrition (Barker) hypothesis for the underlying cause of metabolic syndrome.

Acknowledgments

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References

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