SCIENTIFIC LETTER

The connexin 37 gene polymorphism and coronary artery disease in Ireland

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Coronary artery disease (CAD) and myocardial infarction (MI) are polygenic disorders caused by a complex interaction between environmental and genetic factors. A family history of MI is also an independent risk factor for MI.[1] Several methods have been proposed to identify the underlying genetic cause of CAD, including genetic linkage studies and candidate gene approaches.

CAD and MI are the clinical manifestations of underlying coronary atherosclerosis. The cornerstones of this process are elevated plasma lipid concentrations and inflammation. Central to this process is the relation between endothelial cells (EC) and smooth muscle cells (SMC). Connexins are members of a multigene gap junction family that may mediate EC-SMC communication. These transmembrane channels connect neighbouring cells allowing movement of small molecules and second messengers.[2]

The role of connexin 37 (CX37) and the C1019T polymorphism have been previously investigated in CAD and MI. The functional impact of the C1019T single nucleotide polymorphism has not been determined but it does result in a change in the amino acid sequence (Pro319Ser). A genome-wide search for susceptibility genes for MI identified a novel susceptibility locus on chromosomal region 1p34–36.[3] This region contains the gene coding for CX37. The T allele of the C1019T polymorphism has been found to be significantly associated with the risk of MI in men and with CAD in high risk males,[4] defined as those with hypertension, diabetes mellitus, and hypercholesterolaemia. However, the C allele has been reported to be associated with CAD in Taiwanese men and carotid plaque thickening in Swedish men.[5] These findings need to be replicated in other populations before their clinical significance can be ascertained.

METHODS

We have investigated this polymorphism using family based association methods in an Irish population with premature onset CAD. Recruitment of the study population took place between August 1999 and March 2002. The inclusion criteria are described in detail in a previous publication.[6] Briefly, individuals were white with all four grandparents born in Ireland. Each family was required to have at least one family member affected with proven premature CAD (disease onset $\leqslant$ 55 years for males and $\leqslant$ 60 years for females) and at least one unaffected sibling and/or both parents surviving. Proven CAD was defined by one or more of the following: previous MI; previous unstable angina, typical chest pain with dynamic ECG changes or minor elevation of cardiac markers; or stable angina with angiographic evidence of obstructive coronary disease, $>$ 70% stenosis. Unaffected siblings were required to be $\geqslant$3 years older than the affected sibling at age of diagnosis of CAD and have no evidence of previous CAD. Unaffected siblings were screened for angina or MI using the Rose chest pain on effort and possible infarction questionnaire and a standard 12 lead ECG independently coded using the Minnesota code. All subjects underwent physical examination and provided demographic information regarding their medical history and ischaemic heart disease risk factors.[6]

Written informed consent was obtained from each patient. The study was approved by the research ethics committee of Queen's University Belfast and the investigation conforms to the principles outlined in the Declaration of Helsinki.

As CAD exhibits late onset our study consisted mostly of discordant sibling relationships and smaller numbers of parents and affected child trios. Two family based tests of association, the combined transmission disequilibrium test (TDT)/sib-TDT and the pedigree disequilibrium test (PDT), were used to analyse the data. These tests avoid the problem of population stratification that is found in case–control studies. Genotyping was determined by polymerase chain reaction (PCR) restriction fragment length polymorphism analysis. Oligonucleotide primers, PCR conditions, and restriction digest protocols were adapted from published methods.[5] As a quality control measure 10% of the samples were selected at random for repeat genotyping, no mismatches were identified. Two observers, who were blinded to the subject's disease status, read each gel.

RESULTS

Our study sample comprised 1012 individuals from 386 families (416 affected, 490 unaffected siblings, and 106 parents). We also analysed a subset for the stricter phenotype of premature MI (687 individuals from 258 families). Characteristics of the affected and unaffected siblings are shown in table 1. The prevalence of hypertension was lower in the affected group and is likely to be related to the use of vasoactive medications in this group. The percentage of male patients is higher in the affected than the unaffected groups; this is a reflection of the intrinsic structure of the recruited families.

The T allele frequency in the probands was 0.31 for CAD and 0.30 for MI. For the dataset of CAD, 180 families were informative (39 trios and 141 discordant sibling pairs); for the subset of MI there were 123 informative families (29 trios and 94 discordant sibling pairs). No association was found between the C1019T polymorphism and CAD by either the combined TDT/sib-TDT ($p = 0.90$) or PDT ($p = 0.81$). Similarly, no association was found in the subgroup of patients with MI by either the combined TDT/sib-TDT ($p = 0.94$) or PDT ($p = 0.83$). A retrospective power calculation was performed using the methods described by Spielman and Ewens.[7] In the CAD dataset, 180 families of minimal configuration will have almost 80% power to detect a deviation from 50% to 60% in the rate of allele transmission to affected individuals. Likewise, in the MI dataset, 123

Abbreviations: CX37, connexin 37; CAD, coronary artery disease; EC, endothelial cell; MI, myocardial infarction; PDT, pedigree disequilibrium test; PCR, polymerase chain reaction; SMC, smooth muscle cell; TDT, transmission disequilibrium test

Table 1 Characteristics of siblings

	Affected siblings n = 416	Unaffected siblings n = 490	p Value
Mean (SD) age when CAD diagnosed (years)	45.6 (6.2) (males) 48.8 (6.6) (females)	N/A	N/A
Mean (SD) age at study entry (years)	51.0 (7.5) (males) 52.4 (7.7) (females)	55.1 (8.8) (males) 56.1 (7.7) (females)	<0.001 <0.001
% Male	79.6	47.1	<0.001
% Non-smokers	18.0	42.4	<0.001
% Hypertension	29.6	46.7	<0.001
% Diabetes mellitus	10.6	4.5	<0.001
% Hypercholesterolaemia	93.0	82.9	<0.001

Non-smokers defined as lifelong non-smokers; hypertension defined as personal history of hypertension or systolic blood pressure > 140 mm Hg or diastolic blood pressure > 95 mm Hg; hypercholesterolaemia defined as current treatment with a lipid lowering agent or total serum cholesterol > 5.0 mmol/l; diabetes mellitus defined as a personal history of diabetes or random blood glucose > 11.1 mmol/l.

families of minimal configuration will have approximately 80% power to detect a deviation from 50% to 62.5% in the rate of allele transmission to affected individuals.

DISCUSSION

These results illustrate the ongoing problem of research in the area of complex traits. Many studies have reported positive associations with specific genetic polymorphisms. However, replication of these findings in independent populations remains problematic. A major contributor to this problem is the variation of definition of the clinical phenotype. Regarding CAD, some researchers have included only patients with MI, while others have included those with MI, unstable angina, or chronic stable angina. The diagnosis of MI has also changed in recent years with the advent of more sensitive markers of myocardial necrosis such as the troponins. The age at which premature CAD is defined is arbitrary, as is the decision to use a different age for males than females.

In summary, using a large family based association approach in a well phenotyped Irish population, we have not been able to demonstrate an association of the C1019T polymorphism in the CX37 gene and the presence of either CAD or MI. Unravelling the genetics of CAD poses a major research challenge and will require the concerted effort of cardiologists and molecular biologists, using the complementary approaches of genome-wide linkage analysis and association studies.

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