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

Weihua Meng, Anne E. Hughes, Chris C. Patterson, Christine Belton, Frank Kee and Pascal P. McKeown

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 \( \leq 55 \) yr, females \( \leq 60 \) 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 (\( p = 2.7 \times 10^{-6} \), \( 2.7 \times 10^{-6} \), \( 3.8 \times 10^{-7} \), 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 consistently 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 \( \leq 55 \) years for males and \( \leq 60 \) years for females) and at least 1 unaffected sibling and / or both
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 (>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 informative 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 ($p = 2.7 \times 10^{-6}$, $2.7 \times 10^{-6}$, $3.8 \times 10^{-7}$, 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 prolif-
Table 2

| Risk factor                              | Probands (n = 580) | Unaffected Siblings (n = 758) |
|------------------------------------------|--------------------|------------------------------|
| Age (yr); mean (SD) **                   | 51.3 (8.9)         | 55.9 (7.9)                   |
| Male; n (%) **                           | 467 (80.5%)        | 338 (44.6%)                  |
| Ever smoker; n (%) **                    | 464 (80.0%)        | 432 (57.0%)                  |
| Current smoker; n (%) *                  | 215 (37.1%)        | 221 (29.2%)                  |
| Hypertension treatment; n (%)            | 148 (25.5%)        | 170 (22.4%)                  |
| SBP ≥ 140 mmHg; n (%) **                 | 63 (10.9%)         | 337 (44.5%)                  |
| DBP ≥ 95 mmHg; n (%) **                  | 11 (1.9%)          | 94 (12.4%)                   |
| Total hypertension; n (%) **            | 179 (30.9%)        | 405 (53.4%)                  |
| Diabetes diagnosed; n (%) *             | 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

| SNP name   | Allele | Number of informative families | Observed W* | 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} |
| rs133049   | 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).

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].

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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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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 × 10⁻⁴ |
| 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 × 10⁻⁶.

Table 5 Frequencies of genotypes among 580 probands

| Genotype | rs10757274 | rs2383206 | rs1333049 |
|----------|------------|-----------|-----------|
| AA       | 110        | 262       | 103       |
| AG       | 208        | 103       | 260       |
| GG       | 217        | 204       | 257       |
| (18.9%)  | (45.2%)    | (35.9%)   |

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

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