Chromosome 9p21.3 is associated with early-onset coronary heart disease in the Irish population

Weihua Meng^a, Anne E. Hughes^a, Chris C. Patterson^a, Christine Belton^a, Frank Kee^a and Pascal P. McKeown^{a,b,*}

^a*Centre for Clinical and Population Sciences, Queen's University Belfast, Institute of Clinical Science, Grosvenor Road, Belfast, Northern Ireland, UK*

^b*Regional Medical Cardiology Centre, Royal Victoria Hospital, Grosvenor Road, Belfast, Northern Ireland, UK*

Abstract. Coronary heart disease (CHD) remains a leading cause of death across the world. A region on chromosome 9p21.3 has been recently reported to be associated with CHD. We evaluated 3 SNPs and 3 common haplotypes in the 9p21.3 region in 1494 individuals from 580 Irish families, where at least 1 member had early-onset (males ≤ 55 yr, females ≤ 60 yr) CHD. Genotypes were determined by multiplex SNaPshot technology. Using the combined TDT/S-TDT test, the 3 single nucleotide polymorphisms (SNP), rs10757274, rs2383206 and rs1333049, were strongly associated with early-onset CHD ($p = 2.7 \times 10^{-6}$, 2.7×10^{-6} , 3.8×10^{-7} , respectively). Analysis of haplotypes by the TRANSMIT program also showed that the GGC haplotype was associated with early-onset CHD ($p = 7.9 \times 10^{-7}$). In conclusion, using a family-based approach in the Irish population, we have confirmed previous reports of association between a region on chromosome 9p21.3 and early-onset CHD.

Keywords: Coronary heart disease, chromosome 9p21.3, genetics

1. Introduction

Coronary heart disease (CHD) is a major global health problem. Whilst traditional risk factors play a major role in the development of the disease, there is clear evidence that genetic factors are also important [1, 2]. However, over the years, despite intensive efforts, there has been a failure to replicate genetic associations across populations [3]. Recently, using a genome-wide association study approach, several independent research groups have identified chromosomal regions which are associated with CHD; in particular, a small region spanning 58kb on chromosome 9p21 has consis-

tently been reported to be very significantly associated with CHD [4–7].

In our study, we have investigated this region, using an Irish family based resource, where the probands had early-onset CHD.

2. Materials and methods

2.1. Study subjects

The family samples used in this study have been described in detail elsewhere [8,9]. From the period of August 1999 to October 2004 we recruited 1494 individuals from 580 families. All subjects were Caucasian whose 4 grandparents were born in Ireland. Each family had at least 1 member affected with early-onset CHD (disease onset ≤ 55 years for males and ≤ 60 years for females) and at least 1 unaffected sibling and / or both

*Corresponding author: Dr. Pascal P. McKeown, Centre for Clinical and Population Sciences, Queen's University Belfast, Institute of Clinical Science, Grosvenor Road, Belfast, BT12 6BJ, Northern Ireland, UK. Tel.: +44 28 9063 4825; Fax: +44 28 9031 2907; E-mail: p.p.mckeown@qub.ac.uk.

Table 1
Family structures of 1494 subjects in 580 families

	Number of families	Number of individuals
Proband+ parents + 0 sibling	48	144
Proband+ parents + 1 sibling	9	36
Proband+ parents + 2 sibling	7	35
Proband + 1 sibling	337	674
Proband + 2 sibling	128	384
Proband + 3 sibling	39	156
Proband+4 sibling	8	40
Proband+5 sibling	3	18
Proband+6 sibling	1	7
Total	580	1494

parents surviving. CHD was defined as the presence of 1 or more of the following features: (1) a history of acute myocardial infarction (MI) (as defined by WHO criteria); (2) a history of unstable angina (typical chest pain with dynamic ECG changes or minor elevations in cardiac markers); (3) coronary artery disease angiographically ($\geq 70\%$ luminal stenosis).

Unaffected siblings were required to: (1) be older than the affected sibling was at the onset of CHD; (2) have no symptoms of angina or possible MI by WHO questionnaire assessments [10]; (3) have no history of CHD diagnosed by a doctor; and (4) have a resting 12 lead ECG record showing no evidence of ischaemia or previous MI [11]. The study was approved by the Research Ethics Committee of Queen's University Belfast and informed consent was obtained from all subjects.

2.2. Genotyping

Three single nucleotide polymorphisms (SNP) (rs10757274, rs2383206, rs1333049) were chosen for study, based on previous publications [4,6]. Genotypes were determined using multiplex SNaPshot technology, an ABI fluorescence-based assay allelic discrimination method (Applied Biosystems, USA). Each gel image was read by two independent observers unaware of the subject's disease status. GeneMarker V1.6 (Softgenetics, USA) was used to determine each allele.

2.3. Statistical analysis

The combined TDT/S-TDT test [12,13] was used to assess the presence of association between the 3 SNPs and early-onset CHD by testing for unequal transmission of an allele from parents to affected offspring or unequal sharing of an allele within disease-discordant sibships. The combined TDT/S-TDT combines the TDT with the sibling TDT (S-TDT). Trios are informa-

tive for the TDT at a given locus if there is an affected child and at least one parent is heterozygous. Sib pairs are informative for the S-TDT if there is at least one affected and one unaffected sibling with different genotypes. TRANSMIT was used to assess association using haplotype transmissions to affected offspring in the presence of uncertainty in haplotype assignment. The program makes use of parental genotypes (if available) as well as genotypes from siblings [14,15]. Only results for common haplotypes (estimated frequency $> 1\%$) are presented because of the unreliability of the chi-squared test for rare haplotypes.

3. Results

A total of 1494 individuals from 580 families were included (800 discordant sib-pairs and 64 parent-child trios). The family structures are summarized in Table 1. There are more male probands and more female siblings. With regard to traditional risk factors, the prevalence of smoking and diabetes mellitus is higher in the probands; blood pressure and cholesterol levels were lower in the probands, which may be related to therapeutic intervention (Table 2). Each SNP was strongly associated with early-onset CHD ($p = 2.7 \times 10^{-6}$, 2.7×10^{-6} , 3.8×10^{-7} , respectively) (Table 3). There were over 300 informative families for each of the 3 SNPs. The haplotype analysis is shown in Table 4. The GGC haplotype was associated with early-onset CHD ($p = 7.9 \times 10^{-7}$). The SNP genotype frequencies of the probands are summarized in Table 5.

4. Discussion

Until recently, elucidation of the genetic basis of CHD has been dogged by non-replication of results [3]. The availability of modern genotyping technologies has facilitated the use of genomewide association studies using large, well-characterized case-control studies. These studies have consistently reported a strong association between CHD and a region on chromosome 9p21.3 [4–7]. In the study by Helgadottir and colleagues, about 21% of individuals were homozygous for the risk allele of rs10757278, with a corresponding odds ratio for development of MI of over 2.0 for early-onset cases [5]. At present, the responsible gene in this region has not been identified. Sequencing of the 2 closest genes (tumour suppressor genes, CDKN2B and CDKN2A, which play a role in regulating cell prolifer-

Table 2
Risk factors in probands with premature onset CHD and their unaffected siblings

Risk factor	Probands (<i>n</i> = 580)	Unaffected Siblings (<i>n</i> = 758)
Age (yr); mean (SD) **	51.3 (8.9)	55.9 (7.9)
Male; <i>n</i> (%) **	467 (80.5%)	338 (44.6%)
Ever smoker; <i>n</i> (%) **	464 (80.0%)	432 (57.0%)
Current smoker; <i>n</i> (%) *	215 (37.1%)	221 (29.2%)
Hypertension treatment; <i>n</i> (%)	148 (25.5%)	170 (22.4%)
SBP $\geq$ 140 mmHg; <i>n</i> (%) **	63 (10.9%)	337 (44.5%)
DBP $\geq$ 95 mmHg; <i>n</i> (%) **	11 (1.9%)	94 (12.4%)
Total hypertension; <i>n</i> (%) **	179 (30.9%)	405 (53.4%)
Diabetes diagnosed; <i>n</i> (%) *	53 (9.1%)	39 (5.1%)
Total cholesterol (mmol/l); mean (SD) **	4.9 (1.1)	5.8 (1.0)

* $p < 0.01$, ** $p < 0.001$.

SD – standard deviation, SBP – systolic blood pressure, DBP – diastolic blood pressure.

Table 3
Association tests between 3 SNPs and premature heart disease performed using the Transmission Disequilibrium Test/Sibling Transmission Disequilibrium Test (TDT/S-TDT)

SNP name	Allele	Number of informative families	Observed <i>W</i> *	Expected	Variance	p-value
rs10757274	AG	311	254	300.2	94.6	2.7×10^{-6}
rs2383206	AG	314	252	298.4	96.0	2.7×10^{-6}
rs1333049	CG	324	399	347.7	100.2	3.8×10^{-7}

* $W = X + Y$ where *X* is the number of transmissions of the first-mentioned allele from heterozygote parents to affected siblings (TDT) and *Y* is the number of occurrences of the first-mentioned allele in affected sibs in remaining informative families (S-TDT).

The 3 SNPs are arranged in an order according to their positions in the chromosome 9. The risk alleles are G (rs10757274), G (rs2383206), and C (rs1333049).

eration and apoptosis) has not provided obvious candidate SNPs, which could explain the association with CHD [4,5,16]. Interestingly, a recent study showed that this region may be associated with type 2 diabetes [17]. It is anticipated that it could explain the coexistence of diabetes and CHD in some patients [18].

Compared to case-control studies, family-based studies have some unique advantages. Generally speaking, family members share a common genetic background. Moreover, they are more likely to have been exposed to similar environmental factors which could possibly be related to disease aetiology. The TDT/S-TDT test is also specially designed for complex diseases and, therefore, avoids the effects of confounding due to population stratification [12].

Larson and colleagues have recently reported the results of the Framingham Heart Study 100K project [19]. Using regression models with generalized estimating equations they did obtain a significant result for the chromosome 9 region but they failed to detect this when they used a family based association test (FBAT).

In our study, we have confirmed, using a family-based association approach, a significant association

between SNPs in this region and CHD. The relative large numbers of informative families indicate the reliability of this study. The reported allele frequency for these 3 SNPs is similar in the Caucasian, Chinese and Japanese populations and very different from that found in the sub-Saharan African population [4,20]. The three chosen SNPs are in tight linkage disequilibrium (LD). Haplotype analysis provides more genetic information than analysis on a single locus. The haplotype analysis using the TRANSMIT program in our study also revealed a significant association between the GGC haplotype and early-onset CHD. This haplotype and its complementary haplotype (AAG) have frequencies of 56.3% and 39.7% in the White population (Hapmap data). Investigation of this region in other racial and ethnic groups will be helpful [21].

In summary, using a family-based study in an Irish population, we confirmed a strongly positive association between a region on chromosome 9p21.3 and early-onset CHD. This is the first family-based association study to demonstrate a significant association of this region with early-onset CHD. There are still a lot of unanswered questions concerning this region, which

Table 4
Comparison of observed with expected haplotype transmissions to affected offspring for three common haplotypes (>1%) obtained using the TRANSMIT program, which shows overtransmission of the GGC haplotype

Haplotype	Observed	Expected	Variance	Chi-square (1df)*	p-value
GGC	685.8	634.7	107.0	24.4	7.9×10^{-7}
AAG	489.9	530.8	106.3	15.8	7.2×10^{-5}
GGG	22.2	30.3	11.6	5.71	0.017

*df – degree of freedom.

Combined chi-square test = 26.4 on 3df, $P = 7.9 \times 10^{-6}$.

Table 5
Frequencies of genotypes among 580 probands

rs10757274			rs2383206			rs1333049		
AA	AG	GG	AA	AG	GG	CC	CG	GG
110	262	208	103	260	217	204	257	119
(18.9%)	(45.2%)	(35.9%)	(17.8%)	(44.8%)	(37.4%)	(35.2%)	(44.3%)	(20.5%)

appears to be a gene-poor area of the genome. Further work focusing on this region using collaborative studies is required.

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