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The matrix metalloproteinase-3 (MMP-3) 5A/6A promoter polymorphism is not associated with ischaemic heart disease: Analysis employing a family based approach

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Abstract. Matrix metalloproteinase-3 (MMP-3) has been proposed as an important mediator of the atherosclerotic process. The possible role of the functional -1612 5A/6A polymorphism of the MMP-3 gene in the susceptibility to ischaemic heart disease (IHD) was investigated in a well-defined Irish population using two recently described family based tests of association. One thousand and twelve individuals from 386 families with at least one member prematurely affected with IHD were genotyped. Using the combined transmission disequilibrium test (TDT)/sib-TDT and the pedigree disequilibrium test (PDT), no association between the MMP-3 -1612 5A/6A polymorphism and IHD was found. Our data demonstrate that, in an Irish population, the MMP-3 -1612 5A/6A polymorphism is not associated with IHD.

Keywords: Gene polymorphism, ischaemic heart disease, matrix metalloproteinase-3, stromelysin-1

1. Introduction

Ischaemic heart disease (IHD) is a complex trait, with both genetic and environmental factors contributing to the phenotype. Marenberg and colleagues have reported an increased risk of death from IHD, particularly at younger ages, both in monozygotic twins and, to a lesser degree, in dizygotic twins, when the co-twin had died from IHD [1]. A family history of IHD has also been noted to be a strong independent risk factor for IHD [2,3]. These findings suggest that there is a significant genetic basis to IHD. However, research in this area using case-control association studies of candidate genes has produced conflicting results [4].

The matrix metalloproteinases (MMPs) are a family of zinc- and calcium- dependent enzymes with common functional domains that degrade a wide range of extracellular proteins. Vascular remodelling, defined as any enduring change in the size and/or composition of an adult blood vessel, not only allows adaptation and healing of blood vessels, but also forms the basis of pathological mechanisms in the blood vessel, such as atherosclerosis [5]. Remodelling requires degradation and reorganisation of the extracellular matrix scaffold of the vessel wall; this has led to the suggested involve-

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ment of MMPs in progression of atherosclerosis and atherosclerotic plaque disruption.

MMP-3 (also known as stromelysin-1) is present in atherosclerotic lesions [6]. Local MMP-3 overexpression in the vulnerable shoulder regions of atheroma may contribute to plaque cap weakening and subsequent rupture, a critical factor in the pathogenesis of acute myocardial infarction (MI) [7]. Alternatively, decreased MMP production and/or activity could lead to decreased matrix degradation, favouring more rapid development and progression of an atherosclerotic plaque [8]. A bialleic single nucleotide polymorphism in the MMP-3 gene, 1612 base pairs upstream from the start of transcription, has been identified, with one allele having a cluster of five adenosines (5A) and the other six adenosines (6A), the 5A/6A polymorphism [9]. *In vitro* assays of promoter activity have revealed that the 5A allele has 2-fold higher promoter activity than the 6A allele [10]. Therefore, this polymorphism is a putative genetic risk factor for IHD.

In the present study we investigated the presence of linkage disequilibrium between the MMP-3 -1612 5A/6A polymorphism and IHD using two recently described family-based association methods in a well-defined Irish population. We used the combined transmission disequilibrium test (TDT)/sib-TDT [11] and the pedigree disequilibrium test (PDT) [12]. Both methods have been designed specifically for the study of complex diseases such as IHD, as they overcome the problems of population admixture inherent in case-control methods.

2. Materials and methods

2.1. Study population

The entry criteria used in this study have been described elsewhere [13]. Between August 1999 and March 2002 we recruited 1023 individuals from 388 families. All subjects were Caucasian whose four grandparents were born in Ireland. Each family had at least one member affected with premature IHD (disease onset $\leqslant$ 55 years for males and $\leqslant$ 60 years for females) and at least one unaffected sibling and/or both parents surviving.

The affected individuals were recruited from the cardiology units of the Royal Victoria Hospital and Belfast City Hospital, Northern Ireland. IHD was defined as the presence of one or more of the following features: (1) a history of acute MI (as defined by WHO criteria) [14]; (2) a history of unstable angina (typical chest pain with dynamic ECG changes or minor elevations in cardiac markers); (3) coronary artery disease angiographically ($\geqslant$ 70% luminal stenosis).

Unaffected siblings were required to: (1) be older than the affected sibling was at the onset of IHD; (2) have no symptoms of angina or possible MI by WHO questionnaire assessment [15]; (3) have no history of IHD diagnosed by a doctor; and (4) have a resting 12 lead ECG showing no evidence of ischaemia or previous MI (independently coded using the “Minnesota code” [16], with codes 1.1–1.2 indicating probable MI and codes 1.3, 4.1–4.4, 5.1–5.2, 7.1 indicating possible ischaemia). Phenotyping of parents was not required.

All subjects underwent physical examination and provided demographic information and medical history (including IHD risk factors) using standardised questionnaires.

The study was approved by the Research Ethics Committee of Queen’s University Belfast and informed consent was obtained from all subjects.

2.2. DNA procedures

DNA was extracted from peripheral whole blood using a salting out method [17]. The MMP-3 -1612 5A/6A genotypes were determined by PCR amplification of the region containing the mutation followed by *XmnI* digestion and agarose gel electrophoresis as previously described by Dunleavey et al. [18]. Genotyping was repeated in 10% of the samples, randomly selected as a quality control measure. Each gel was read by 2 observers unaware of the subject’s disease status.

2.3. Statistical analysis

Two family-based association tests, the combined TDT/sib-TDT and the PDT, were used to assess the presence of linkage disequilibrium between the MMP-3 -1612 5A/6A polymorphism and IHD. Both tests determine the presence of linkage disequilibrium by testing for unequal transmission of an allele from parents to affected offspring or unequal transmission of an allele to affected offspring within disease-discordant sibships.

The combined TDT/sib-TDT [11] combines the TDT with the sib-TDT. Trios (both parents and affected offspring) are informative for the TDT test if there is an affected child and at least one parent heterozygous at the marker. Disease discordant sib pairs are informative for the sib-TDT if there is at least one affected and one unaffected sibling with different marker geno-

Table 1
Family structures

Structure	Number of families	Number of individuals
1 affected sib & 1 unaffected sib	206	412
1 affected sib & 2 unaffected sibs	81	243
1 affected sib & 3 unaffected sibs	17	68
1 affected sib & 4 unaffected sibs	2	10
1 affected sib & 5 unaffected sibs	1	6
1 affected child & both parents	41	123
1 affected child, 1 unaffected sib & both parents	6	24
1 affected child, 2 unaffected sibs & both parents	3	15
2 affected sibs & 1 unaffected sib	13	39
2 affected sibs & 2 unaffected sibs	7	28
2 affected sibs & 3 unaffected sibs	3	15
2 affected sibs & 4 unaffected sibs	2	12
2 affected children & both parents	2	8
2 affected children, 1 unaffected sib & both parents	1	5
3 affected sibs & 1 unaffected sib	1	4
Total	**386**	**1012**

Table 2
Characteristics of siblings (SD = standard deviation; N/A = not applicable) (Non-smokers defined as lifelong non-smokers; hypertension defined as personal history of hypertension or systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg; hypercholesterolaemia defined as current treatment with a lipid-lowering agent or total serum cholesterol > 5.0 mmol/l; diabetes mellitus defined as personal history of diabetes or random blood glucose > 11.1 mmol/l.)

	Affected siblings $N = 416$	Unaffected siblings $N = 490$	P value
Age when IHD diagnosed, years (mean ± SD)	45.6 ± 6.2 (males)	N/A	N/A
	48.8 ± 6.6 (females)		
Age at study entry, years (mean ± SD)	51.0 ± 7.5 (males)	55.1 ± 8.8 (males)	<0.001
	52.4 ± 7.7 (females)	56.1 ± 7.7 (females)	<0.001
Male sex (%)	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

types. Only one trio or discordant sib pair can be included from each family to ensure that the analyses give valid tests of association. In families with multiple phenotypically discordant sib pairs, the sib pair with the maximally discordant genotype is selected [19].

The PDT [12] allows the use of data from related trios and discordant sib pairs from extended pedigrees. Informative extended pedigrees contain at least one informative trio and/or discordant sib pair as described for the combined TDT/sib-TDT.

Prospective power calculations for family-based association studies of complex diseases are problematic, as they require a model of inheritance to be specified and the number of informative families can be difficult to predict. We therefore assessed power retrospectively.

The independent samples t-test was used to compare means for quantitative variables and the chi-squared test was used for qualitative variables. All statistical tests were performed at the 5% significance level (two-tailed).

3. Results

Three hundred and eighty eight families were recruited. Two families and two siblings from a third family were subsequently excluded from the study, due to incompatible genotypes. Therefore, our study sample comprised 1012 individuals drawn from 386 families. The structure of these families is shown in Table 1. Characteristics of the affected and unaffected siblings are shown in Table 2.

The genotype frequencies of the probands were: 5A5A 0.29, 5A6A 0.52 and 6A6A 0.19, giving a 5A allele frequency of 0.55.

Combined TDT/sib-TDT: After genotyping and after selection of a single discordant sib pair or trio per family, 156 discordant sib pairs and 59 transmissions in 45 trios were informative for analysis. There was no statistically significant transmission of either allele to affected individuals ($p = 0.14$, Table 3).

PDT: After genotyping, 201 families were informative for PDT analysis. There was no statistically sig-

Table 3
Combined TDT/sib – TDT analysis. ($Z = 1.48$, $p = 0.14$)

	Transmission of 5A allele to affected individuals	
	Observed	Expected
TDT	31	29.5
Sib-TDT	171	161.5
Combined TDT/sib-TDT	202	191

nificant excess transmission of either allele to affected individuals ($p = 0.15$).

4. Discussion

We found no association between the -1612 5A/6A polymorphism of the MMP-3 gene and premature onset IHD. There is an abundance of evidence in the literature implicating MMP-3 in the pathogenesis of atherosclerosis. Enhanced MMP activity may contribute to weakening of the atherosclerotic cap and subsequent rupture [20], a process that may lead to coronary artery thrombosis, resulting in MI, unstable angina, or sudden death. MMPs, including MMP-3, have been demonstrated in coronary atherosclerotic plaques, particularly at the regions considered prone to rupture [6,7,20]. Plaque rupture may also occur subclinically, with the subsequent repair mechanisms and remodelling leading to the development and progression of atherosclerotic plaques without a documented acute IHD event [21]. Alternatively, decreased MMP production and/or activity could favour more rapid development and progression of an atherosclerotic plaque through reduced extracellular matrix remodelling [8].

There are several potential reasons why we did not find an association between the 5A/6A polymorphism and IHD. Firstly, the 5A/6A polymorphism may not be associated with IHD. Secondly, this may reflect a type II error, that is, there is an association between the 5A/6A polymorphism and IHD, but our methods failed to detect it. The tests used in this study do appear to be reasonably powered. For example, a retrospective calculation of power estimated that the 201 families of minimal configuration to be informative (either one heterozygous parent and one affected child for the TDT or one affected sibling and one unaffected sibling with different genotypes for the sib-TDT) afforded over 80% power to detect a deviation of allele transmission from 50 to 60 percent using the combined sib-TDT/TDT (two-tailed test) [11]. Both the 6A and 5A alleles have been implicated in atherosclerosis [9,22–24]. The 6A allele, associated with decreased MMP-3 activity, has been linked to progression of coronary and carotid atherosclerosis [9,22,23,25–28]. In contrast, the 5A allele, with increased MMP-3 activity, has been linked to MI [24,29]. Both are biologically plausible, as decreased MMP activity could lead to an increased connective tissue content and size of the atherosclerotic plaque, whereas increased MMP activity, particularly in the shoulder region of plaques could result in plaque rupture and MI. In a study of 1,240 individuals undergoing coronary angiography, Beyzade and colleagues reported that those carrying the 6A/6A genotype had a greater number of coronary arteries with a significant stenosis, whereas the 5A/5A and 5A/6A genotypes were associated with an increased risk of MI; this group also identified six novel polymorphisms in the MMP-3 gene but none of these polymorphisms was found to have significant effects on the extent of coronary atherosclerosis or MI risk [30]. The affected individuals in our study were clinically heterogeneous, with some defined on the basis of a history of an acute coronary syndrome, some with stable angina pectoris and angiographic evidence of coronary atherosclerosis, and others with both types of IHD presentation. In addition, some studies have reported an association of the 5A/6A polymorphism in subgroups such as women and smokers [29,31]. The family based tests of association used in this study do not cope with additional phenotypic information, thus prohibiting such subgroup analysis. Finally, we cannot exclude population heterogeneity and therefore our findings apply only to an Irish population as studied.

In summary, using two recently described family based association tests, we have demonstrated no association between the MMP-3 -1612 5A/6A polymorphism and premature onset IHD.

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