Deoxyribonucleic acid polymorphism of the apoprotein AI-CIII-AIV gene cluster and coronary heart disease in non-insulin-dependent diabetes

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Abstract

The prevalence of an uncommon allelic variant (S2) of the apoprotein AI-CIII-AIV gene cluster was determined in non-insulin-dependent diabetics with or without evidence of coronary heart disease and in controls. Frequencies of the S2 allele were 14% for diabetics with coronary heart disease compared with 2% for non-diabetics with no clinical evidence of occlusive vascular disease. No subject with the S2 allele was detected among a further group of matched diabetics without clinical features of macrovascular disease.

The results suggest that a genetic component contributes to the susceptibility to coronary heart disease in non-insulin-dependent diabetics. Whether the observed deoxyribonucleic acid variant is aetiological for atherosclerosis or in linkage disequilibrium with other atherogenic loci on chromosome 11 remains to be clarified.

Introduction

Recombinant deoxyribonucleic acid (DNA) probes have been used to identify DNA variants of the apoprotein AI-CIII-AIV gene

Non-insulin-dependent diabetics have roughly twice the incidence of coronary heart disease compared with age matched non-diabetic subjects.⁵ Though the prevalence of various environmental risk factors for atherogenesis in diabetes has been intensively studied,⁶ the role of inherited factors has been largely ignored. We therefore decided to study the distribution of the apoprotein CIII genotype in a group of carefully defined non-insulin-dependent diabetics with evidence of coronary heart disease to see whether similar genetic determinants occur in this important subgroup of patients with arterial disease. Recognising major genes concerned in the pathogenesis of atherosclerotic arterial disease would considerably expand our understanding of this process and, by identifying susceptible people at an early age, may allow the introduction of preventive measures.⁷

Patients and methods

Table I gives the clinical details of the 102 white subjects studied. All were recruited from either the Whittington or St Bartholomew's Hospital, and all diabetics were classified as non-insulin-dependent (type II) based on World Health Organisation criteria.⁸ Of the 47 patients who presented with evidence of coronary heart disease before the age of 60, 35 were survivors of

TABLE I—Clinical details of patient groups

Study group	No in group	No of men	Mean age (years) (SD)	No (%) with ideal body mass (>120%)	No (%) with hypertension	Mean duration of diabetes (years) (SD)	Mean serum cholesterol (mmol/l) (SD)	Mean serum triglyceride (mmol/l) (SD)
Non-insulin-dependent diabetics with coronary heart disease	47	26	59.4 (8.42)	20 (43)	10 (21)	9.4 (6.20)	7.1 (1.02)*	2.4 (1.01)†
Non-insulin-dependent diabetics with no clinical evidence of heart disease	20	8	61.0 (3.80)	8 (40)	3 (15)	11.2 (5.30)	6.9 (1.21)	1.6 (0.37)
Non-diabetic controls	35	18	54.2 (1.27)	7 (20)	5 (14)		6.2 (0.61)	1.4 (0.41)

*Compared with non-diabetic controls $p < 0.01$ (unpaired t test).

†Compared with both other groups $p < 0.01$ (unpaired t test).

loci that associate with coronary atherosclerosis and may represent potential genetic markers for this common disorder. In a group of young survivors of myocardial infarction 21% possessed an uncommon genotype of the apoprotein CIII gene compared with only 4% of normotriglyceridaemic controls.¹ Other studies of normolipaeamic patients with angiographically defined obstructive coronary artery disease found a variant genotype frequency of between 18% and 24%.^{2,3} One variant allele (S2) is identified by an additional cleavage site for the restriction enzyme Sst I and hybridisation with either an apoprotein AI or CIII gene probe.⁴

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myocardial infarction, based on clinical history, typical electrocardiograms, and cardiac enzyme changes. The remaining 12 patients had been investigated by coronary arteriography because of persistent chest pain and were found to have severe occlusive coronary artery stenosis requiring bypass surgery. A second group of 20 non-insulin-dependent diabetics with no clinical evidence of vascular disease or hypertriglyceridaemia at the time of study were also genotyped. All gave a negative response to an angina questionnaire,⁹ none had a history of coronary heart disease, and all had palpable peripheral pulses and normal resting electrocardiograms. A group of 35 age matched non-diabetic patients attending the medical outpatient department (rheumatology and chest clinics) served as further controls.

DNA analysis—DNA was isolated from leucocytes in 10 ml venous blood as described by Kunkel *et al.*¹⁰ Analysis of apoprotein CIII genotypes was performed as described.¹

Statistical analysis—The association of gene related polymorphisms with diabetic coronary heart disease was analysed by a 2×2 contingency table comparing the polymorphism genotype with the disease group. A χ^2 test of significance was then applied.

Results

After digestion with the restriction endonuclease Sst I three gene related fragments were visualised by hybridisation with an apoprotein AI probe

(figure). The 5.7 kilobase pair (kbp) fragment was invariant and the polymorphism was characterised by the presence of either a 4.2 kbp fragment (S1 allele) or a 3.2 kbp fragment (S2 allele). Subjects were genotyped S1S1 if they possessed only the 5.7 and 4.2 kbp fragments and S1S2 if they possessed all three.

Autoradiograph of genomic DNA digested with restriction enzyme Sst I and hybridised with phosphorus labelled AI gene probe.

There was a significant increase in the prevalence of the S2 allele among the non-insulin-dependent diabetic group with coronary heart disease compared with the non-diabetic controls (table II). The frequency of the S2 allele was significantly higher among the normolipaemic non-insulin-dependent diabetics with evidence of coronary heart disease compared with both normolipaemic non-diabetics ($p < 0.01$) and normolipaemic diabetics without evidence of vascular complications ($p < 0.001$). Frequencies of the apolipoprotein CIII allele were similar for both the diabetic survivors of myocardial infarction and those patients with angiographically defined coronary artery disease (data not shown). Of the diabetics with coronary heart disease, 20 (43%) had fasting hypertriglyceridaemia at the time of study and six of these possessed the S2 allele.

TABLE II—Apolipoprotein CIII genotypes and frequencies of alleles

Study group	No in group	No (%) with genotype		Allele frequency	
		S1S1	S1S2	S1	S2
(A) Diabetics with coronary heart disease	47	34 (72)	13 (28)	0.86	0.14
(B) Hypertriglyceridaemic diabetics	20	14 (70)	6 (30)	0.82	0.18
(C) Normotriglyceridaemic diabetics with coronary heart disease	27	20 (74)	7 (26)	0.87	0.13
(D) Normotriglyceridaemic diabetics without vascular complications	20	20 (100)	0	1.00	0
(E) Non-diabetic controls	35	34 (97)	1 (3)	0.98	0.02

Allele frequencies: (A) v (E) $p < 0.01$; (A) v (D) $p < 0.001$; (B) v (E) $p < 0.001$; (C) v (D) $p < 0.001$; (C) v (E) $p < 0.01$ (χ^2 test).

Discussion

We have studied a variant of the apolipoprotein AI-CIII-AIV gene cluster which associates with coronary heart disease in non-insulin-dependent diabetics and provides evidence for a genetic component in the aetiology of this important long term diabetic complication. At present it is not clear whether polymorphisms at this gene cluster are aetiological for atherosclerosis. DNA variation at this locus might predispose to coronary atherosclerosis by altering plasma lipid concentrations. Possession of the S2 allele, however, does not appear to influence concentrations of circulating serum apolipoprotein AI or apolipoprotein CIII.⁴ Alternatively the observed association with coronary heart disease in non-insulin-dependent diabetics may

arise from linkage disequilibrium with other atherogenic loci on chromosome 11.

Three further points emerge from our study. The results from the non-insulin-dependent diabetics with coronary heart disease who were normolipaemic at the time of study suggest that the observed increase in frequency of the S2 allele was independent of any association with diabetic hypertriglyceridaemia. No significant differences in the frequencies of the S2 allele were observed between normotriglyceridaemic and hypertriglyceridaemic diabetics with clinical evidence of vascular disease. Nevertheless, of all subjects with diabetes and coronary heart disease, 20 (43%) had fasting hypertriglyceridaemia at the time of study and six of these patients were heterozygous for the S2 allele. This supports previous data on the increased frequency of the S2 allele in diabetic hypertriglyceridaemia.¹¹ Finally, no significant difference in frequencies of alleles was found between normotriglyceridaemic diabetics without clinical evidence of vascular disease and the non-diabetic controls. Hence there appears to be no disease association between diabetes alone and this DNA variant of the apolipoprotein CIII gene.

Though our observations provide evidence for at least one common genetic determinant for coronary heart disease in both diabetics and non-diabetics, we cannot exclude the possibility of differences in the contribution of other loci to the predisposition to coronary heart disease in diabetes.

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100 YEARS AGO

Attention has been called by a contemporary to a system of fraud which has been practised in connection with the working of the new French law which enjoins that all packages containing butter substitutes shall be specially marked with words denoting the admixture, the term usually employed being *graisse alimentaire*. This law has led to the discovery that a large amount of the "guaranteed" French butter is either butterine or an admixture. Manufacturers in France who are now compelled by French law to brand these goods do so, but large quantities are marked in addition "guaranteed pure butter," and when the goods arrive in this country the words *graisse alimentaire* are removed. One of the southern railway companies has, it is said, actually fixed a tariff for the commission of this fraud, and other companies allow it to be perpetrated by the owner while the goods are in transit. Such frauds should be summarily suppressed, and, if necessary, the law should be strengthened in order that the perpetrators may be severely punished. (*British Medical Journal* 1887;ii:369.)