Renin-Angiotensin System Polymorphisms and Coronary Events in Familial Hypercholesterolemia

Anthony S. Wierzbicki, Michelle Lambert-Hammill, Peter J. Lumb, Martin A. Crook

Abstract—The role of renin-angiotensin system polymorphisms as risk factors for coronary heart disease (CHD) is controversial. This study investigated their role in patients with heterozygous familial hypercholesterolemia (FH). Polymorphism frequencies for angiotensin-I–converting enzyme insertion/deletion (ACE I/D), angiotensinogen M235T, and angiotensin-II type I receptor (AG2R) A1166C were determined in 112 patients with FH and 72 patients with polygenic hypercholesterolemia, of whom 26.7% and 41.6%, respectively, had established CHD. None of the polymorphisms were associated with risk of CHD in patients with polygenic hypercholesterolemia in this study. Logistic regression analysis of risk factors for CHD in patients with FH identified male sex (odds ratio [OR]=3.03; 95% CI, 3.07 to 3.72; \( P=0.05 \)), smoking (OR=2.91; 95% CI, 2.16 to 4.24; \( P=0.05 \)), diastolic blood pressure (OR=3.70; 95% CI, 3.43 to 3.97; \( P=0.02 \)), plasma glucose (OR=3.31; 95% CI, 3.10 to 3.52; \( P=0.04 \)), and the AG2R A1166C polymorphism as risk factors. The OR for the AG2R A1166C polymorphism was 2.26 (95% CI, 1.26 to 3.72; \( P=0.06 \)) and increased to 3.10 (95% CI, 1.20 to 7.52; \( P=0.04 \)) after adjustment for other risk factors. The AG2R A1166C polymorphism may interact with severe hypercholesterolemia and other risk factors to increase risk of CHD in FH patients. (Hypertension. 2000;36:808-812.)

Key Words: familial hypercholesterolemia ■ coronary artery disease ■ renin-angiotensin system ■ genetics

Familial hypercholesterolemia (FH) is an autosomal dominant disorder of clearance of LDL cholesterol and is associated with premature coronary artery disease. It clinical features include hypercholesterolemia, tendon xanthomata, arcus, and premature atherosclerosis.\(^1\) Patients with heterozygous hypercholesterolemia have significant excess risk at younger ages, although this tends to fall with age to levels 3- to 8-fold greater than those in age- and sex-matched controls.\(^1,2\) The age of onset of coronary heart disease (CHD) in individual families is similar but only moderately correlated with LDL cholesterol concentration and type of mutation.\(^3\) Hence, it is likely that genetic factors beyond the LDL receptor play a role in determining the incidence of CHD in heterozygous FH.

The renin-angiotensin system plays a critical role in the control of blood pressure, which is a significant risk factor for atherosclerosis and cardiovascular disease. Recently, polymorphisms in the 3 genes in the renin-angiotensin system—angiotensin-I–converting enzyme deletion/insertion (ACE I/D), the angiotensinogen gene methionine-235 threonine (AGT M235T), and the angiotensin II type I receptor (AG2R) A1166C—have been postulated as risk factors for CHD in patients with polygenic hyperlipidemia.\(^4,5\) However, the role of these polymorphisms in cardiovascular disease in FH has never been explored. This study examined the role of renin-angiotensin polymorphisms in determining the risk of CHD in patients with FH or polygenic hyperlipidemia.

Methods

Subjects

One hundred twelve patients with clinical heterozygous FH aged >40 years and 72 patients with polygenic hyperlipidemia were recruited from the lipid clinics of Guy’s and St Thomas’ hospitals with local hospital ethical committee permission and after individual informed medical consent had been obtained. The diagnosis of FH was made by Simon Broome Register criteria\(^1,2\): the presence of an autosomal dominant family history of CHD before age 60 years in first-degree or age 50 years in second-degree relatives, LDL >5.7 mmol/L (220 mg/dL), and the presence of tendon xanthomata for a definite, as opposed to a probable, diagnosis. The diagnostic criteria for polygenic hyperlipidemia were absence of history of CHD in a first- or second-degree relative before age 70 years. Patients with hypercholesterolemia and triglycerides >5 mmol/L (442 mg/dL) with positive family histories for CHD were excluded on grounds of possible familial combined hyperlipidemia.

All patients had been reviewed in the clinic for at least 6 years and had undergone exercise testing a minimum of 5 times per year; those with suboptimal exercise tolerance underwent thallium cardiac scintigraphy or stress echocardiography. Coronary artery disease was diagnosed on the basis of confirmed cardiac event, angioplasty, coronary bypass surgery, or angina with significant lesions visible on angiography.
Clinical Risk Factors
Cigarette smoking was certified by self-assessment at the time of the study, and subjects were classified as nonsmoker, ex-smoker, or current smoker. Body mass index was calculated as weight/height². Hypertension was diagnosed by either treatment or blood pressure >140 mm Hg systolic and >90 mm Hg diastolic. Tendon xanthomata and arcus were assessed clinically.

Biochemical Analyses
A full biochemical profile, including urea and electrolytes, liver function tests, thyroid function test, creatine kinase, fasting lipids (total, LDL, and HDL cholesterol and triglycerides), and lipoprotein(a) (Lp(a)), was performed after a 16-hour fast. Baseline lipids were assessed after subjects had discontinued drug therapy for 4 weeks. Lipids were measured by automated techniques on a Vitros 950 analyzer and apolipoproteins on a Behring BN2 analyzer. Fasting plasma homocysteine was measured by high-performance liquid chromatography.

Determination of Genotypes
DNA was extracted from fresh lymphocytes by a standard method (Puregene, [Gentra] Flowgen). Polymorphisms in genes including the ACE I/D, AGT M235T, and AG2R A1166C were assessed by amplification of the ACE gene in 5% dimethyl sulfoxide and by independent confirmation with the use of an I allele–specific primer pair. Restriction fragments were separated by electrophoresis on 2% to 4% agarose gels and typed by 2 independent observers blinded to the clinical data. Genotype frequencies were compared with 100 randomly selected healthy control samples to assess significant sampling errors in genotype determination. A 2-tailed P value <0.05 was considered significant.

Characterization of Subjects
A total of 184 subjects were included in the study. Patient characteristics are shown in Table 1. The median follow-up for patients with FH was 7.5 years (range, 6 to 25 years). FH patients were 9.2 years younger and had established CHD 11 years earlier than patients with polygenic hyperlipidemia. They had higher total and LDL cholesterol concentrations than polygenic patients and had higher rates of smoking in men with CHD (P<0.001). The incidence of CHD was slightly lower in the FH population than in patients with polygenic hyperlipidemia, although this was not significant. All other differences between the 2 groups were not significant.

Results
Risk Modeling

Logistic regression modeling identified male sex (OR = 3.03; 95% CI, 3.07 to 3.72; P < 0.05), smoking (OR = 2.91; 95% CI, 2.16 to 4.24; P < 0.05), diastolic blood pressure (OR = 3.70; 95% CI, 3.43 to 3.97; P < 0.02), plasma glucose (OR = 3.31; 95% CI, 3.10 to 3.52; P < 0.04), and the AG2R1166C allele (OR = 3.11; 95% CI, 1.20 to 7.52; P < 0.04) as significant risk factors for CHD in patients with FH. Other risk factors including total (or LDL) cholesterol (OR = 2.29; 95% CI, 0.89 to 3.20; P = 0.09) and Lp(a) (OR = 1.99; 95% CI, 0.70 to 3.26; P = 0.12) showed a slight association in the environmental risk factor model but did not reach statistical significance and therefore were omitted in combined environmental-genetic interaction modeling.

Genotype Analyses

The results of genotype analysis are shown by genotype and allele group in Tables 2 and 3. Allele frequencies in the groups without CHD did not differ significantly from those in the healthy control group. All allele frequencies were in groups without CHD did not differ significantly from those in the healthy control group. All allele frequencies were in Tables 2 and 3. Allele frequencies in the AG2R A1166C polymorphism were increased to 3.10 (95% CI, 1.20 to 7.52; P = 0.04).

Discussion

A number of factors, including age, sex, smoking, and HDL concentrations, have previously been identified as risk factors for CHD in FH in cross-sectional studies.1–3,13–18 Logistic regression modeling of this admittedly small population identified risk factors similar to those in these large studies, indicating that the population studied was likely to be representative.13 In this study, as in the large cohort study of 526 patients in the United Kingdom with heterozygous FH, LDL was a poor predictor of the risk for CHD,1 in contrast to epidemiological studies of patients with polygenic hyperlipidemia. In addition, data from a cohort of 263 patients with identical LDL receptor mutations have shown that LDL was only a risk factor for CHD in women, and this study included relatively few women.16 Patients included in this study were identified by clinical diagnosis with the use of the Simon Broome Registry criteria. Previous studies in a similar population in North London had indicated that the prevalence of LDL receptor mutations in this heterogeneous population was likely to be 20% in tendon xanthoma–negative patients and 50% in tendon xanthoma–positive patients, with an overall average of 33%.17 Data on the study population are unavailable at this time. Although this population is heterogeneous, clinically it has the phenotype of familial hypercholesterolemia. Such populations have been used as the basis of epidemiological studies to identify additional risk factors for early CHD in FH, eg, the Simon Broome Register Project,1,2 and in other studies of genetic risk factors for CHD in FH.18 The study was small but similar in size to other studies of this disorder16,18 and derived its power from sampling the extremes of the distribution curve due to the 5- to 9-fold excess risk of CHD in patients with FH, particularly men, before age 50 years (even on treatment) compared with unaffected patients with FH.1,2,19

This study investigated the role of renin-angiotensin polymorphisms, which have been posited as risk factors in polygenic hyperlipidemia in a heterogeneous white popula-
tion with FH. Allele frequencies in this study were representative of white populations and did not show significant sampling errors when compared with data from other similar populations.\textsuperscript{13,18}

The role of renin-angiotensin system polymorphisms in CHD in patients with polygenic hyperlipidemia is controversial, with conflicting results from different studies. The ACE-D allele is associated with higher ACE levels,\textsuperscript{20} and meta-analyses tend to support a weak role for these polymorphisms in CHD, with a relative risk of 1.1 to 1.3.\textsuperscript{21–23} In this study no association was found between any renin-angiotensin system polymorphism and risk of CHD in the patients with polygenic hyperlipidemia.

Few studies have explored the role of the renin-angiotensin system as genetic risk factor in FH. One previous study in a heterogeneous US cohort that included patients with both FH and familial defective apolipoprotein B\textsubscript{3500} identified male sex (OR = 3.38), smoking (OR = 3.71), and ACE DD genotype (OR = 2.21) as risk factors for CHD.\textsuperscript{18} That study had an ACE-D allele frequency of 0.55, similar to this study, but the significant association with CHD was only found in men, in whom the D allele frequency was increased to 0.70. The study had a power of 80\% to detect a difference of 15\% in allele frequencies. In contrast, this study, of similar power, while confirming the relative strength of male sex (OR = 3.03) and smoking (OR = 2.91) as risk factors, also identified diastolic blood pressure (OR = 3.70) and glucose (OR = 3.31) as significant risk factors. However, it was unable to demonstrate an association of ACE genotype with risk of CHD overall or in men or women after correction for other risk factors. When the results of both studies on ACE genotypes in FH are combined, no association of CHD with ACE genotype is seen ($\chi^2$ = 1.18; $P$ = 0.27; OR = 1.35; 95\% CI, 0.83 to 2.19 for ACE-D). This suggests that larger studies may be needed to clarify the role of ACE genotypes as risk factors for CHD in patients with FH.

The interaction of renin-angiotensin system polymorphisms may be complex since the ACE-D allele may interact with the AG2R 1166C allele to increase risks of CHD.\textsuperscript{6} AG2R polymorphisms were not assessed in the previous study of CHD in FH patients, yet this study did find an association of CHD with the AG2R genotype, with a relative risk of 2.26 (95\% CI, 1.26 to 3.72) increased to 3.10 (95\% CI, 1.20 to 7.52) after adjustment for other environmental risk factors, including age, sex, blood pressure, and smoking. Analysis by sex, although limited by small numbers, particularly in women, showed that the principal effect of the AG2R polymorphism was seen in men (OR = 3.56; 95\% CI, 1.95 to 14.04; $P$ = 0.005) compared with women (OR = 1.20; 95\% CI, 0.20 to 7.19; $P$ = 0.64). This suggests that the effects of the polymorphism are greater in higher-risk individuals. The study was too small for formal genetic tests of interaction, but the use of a multiplicative model for ACE and AG2R polymorphisms, with double homozygotes being assigned a score of 1 and 4, respectively, showed no association with CHD above that for the AG2R polymorphism alone, although it was limited by the small number of AG2R C\textsubscript{1166} homozygotes.

Hypercholesterolemia has been associated with endothelial dysfunction,\textsuperscript{24} increased oxidative stress,\textsuperscript{24} increased expression of AG2R,\textsuperscript{25} and a left shift in the angiotensin II dose-response curve,\textsuperscript{26} some of which may be corrected by AG2R antagonists in animal models.\textsuperscript{27} The functional consequence of severe hypercholesterolemia with increased angiotensin II production (via ACE) and increased receptor expression or sensitivity could be increased rates of CHD. The AG2R A1166C polymorphism, which was a significant risk factor for CHD in this study, has been associated with increased coronary arterial vasoconstriction,\textsuperscript{28} aortic stiffness,\textsuperscript{29} and arterial angiotensin II responsiveness,\textsuperscript{30} all of which could predispose to increased risk of CHD. Calculation of population attributable risk using an assumption of a 3.1-fold excess risk for the 1166C allele would suggest that the AG2R genotype may account for up to 16\% of attributable risk for CHD in patients with FH. However, this should be treated with caution because the ACE-D polymorphism in the study by O’Malley et al\textsuperscript{18} would be expected to account for 41\%. Reports of the association of AG2R polymorphisms with CHD are conflicting,\textsuperscript{21} but if the full AG2R phenotype predisposing to CHD is dependent on LDL concentration for expression, then effects might be less in normolipidemic as opposed to severely hypercholesterolemic populations. Similarly, lesser effects would be expected in nonsmokers and women. The contrasting findings of this study in polygenic hypercholesterolemia as opposed to FH would support such a hypothesis. None of the meta-analyses of AG2R polymorphisms to date have investigated the association of the CHD phenotype with LDL concentration as a covariable.\textsuperscript{21,22} However, the possibility of type I error due to sampling differences, as may have occurred with ACE-I, cannot be completely excluded in studies of this size, especially with polymorphisms with highly asymmetric allele distributions. Given the conflicting results with respect to ACE genotype and risk of CHD in FH to date, larger studies will be required in the FH population to confirm whether the C1166 allele is a risk factor for CHD in this group.

In summary, this study showed no association of ACE-D or AGT M235T with CHD in patients with FH or in patients with polygenic hyperlipidemia. However, an association was demonstrated for the AG2R A1166C polymorphism in FH patients. Thus, AG2R polymorphisms may interact with hypercholesterolemia and other cardiovascular risk factors to increase the risk of CHD in FH.

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