Abstract—A positive family history is a recognized cardiovascular risk factor, and genome-wide scans may reveal susceptibility loci for coronary artery disease. The acute coronary syndrome, consisting of myocardial infarction and unstable angina, is the most important manifestation of coronary disease and is characterized by atherosclerotic plaque disruption and coronary thrombosis. From \( \approx 6000 \) hospital admissions to cardiology units, we identified affected sibling pairs (\( n = 61 \)) who had documented acute coronary syndrome before the age of 70 years. A 10-cM resolution genetic map and MAPMAKER/SIBS were used for genome-wide linkage analysis. One locus on chromosome 2q36-q37.3 showed linkage with a lod score of 2.63 (\( P < 0.0001 \)). Separate multipoint fine-mapping of this locus with independent markers replicated the linkage results (lod 2.64). Two other regions on chromosomes 3q26-q27 and 20q11-q13 showed lod scores in excess of 1.5 (\( P < 0.005 \)). This genome scan in acute coronary syndrome suggests 1 locus that encompasses the gene encoding the insulin receptor substrate-1 gene. Two other potential loci were identified. These data imply that a limited number of potent susceptibility genes exist for the acute coronary syndrome. Such genes are likely to be relevant to the combined processes of atherosclerosis, plaque instability, and coronary thrombosis. (Arterioscler Thromb Vasc Biol. 2002;22:874-878.)

Key Words: genome scan  ■  coronary disease  ■  mapping  ■  myocardial infarction  ■  insulin receptor substrate-1

A positive family history of coronary heart disease is a well-recognized cardiovascular risk factor.\(^1\) This is explained in part by the familial aggregation of individual quantitative cardiovascular risk factors, such as lipids, blood pressure, and body weight.\(^2\) Family patterns of cardiovascular risk are likely to result from the effects of shared genes and environment.\(^3\) The role of genes has been established in certain rare mendelian diseases resulting from major genetic abnormalities affecting lipid metabolism\(^4\) and blood pressure.\(^5\) However, genetic influence on common cardiovascular disease is presumed to result from the combined effects of a number of polygenes. The number and nature of relevant polygenes has not been defined but may be revealed through genome scans. Comprehensive gene maps constituting highly polymorphic and evenly spaced markers have been developed to interrogate all 22 autosomes and the X chromosome. Such maps have been applied to linkage studies of conventional cardiovascular risk factors, such as blood pressure,\(^6\) body weight,\(^7\) lipids,\(^8\) and diabetes.\(^9\) However, there is no certainty that genes influencing these individual risk factors will necessarily be those linked with coronary artery disease, per se.

The acute coronary syndrome refers to myocardial infarction and unstable angina.\(^10\) In both, the pathophysiology of coronary atherosclerosis and thrombosis involves interactions between the vessel wall and the circulation.\(^11,12\) In the vessel wall, this process encompasses abnormalities of endothelial function, extracellular matrix, smooth muscle function and growth, and, possibly, the integrity of the elastic laminae. In the circulation, lipid profiles, hemodynamic characteristics, platelets, clotting factors, and white cells have been implicated. Potentially thousands of genes are relevant to this apparently complex disease. However, many risk factors are intercorrelated, raising the possibility of a higher level of genetic control by a small number of “master” genes that control fundamental physiological systems.

Many investigators have chosen to study candidate genes, but the results have been inconsistent,\(^13\) and a candidate-by-candidate approach is relatively inefficient. The advantage of a genome-wide scan is that it takes a relatively unbiased, efficient, and comprehensive approach to genetic discovery. A recent genome-wide analysis of a heterogeneous group of coronary artery disease phenotypes has suggested 2 loci on chromosome 2 and the X chromosome.\(^14\) To date, no specific genome study of the acute coronary syndrome has been reported. The Acute Myocardial Infarction Genetic Origins (AMIGO) Study was established to perform a genome-wide scan.
linkage analysis and to identify etiologic chromosomal regions.

Methods

We screened admissions to the cardiology and coronary care units of 8 metropolitan Melbourne hospitals. Ethics committee approval was obtained from all institutions, and informed consent was obtained from all participants. Subjects were entered if they reported a “heart attack” before the age of 70 years and if they had siblings with the same medical history. Recruitment was limited to white subjects. Because phenotyping certainty can augment the power of linkage analyses,15 we sought careful documentation of chest pain, cardiac enzymes, ECGs, and the results of coronary angiography from medical records. Approximately 25% of the subjects (see below) were excluded because the evidence was insufficient or contradictory. We included 122 subjects (61 sibling pairs) who had evidence of acute coronary syndrome. All but 2 subjects had experienced an episode of typical ischemic pain lasting for at least 15 minutes that was unresponsive to sublingual nitrates and associated with other symptoms. In 110 individuals, the diagnosis of myocardial infarction was confirmed on the basis of 1 of the following: (1) evolutionary ST-T changes in at least 2 contiguous leads or the development of new Q waves on the ECG, (2) elevation of the serum creatine kinase level to more than twice the upper limit of normal for the laboratory, (3) angiographic evidence of occlusion in the ischemia-related coronary artery, or (4) evidence of infarction confirmed at the time of cardiac surgery. In another 10 subjects, a diagnosis of unstable angina was made. All subjects with unstable angina had angiographic evidence of hemodynamically significant coronary artery stenosis. Of the 2 patients without documented prolonged ischemic pain, one presented with worsening exertional angina and had triple-vessel disease with occlusion of the right coronary artery, and the other had presented after cardiac arrest and was found to have angiographic evidence of hemodynamically significant coronary artery stenosis.

Participants completed a validated questionnaire pertaining to coronary risk factors at the time of their first reported acute coronary syndrome. We did not measure risk factors at the time of recruitment, because these were considered unreliable measures of the premorbid condition. Participants provided a 20-mL blood sample for DNA analysis.

Analysis of Screening and Recruitment

To examine the process of screening and recruitment, we analyzed those patients admitted over an 18-month period (March 1993 to October 1994) at 2 AMIGO-participating hospitals. In this subgroup, screening data on all subjects were used to identify affected sibling pairs and also patients reporting myocardial infarction but without a sibling history of myocardial infarction. The latter group were sampled at random for comparison with siblings to check for evidence of potential selection bias.

Genome-Wide and Fine Mapping

We used the ABI PRISM Human Linkage Mapping Set, version 2 (Perkin-Elmer), which constitutes 400 microsatellite markers with an ~10-cM resolution. All microsatellites were amplified according to manufacturer’s instructions by using polymerase chain reaction robots; the resulting products were visualized on ABI PRISM 377 gene sequencers (Perkin-Elmer), and alleles were sized by using GeneScan Analysis, version 2.1, and Genotyper, version 2.0 (Perkin-Elmer). We repeated the linkage analysis of a suggestive region toward the q telomere of chromosome 2 (see Results) with a total of 7 microsatellite markers (D2S336, D2S2202, D2S338, D2S2253, D2S125, D2S395, and D2S140).

Linkage Analyses and Error Checking

The estimates of allele frequencies were derived from the sample. We also had available allele frequency estimates from a separate sample of 548 healthy adults from Melbourne who had taken part in the Victorian Family Heart Study (VFHS).1 The allele frequencies were similar in both groups, indicating that the AMIGO sample allele frequencies were representative of the population. Substitution of VFHS frequencies achieved similar linkage results (data not shown). MAPMAKER/SIBS, version 2.0,17 was used to perform a multipoint linkage analysis by using the information from all genetic markers jointly to infer the full probability distribution of identifying by descent (IBD) status across the 22 autosomes and the X chromosome. The analysis is nonparametric and model free, and it estimates the maximum likelihood (ML) values of the allele-sharing proportions along the genome and computes a maximum lod score z value at each location. The likelihood of the observed data arising under these ML values is compared with the likelihood under random mendelian segregation.16 ML estimates for the IBD-sharing probabilities were estimated under the assumption of dominance variance with the use of triangle inequalities (Holmans18). The guidelines proposed by Lander and Kruglyak19 were used to determine the genome-wide significance of linkage data. Genotyping and pedigree errors can contribute to false-positive or false-negative linkage results. For an indication of possible genotyping errors, we checked all data sets for double recombinants by using the program SIBLODERR,20 which is based on previously published algorithms.21 On the genome-wide scan mapping data for chromosome 2, we detected 57 genotyping errors, which equates to a low genotyping error rate of 0.8%. The data appear to be robust because a subsequent analysis of the data after the removal of these errors led only to a modest lod increase of 0.05. Similar results were obtained for the other chromosomes in the genome scan. Eight genotyping errors were detected in the fine-mapping data, but their correction resulted in no change to the lod score.

Statistical Analyses

Data are presented as mean±SD. The χ2 or Fisher exact tests were used to compare proportional differences in qualitative variables between groups. Statistical significance was accepted at a value of P<0.05.

Results

Analysis of Screening and Recruitment

A total subset of 1990 consecutive admissions were screened over the 18-month period. These patients had a mean age of 63 years, and 70% were male. Of the screened admissions, 1125 (57%) patients reported a history of myocardial infarction before the age of 70 years. A history of myocardial infarction in a sibling before the age of 70 years was reported by 452 (22%) of the subjects admitted to the hospital. Sixteen percent of all admissions reported a history of both personal and sibling myocardial infarction. This amounted to 309 potential pairs, 243 of whom were excluded because 1 of the siblings was dead, could not be contacted, or was unavailable to participate. Of the remaining 66 sibling pairs, 23 pairs were excluded because 1 or both siblings did not meet age or ethnicity criteria. A further 17 pairs were excluded because careful checks of medical records revealed insufficient evidence to confirm the diagnosis of acute coronary syndrome as defined in Methods. Therefore, as a result of 18 months of screening at these 2 of our 8 recruiting centers, 26 affected sibling pairs were enrolled. This equates to ~1 eligible sibling pair for every 100 admissions to cardiology and coronary care units.

From all sites, we recruited a total of 61 affected sibling pairs for the linkage analyses. We compared the characteristics of these 122 siblings with those of 91 control subjects who reported a history of myocardial infarction but had no
TABLE 2. Peak LOD Scores for Each Chromosome Examined in the Genome Scan

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Peak LOD Score</th>
<th>Chromosome</th>
<th>Peak LOD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.20</td>
<td>13</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>2.63</td>
<td>14</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>1.76</td>
<td>15</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>0.97</td>
<td>16</td>
<td>1.30</td>
</tr>
<tr>
<td>5</td>
<td>0.63</td>
<td>17</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>1.48</td>
<td>18</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.00</td>
<td>19</td>
<td>0.32</td>
</tr>
<tr>
<td>8</td>
<td>0.34</td>
<td>20</td>
<td>1.57</td>
</tr>
<tr>
<td>9</td>
<td>0.11</td>
<td>21</td>
<td>0.82</td>
</tr>
<tr>
<td>10</td>
<td>0.40</td>
<td>22</td>
<td>0.01</td>
</tr>
<tr>
<td>11</td>
<td>0.04</td>
<td>X</td>
<td>0.66</td>
</tr>
<tr>
<td>12</td>
<td>0.13</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

TABLE 1. Summary of the Coronary Risk Questionnaire Data

<table>
<thead>
<tr>
<th></th>
<th>Affected Siblings</th>
<th>Subjects Without Affected Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>122</td>
<td>91</td>
</tr>
<tr>
<td>Male:Female</td>
<td>95:27</td>
<td>77:14</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.9 (SD, 9.5)</td>
<td>59.8 (SD, 10.9)</td>
</tr>
<tr>
<td>Parental history of AMI</td>
<td>76 (62%)</td>
<td>40 (44%)*</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>52 (43%)</td>
<td>49 (54%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>62 (51%)</td>
<td>48 (53%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>74 (61%)</td>
<td>65 (72%)</td>
</tr>
<tr>
<td>Stroke</td>
<td>15 (12%)</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>14 (12%)</td>
<td>11 (12%)</td>
</tr>
</tbody>
</table>

*p < 0.008.

sibling history of myocardial infarction. Table 1 shows that apart from the greater frequency of reported parental myocardial infarction in the siblings, there was no significant difference in the cardiovascular risk profile between the 2 groups. In none of our subjects was a diagnosis of familial hypercholesterolemia present.

Genome Scan and Fine Mapping

A complete genome scan was achieved in 53 sibling pairs, with 8 pairs not complete because of problems with DNA amplification. The multipoint linkage analysis revealed peak lod scores for each chromosome, as shown in Table 2. Three regions exceeded a lod score of 1.5 (P < 0.005). These were found on chromosomes 2 (at 2q36-q37.3), 3 (at 3q26-q27), and 20 (at 20p11-p13). Only the peak on chromosome 2 exceeded the lod threshold19 of 2.2, with a peak of 2.63 (P < 0.0002, Figure). To confirm that this peak toward the q telomere was not a spurious result dependent on a few markers, we performed a further linkage analysis of this region in all 61 sibling pairs with 7 markers, 2 of which were telomeric to the original D2S125 marker. As shown in the Figure, a lod peak of 2.64 was obtained.

Discussion

We report a locus for acute coronary syndrome on chromosome 2q36-q37.3. This locus achieved a lod peak of 2.64 and spans several outstanding candidate genes, including those genes encoding the insulin receptor substrate-1 (IRSI) and the HDL cholesterol–binding protein (HDLBP). This region has also been identified as the locus NIDDM1, which is linked with type 2 diabetes mellitus,9 in which the gene CAPN10 has been implicated recently.22 However, only 1 pair of siblings was concordant for diabetes in the present study; accordingly, diabetes, per se, does not explain the results of our analysis. Two other genetic regions on chromosomes 3q26-q27 and 20q11-q13 achieved lod peaks of > 1.5. The region of chromosome 3 has been linked to genome-wide scans to lipoprotein variation23 and contains genes encoding apoD (a significant glycoprotein component of HDL) and the glucose transporter 2 (part of the glucose sensor in pancreatic B cells). The region on chromosome 20 has been linked with maturity-onset diabetes of the young.24

To report lod peak genome-wide significance, we have used the guidelines of Lander and Kruglyak,19 recognizing that they have been criticized as being too stringent regarding single genome scans.14 The locus on chromosome 2 exceeds the threshold lod of 2.2, but peaks on chromosomes 3 and 20 do not. However, we should urge caution in disregarding the 2 lesser peaks (that exhibit a conventional P value of = 0.005) at this stage. Their importance will be determined only by testing in other independent studies.

Although the present study contained only 61 sibling pairs, this does not predispose to false-positive (type I) errors. We cannot attribute our positive findings to the size of the study, and we can conclude that the effects of 1 locus on chromosome 2 and possibly 2 others on chromosomes 3 and 20 are sufficiently robust to be detected with as few as 61 sibling pairs in our population. However, the sample size is directly relevant to the likelihood of false-negative (type II) errors. This is relevant to estimating how many loci might confer an important predisposition to the acute coronary syndrome. Nevertheless, we do not believe that the present study is seriously underpowered. It has been estimated that between 32 and 119 sibling pairs are required to achieve satisfactory statistical power for conditions that show sibling risk ratios between 5.0 and 2.0, respectively.25 The independent relative risk for coronary heart disease in first-degree relatives has been reported to be between 2.0 and 3.9,26 and to be as high as 15.0 for female monozygotic twins with coronary disease before the age of 65 years.27 The proportion of genetic risk attributable to individual loci depends on the number of genes involved and their relative importance. In addition to sample size, phenotypic and genotypic accuracy is important for statistical power.15 We took great care to exclude phenotypic and genotypic sources of error. Nevertheless, we believe that we cannot make a reliable estimate of the total number of loci that might contribute to coronary artery disease.

Our analysis of screening and recruitment revealed that despite the very high rates of exclusion, it is possible to select carefully and to avoid significant bias in cardiovascular risk factor profiles. We based this conclusion on the findings of a validated questionnaire that sought information about the premorbid situation. Although it might have been possible to measure coronary risk factors at the time of recruitment, such postmorbid data are not reliable indices of the premorbid...
situation because they are prone to bias as a result of lifestyle changes and treatment interventions after the acute coronary syndrome. The comparison of affected subjects with or without affected siblings found no obvious differences in exposure to conventional cardiovascular risk factors, other than increased rates of reported positive parental history of myocardial infarction. Our analysis also highlighted the potential for classification inaccuracy in relying simply on reported histories of myocardial infarction and justified the care taken in phenotypic characterization.

The linkage approach taken in the present study has the advantage of achieving an unbiased estimate of the genetic region or regions that might influence predisposition to the acute coronary syndrome. Another approach using case-control association analyses has been used in relation to 1 candidate in this region, the IRS1 gene (see below). Although further association studies of such candidates could have been undertaken, our linkage result would not be changed by a positive or negative case-control analysis. Just as important, case-control studies have their own problems and limitations. They are specifically prone to false positives as a result of population stratification. Similarly, a negative result would not be informative because other IRS1 polymorphisms might be relevant in our population or because other candidates in the region might explain the linkage result. Instead, the next logical approach is to confirm and refine the linkage results by larger studies in independent populations such as those already under way, and then to begin a more comprehensive analysis of candidates by using methods such as those based on single nucleotide polymorphisms.

One particular advantage of the genome-wide approach is that the current incomplete understanding of the pathogenesis of the acute coronary syndrome does not constrain research focus. Indeed, genome scans have the potential to reveal novel genetic origins and pathways. Notwithstanding the statistical power issues discussed above, it is perhaps surprising that only 3 loci emerged from this linkage analysis, given the number of risk factors that contribute to coronary disease. However, there is no reason to presume that the effects of all of the genes affecting each of the known coronary risk factors will translate into detectable influences on the acute coronary syndrome. Myocardial infarction and unstable angina usually involve the combination of coronary artery atherosclerosis, plaque instability and rupture, and coronary thrombosis. On this basis, our linkage analysis might be expected to reveal genes that influence all of these elements. Therefore, candidates on chromosome 2 and the other 2 loci will be those that control fundamental metabolic and physiological processes. The IRS1 gene that encodes the insulin receptor substrate-1 is of special interest because it is believed to be important in determining insulin sensitivity and related metabolic syndromes, including impaired endothelium-dependent vascular function. More important, several human IRS1 polymorphisms have been identified and have been associated with coronary artery disease. Despite the attractiveness of IRS1, the present study does not prove its involvement. Other genes in the region, many of which are yet to be defined, might account for the observed linkage. For example, the HDL-binding protein has an important role in the removal of excess cellular cholesterol.

In conclusion, this genome scan for acute coronary syndrome has identified 3 potential chromosomal loci. One of these, a locus suggested to be on chromosome 2, is outstanding in terms of its relative significance and the nature of candidate genes such as IRS1 that are in the region. Important coronary artery risk loci may have been missed in this genome-wide scan because these loci do not lead to the acute coronary syndrome in this population or because the power of the study was too low to detect smaller effects. Further studies must follow to extend these observations and to test these new genetic hypotheses in other geographical and racial groups. Such studies will need to devote substantial care and resources to ensure robust analyses. Nevertheless, the prospect of defining a limited number of genetic factors that are linked directly to the acute coronary syndrome, per se, rather than to surrogate risk factors is a compelling challenge.

Acknowledgments

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Genome-Wide Linkage Analysis of the Acute Coronary Syndrome Suggests a Locus on Chromosome 2


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