Candidate Gene Association Studies in Abdominal Aortic Aneurysm Disease: A Review and Meta-Analysis

A.R. Thompson,1,2* F. Drenos,1 H. Hafez2 and S.E. Humphries1

1Centre for Cardiovascular Genetics, British Heart Foundation Laboratories, Royal Free & University College London Medical School, 5 University Street, London WC1E 6JJ, UK, and 2Department of Vascular Surgery, St Richards Hospital, Chichester, West Sussex PO19 6SE, UK

Background. Candidate gene analysis has been frequently used in attempts to understand the pathological processes involved in many aspects of AAA disease.

Methods. This paper sets out a systems approach to reviewing AAA candidate gene analysis studies, whilst, explaining the key principles and design limitations of this universally applied technique. In addition, we have performed a meta-analysis of six gene polymorphisms (ACE I/D, MTHFR +677 C>T, MMP9−1562 C>T, IL1β/3953 C>T, eNOS 4a/4b & TIMP1−434 C>T) reported in multiple case control studies.

Results and conclusions. Three of these polymorphisms were associated with a significant risk of AAA, ACE RR 1.33 [95% CI 1.20−1.48], MTHFR RR 1.14 [1.08−1.21] and MMP9 RR 1.09 [1.01−1.18]. These differences have been previously reported as equivocal, within a context of contradictory studies and as such this meta-analysis provides new evidence for their involvement in AAA disease. The plausibility of these findings is discussed within the context of a systems approach to the pathology of AAA disease.

© 2007 European Society for Vascular Surgery. Published by Elsevier Ltd. All rights reserved.

Keywords: Abdominal aortic aneurysm; Candidate gene analysis; Single nucleotide polymorphism.

Background

Abdominal aortic aneurysm (AAA) is a common life threatening condition predominantly affecting men of retirement age. Despite this late presentation it has been estimated that around 15% of AAA patients have a family history of AAA disease. The evidence supporting an inheritable component to AAA disease has recently been reviewed by Sanford et al. and will not be discussed in detail.1 This article reviews the impact of candidate gene analysis in understanding AAA aetiology and uses meta-analysis to help interpret the results of studies to date.

The Search for Susceptibility Genes

A PubMed literature search with the terms “aneurysm” and “polymorphism” identified 168 studies. Of these, there were 26 case-control and 5 aneurysm expansion studies, pertaining to candidate gene analysis in AAA disease, looking at 78 different single nucleotide polymorphisms (SNPs). In reviewing the studies, summarised in Table 1, reporting positive associations, this paper will consider biological plausibility, population characteristics, concordance with associated phenotypes (hypertension & other atheromatous disease processes), the strength of any association and the evidence for a gene dose effect. In addition, we have performed a fixed effects meta-analysis in six SNPs investigated in more than one case/control study [ACE I/D, MTHFR +677 C>T, IL1β +3953 C>T, TIMP1−434 C>T, NOS3 4a/4b, MMP9−1562 C>T].2

*Corresponding author. A. R. Thompson, UCL, Cardiovascular genetics, 5 University Street, London WC1E 6JJ, UK.
E-mail address: rmhaath@ucl.ac.uk (A. R. Thompson)
### Table 1. Summary table of 26 case control (1A), and 5 aorta expansion (1B), candidate gene association studies in AAA disease

<table>
<thead>
<tr>
<th>Table 1A Autoimmunity</th>
<th>Gene</th>
<th>SNP</th>
<th>Author</th>
<th>Year</th>
<th>Cases/controls</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmunity</td>
<td>HLA</td>
<td>DQA1, DQB1, DRB1, DRB3-5</td>
<td>Ogata et al</td>
<td>2006</td>
<td>387/426</td>
<td>ns outside of subgroup analysis</td>
</tr>
<tr>
<td>Collagen</td>
<td>COL3A1</td>
<td>+581T&gt;C</td>
<td>Ogata et al</td>
<td>2005</td>
<td>387/425</td>
<td>ns</td>
</tr>
<tr>
<td>ELN</td>
<td></td>
<td>+422G&gt;A</td>
<td>Ogata et al</td>
<td>2005</td>
<td>387/425</td>
<td>ns outside of subgroup analysis</td>
</tr>
<tr>
<td>Homocysteine metabolism</td>
<td>MTHFR</td>
<td>+677C&gt;T</td>
<td>Brunelli et al</td>
<td>2000</td>
<td>58/60</td>
<td>*TT genotype in AAA patients p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strauss et al</td>
<td>2003</td>
<td>74/71</td>
<td>ns outside of subgroup analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strauss et al</td>
<td>2005</td>
<td>428/282</td>
<td>ns</td>
</tr>
<tr>
<td>Chemokines</td>
<td>CCR5</td>
<td>△32</td>
<td>Ghilardi et al</td>
<td>2004</td>
<td>70/172</td>
<td>*Δ32 allele in AAA patients p = 0.002. Difference in genotype distribution p = 0.009</td>
</tr>
<tr>
<td>Inflammatory mediators</td>
<td>HO-1</td>
<td>(GT)n</td>
<td>Schillinger et al</td>
<td>2002</td>
<td>70/61</td>
<td>*</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td>−1082G&gt;A, −592C&gt;A</td>
<td>Bown et al</td>
<td>2003</td>
<td>100/100</td>
<td>−1082A allele in AAA patients</td>
</tr>
<tr>
<td>IL-1A</td>
<td></td>
<td>−899C&gt;T, +4845G&gt;T</td>
<td>Marculescu et al</td>
<td>2005</td>
<td>135/270</td>
<td>ns</td>
</tr>
<tr>
<td>IL-1Beta</td>
<td></td>
<td>+3953C&gt;T</td>
<td>Bown et al</td>
<td>2003</td>
<td>100/100</td>
<td>ns</td>
</tr>
<tr>
<td>IL-1Beta</td>
<td></td>
<td>−511C&gt;T, −31C&gt;T, +3954C&gt;T</td>
<td>Marculescu et al</td>
<td>2005</td>
<td>135/270</td>
<td>ns</td>
</tr>
<tr>
<td>IL-1RN</td>
<td></td>
<td>+2018C&gt;T</td>
<td>Marculescu et al</td>
<td>2005</td>
<td>135/270</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td>−174G&gt;C</td>
<td>Bown et al</td>
<td>2003</td>
<td>100/100</td>
<td>ns</td>
</tr>
<tr>
<td>TGF beta1</td>
<td></td>
<td>−509T&gt;C</td>
<td>Ogata et al</td>
<td>2005</td>
<td>387/425</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BsmI</td>
<td>Massart et al</td>
<td>2004</td>
<td>99/225</td>
<td>ns</td>
</tr>
<tr>
<td>TNF alpha</td>
<td></td>
<td>−308G&gt;A</td>
<td>Bown et al</td>
<td>2003</td>
<td>100/100</td>
<td>ns</td>
</tr>
<tr>
<td>PAF-AH</td>
<td></td>
<td>+994G&gt;T</td>
<td>Unno et al</td>
<td>2002</td>
<td>131/106</td>
<td>*T allele in AAA patients p = 0.015. Difference in genotype distribution p = 0.012</td>
</tr>
<tr>
<td>Candidate Genes in AAA</td>
<td>Mediators of extracellular matrix protein degradation</td>
<td>MMP1</td>
<td>−1607G&gt;G, GG</td>
<td>Ogata et al [Ogata, 2005]</td>
<td>2005</td>
<td>387/425</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------------------</td>
<td>------------</td>
<td>-------------</td>
<td>----------------------------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>MMP3</td>
<td>−1612 5A/6A</td>
<td>Yoon et al [Yoon, 1999]</td>
<td>1999</td>
<td>47/174</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MMP9</td>
<td>(CA)n</td>
<td>Yoon et al [Yoon, 1999]</td>
<td>1999</td>
<td>47/174</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>PAI-1</td>
<td>−675 4G/5G</td>
<td>Yoon et al [Yoon, 1999]</td>
<td>1999</td>
<td>47/176</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>TIMP1</td>
<td>+323C&gt;T, +434C&gt;T</td>
<td>Wang et al [Wang, 1999]</td>
<td>1999</td>
<td>84/51</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>TIMP3</td>
<td>−1296T&gt;C</td>
<td>Ogata et al [Ogata, 2005]</td>
<td>2005</td>
<td>387/425</td>
<td>ns outside of subgroup analysis</td>
</tr>
<tr>
<td></td>
<td>NOS3</td>
<td>+894G&gt;T, −786T&gt;C, 4A/4B</td>
<td>Fatini et al [Fatini, 2005]</td>
<td>2005</td>
<td>250/250</td>
<td>† 573G allele in AAA males p = 0.0374 ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4a/b</td>
<td>Hamano et al [Hamano, 1999]</td>
<td>1999</td>
<td>125/153</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/D</td>
<td>Fatini et al [Fatini, 2005]</td>
<td>2004</td>
<td>250/250</td>
<td>† D allele in AAA patients p = 0.001. Difference in genotype distribution p = 0.002 ns</td>
</tr>
</tbody>
</table>

*Continued on next page*
### Table 1 (continued)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Author</th>
<th>Year</th>
<th>Cases/controls</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+1730A&gt;G</td>
<td>Massart et al[Massart, 2004 62 /id]</td>
<td>2004</td>
<td>99/225</td>
<td>† +1730 G allele in AAA patients p &lt; 0.05. Difference in genotype distribution p &lt; 0.05</td>
</tr>
<tr>
<td>Table 1B</td>
<td>Mediators of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extracellular matrix</td>
<td>MMP2</td>
<td>Eriksson et al[Eriksson, 2005 21 /id]</td>
<td>2005</td>
<td>455</td>
<td>ns</td>
</tr>
<tr>
<td>protein degradation</td>
<td>MMP3</td>
<td>Eriksson et al[Eriksson, 2005 21 /id]</td>
<td>2005</td>
<td>455</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MMP9</td>
<td>Eriksson et al[Eriksson, 2005 21 /id]</td>
<td>2005</td>
<td>455</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MMP12</td>
<td>Eriksson et al[Eriksson, 2005 21 /id]</td>
<td>2005</td>
<td>455</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>PAI-1</td>
<td>Eriksson et al[Eriksson, 2005 21 /id]</td>
<td>2005</td>
<td>455</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Cystatin C</td>
<td>Eriksson et al[Eriksson, 2004 22 /id]</td>
<td>2004</td>
<td>424</td>
<td>5G/5G genotype is associated with † growth rate ns</td>
</tr>
<tr>
<td>Rennin angiotensin system</td>
<td>ACE I/D</td>
<td>Yeung et al[Yeung, 2002 599 /id]</td>
<td>2002</td>
<td>58</td>
<td>ns</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>APOE APOE*2,3,4</td>
<td>Gerdes et al[Gerdes, 2000 598 /id]</td>
<td>2000</td>
<td>57</td>
<td>ns</td>
</tr>
</tbody>
</table>

### Abbreviations
- HLA, Human leukocyte antigen
- COL, Collagen
- ELN, Elastin
- PON, Paraoxonase
- CCR, Chemokine Receptor
- HO, Heme-oxygenase
- IL, Interleukine
- TGF, Transforming Growth Factor
- PAF-AH, Platelet-Activating Factor Acetylhydrolase
- MMP, Matrix Metalloproteinase
- PAI, Plasminogen Activator Inhibitor
- TIMP, Tissue Inhibitor of MMP
- NOS, Nitric Oxide Synthetase
- ACE, Angiotensin Converting Enzyme
- ER, Oestrogen Receptor
- PR, Progesterone Receptor
- ns, no statistically significant difference (p > 0.05)
- RR, Relative Risk.
* Indicates controls and cases have undergone imaging of the aorta.
Candidate Genes in AAA

A systems approach

Candidate gene analysis does not stand up without a context with which to demonstrate biological plausibility. A systems approach provides that context and can help in the interpretation of results. An example of this, in a heterogeneous pathology, can be seen in Marfan’s syndrome and related disorders. It has long been known that defects in the fibrillin 1 gene are largely responsible for the autosomal dominantly inherited disorder of Marfan’s syndrome. However the discovery that disorders of similar phenotype, including some familial thoracic ascending aortic aneurysms, are caused by defects in transforming growth factor beta receptor (TGFBR) 1 and 2 (Loeys-Dietz Syndrome), together with animal model work on TGFβ, has led to the realisation that this spectrum of thoracic aneurysmal diseases are encompassed by the TGFβ pathway. To assist in the interpretation of candidate gene analysis in AAA disease, Fig. 1 outlines the basic pathways of AAA disease progression through four stages.

Aneurysm initiation

A number of risk factors for AAA disease are thought to influence aneurysm initiation. Most of these are common to other forms of cardiovascular disease, such as, hypertension (haemodynamic stress), atheroma, smoking, male gender, age (time) and a possible connective tissue disorder. These “risk factors” may have a greater influence later in aneurysm development, or at more than one stage, but for the purpose of this paper it is convenient to consider them in the initial stages.

The renin-angiotensin system (RAS) is known to affect the cardiovascular system at multiple levels. Despite many functional SNPs in multiple genes being studied in ischaemic heart disease (IHD), only the angiotensin converting enzyme insertion/deletion (ACE I/D) polymorphism has been looked at, to date, in AAA disease. An insertion polymorphism of 287 base pairs (bp), in the ACE gene, is associated with a 50% decrease in ACE serum and tissue levels, as measured in vitro, and manifests in vivo as raised Hcy levels. Despite obvious connections between AAA disease and atheromatous disease, a causal role of Hcy in AAA pathogenesis remains unproven. Bruno Massart et al., hypothesised that the delay in onset of AAA disease in women is due to the protective effect of sex steroids. An association between the oestrogen receptor β+1730 G allele polymorphism and AAA disease was reported. However, the significance of this allele on oestrogen receptor activity is unknown and with such small numbers, this hypothesis is in need of further evidence.

The homocysteine (Hcy) level is a risk factor for the development of atherosclerotic disease as well as being able to induce elastolysis in vitro through the action of MMP2. Methylene tetrahydrofolate reductase (MTHFR) is the primary enzyme of Hcy metabolism. The MTHFR +677C>T polymorphism causes an alanine to valine change in the protein which results in a 70% reduction in enzyme activity, as measured in vitro, and manifests in vivo as raised Hcy levels. Despite obvious connections between AAA disease and atheromatous disease, a causal role of Hcy in AAA pathogenesis remains unproven. Bruno Massart et al., demonstrated a higher frequency of the MTHFR +677 TT genotype in a small cohort of AAA patients. Three other studies have also looked at this relationship. Strauss et al., were able to show a significant increase in the rare T allele frequency in the AAA group. However numbers were small and the control group was not matched for age, sex or parameters of atherosclerotic disease. Sofi et al., and Ferrara et al., were able to show a higher T allele frequency in 438 and 88 AAA patients versus 438 and 44 controls, respectively. The study by Ferrara et al., although it reported these findings, was designed to detect a difference in the MTHFR +677C>T allele frequency between a young and an old group of patients. For this reason, patients between the ages of 60–64 were not included, limiting
Inherent tissue defect  
Male  
Smoking  
Haemodynamic stresses  
Atherosclerosis  
Time

Systems targeted by candidate gene analysis in AAA disease
- Elastic fibre biology  
- Steroid Hormones  
- Renin angiotensin system  
- Nitric oxide pathway  
- Homocysteine metabolism  
- Lipid metabolism

Fig. 1. A schematic representation of possible AAA pathogenesis, displaying progression through four distinct phases (Initiation, formation, growth and rupture), contained within the blue shaded area. The targets of candidate gene analysis and the likely site(s) of interaction with AAA pathogenesis are shown in the green shaded area.
numbers in the study. Interestingly, despite small numbers, there was an increase in the allele frequency in the younger cohort of AAA patients ($p < 0.004$). Patients and controls in the study by Sofi et al.,22 were well matched for age and sex but there was more than double the preponderance of hypertension, smoking and dyslipidaemia in the AAA cohort. Another report by Jones et al.,24 was unable to confirm these findings. Meta-analysis of these studies reveals a significant effect in favor of the T allele variant, increasing the risk of AAA disease (RR 1.14 [95%CI 1.08–1.21]) [Fig. 2B].

Nitric oxide (NO) has multiple effects on vessel wall biology that could be important in AAA pathogenesis, including vasodilatation and inhibiting smooth muscle migration/proliferation.25 The Nitric Oxide Synthase 3 (NOS3) $\text{þ894G > T (Glu298Asp)}$ polymorphism has been associated with reduced NO production.26 Fatini et al.,27 reported a highly significant difference ($p < 0.0001$) in the NOS3 $\text{þ894 T allele frequency}$ in a group of 250 AAA patients versus age/sex matched controls. Significant differences were also present in atheromatous risk factors between the patients and controls. The implication is that the rarer T allele reduces NO tissue levels, contributing to AAA formation. Meta-analysis of NOS3 4a/4b frequency in two case/control studies showed no significant difference (data not shown).

Chemokines are instrumental to the early response of the arterial wall to injury. The 32 bp deletion in the chemokine receptor 5 gene (CCR5) decreases receptor expression, inhibiting leukocyte recruitment to the area and reducing any inflammatory infiltrate.28 Gigliardi et al.,29 report an association between AAA disease and the 32 bp deletion in CCR5. Additional comparison with peripheral vascular disease (PVD) and carotid disease patients showed no differences to the controls, supporting the separation of AAA disease from other atheromatous diseases. It is proposed that the CCR5 $\text{32 polymorphism results}$ in a decrease in the Th1 response and in doing so promotes the Th2 response dominant in AAA disease. This differentiation between Th1 and Th2 leukocytes is believed to be a trigger in determining whether atheromatous conditions lead to occlusive or aneurysmal disease.30 The role of CCR5 in this is unproven.

By far the greatest area of research interest in AAA disease of recent years, including candidate gene analysis, is the role of matrix metalloproteinase’s (MMP) and their mediators, in elastin degradation. It has become clear that this is a vital step in the early stages of AAA formation.19 MMP-1, a collagenase, and, MMP-2 and MMP-9, elastases, are implicated in the pathogenesis of AAA disease and atherosclerosis.31,32 The

![Fig. 2. A box whisker plots displaying the results of meta-analysis of case control studies in AAA disease, ACE I/D (2A), MTHFR $\text{+677C > T (2B), and MM9–1562C > T (2C). The meta-analysis was performed using the “Inter-cooled Stat 9.2 (STB-38:sbe16)” package by Sharp and Sterne. [CI, Confidence Interval].}]

Eur J Vasc Endovasc Surg Vol 35, January 2008
MMP9 gene has a polymorphism (−1562C > T) in the promoter region, in which the rare T allele is associated with an almost 2-fold higher promoter activity, which appears to be due to a weaker binding of the transcription repressor protein to the promoter. It is therefore a very plausible candidate for AAA formation. Jones et al., reported an increased frequency in the TT and CT genotypes in 414 AAA cases versus 203 controls. However, Ogata et al., were unable to demonstrate an association between MMP9 genetic variation and AAA in two separate populations. Meta-analysis of these three groups (Fig. 2C) does confirm a significant, albeit small, allelic dose effect (RR 1.09 [95% CI 1.01–1.18]).

Unno et al., proposed a link between platelet activating factor (PAF) induction of MMP1, MMP-2 & MMP-9 transcription and AAA. The activity of PAF is controlled by PAF acetylhydrolase (PAF-AH). A well-known polymorphism (+994G > T) results in lower enzyme activity and increased levels of PAF. Previous studies have connected the +994G > T polymorphism to coronary artery disease, stroke and peripheral arterial disease. We found a significant association of the rare T allele and AAA with a strong genotype dose effect (GT + TT odds ratio for AAA of 2.48 (1.36–4.65). Tissue inhibitors of metalloproteinases (TIMPs) are major inhibitors of MMPs. Wang et al., reported a decrease in the rare A allele for TIMP2 + 573 SNP in AAA patients suggesting it may be protective against AAA. No explanation of the potential action of the TIMP2 + 573G > A SNP is put forward. Although found within the coding region, this base pair change does not result in an amino acid change. Meta-analysis of TIMP1 + 434C > T frequency in two case/control studies showed no significant difference (data not shown).

It is convenient to think of aneurysm growth as a separate process, with some of the initiating steps in aneurysm formation being unnecessary for continued expansion. However, it is likely that many of the chronic inflammatory processes represented in aneurysm formation are also important to aneurysm growth and rupture. The influences of inflammatory mediators are likely to be seen on multiple levels and for this reason are also less likely to be confined to AAA. Bown et al., demonstrated a difference in the IL-10 −1082G > A polymorphism in a small group of AAA patients compared to age and sex matched controls. IL-10 is a potent anti-inflammatory cytokine, inhibiting macrophage function and T cell antigen presentation. The IL-10 −1082A allele reduces IL-10 activity. This anti-inflammatory action could have an effect on AAA. Meta-analysis of IL-1β + 3954C > T frequency in two case/control studies showed no significant difference (data not shown). Hemeoxygenase-1 (HO-1) is expressed in vascular smooth muscle cells and has a potent anti-inflammatory and antioxidant capacity. Schillinger et al., reported an association between the HO-1 short (<25) GT repeats and protection from AAA. This association was significant but showed only a small allele dose effect.

Aneurysm growth

In addition to case-control studies, focusing on likely pathological processes in AAA growth, actual expansion data has been used in direct association studies with candidate genes (Table 1B). Largely this has been performed using the UK small aneurysm trial data. Initial analysis suggested a significant association between growth and plasminogen activator inhibitor (PAI) 1 -675 4G/5G. However, after the same group later accounted for the non-uniform nature of aneurysm growth the reported association was lost. Although the UK small aneurysm trial has produced the most comprehensive growth data to date (Gerdes et al.,), Yeung et al., it lacks early AAA growth data, between 3 and 4 cm. With the establishment of AAA screening this data should become more available and gene association studies may yet have an influence in this area.

Review of the mechanisms leading to AAA rupture was considered outside the scope of this paper and has been extensively reviewed by Choke et al.,

Discussion

Initial attempts to determine the genetic background of AAA have been on a small scale compared to the efforts seen in other forms of vascular pathology, namely hypertension, atheromatous plaque morphology and IHD. As a consequence, candidate gene analysis has often mimicked studies performed in these pathologies as opposed to being tailored to AAA. This has resulted in candidate genes for AAA, such as those identified through gene linkage analysis, and genes involved in elastic fibre biology, being largely under investigated. However, studies to date have provided both some credible associations, as well as highlighting specific challenges to candidate gene analysis in AAA.

The distribution of AAA in the population is likely to reflect multiple common mutations with a small effect. It is important to realize this when designing candidate gene studies, as small effects will require much greater power to detect a difference, especially if the causal mutation is rare. Fig. 3 displays how...
these factors combine to provide estimates of required sample size.\textsuperscript{52} When these factors are taken into account it is clear that few of the studies in Table 1 were sufficiently powered. For example meta-analysis of the ACE I/D polymorphism suggests a truer risk ratio of 1.33 (95% CI 1.20–1.48) for the D allele. Referring to Fig. 3 it can be seen that despite, a favorable allele frequency of over 30\%, at this hazard ratio about 700 cases and 2100 controls are need for 95\% power at the 80\% level.

AAA disease is also problematic for candidate gene analysis because it is susceptible to confounding influences of genetic stratification. In other words, it is difficult to allow for common risk factors which may have genetic overlap with other disorders. Studies have gone to various degrees in attempts to exclude, in particular, atheromatous influences. All AAA candidate gene studies have made representation of risk factors for atheroma in patient and control arms. Several have used additional comparison with PVD, IHD and carotid artery disease cohorts.\textsuperscript{24,34,43} Although a direct causal link between AAA disease and atheroma has not been proven there is concern that shared pathways for these pathologies may decrease the specificity of studies.\textsuperscript{30} Where associations have already been made between a SNP and vascular disease, such as with ACE I/D and MTHFR +677C > T, it is imperative that these confounding factors are accounted for before making associations with AAA. Common risk factors such as hypertension and smoking are the likely pathways through which such associations may exist and it is therefore reasonable to require that they are approximately matched in control groups. Secondly there are also notable differences in the efforts made in studies to exclude AAA from the control groups. Nine of 26 case/control studies (Table 1) imaged the aorta in the control group, excluding a potential 5\% falsely identified as not having AAA disease.\textsuperscript{53} This may constitute up to a 4.7\% loss in sensitivity prior to any final analysis. In many of the studies very small effects are seen and it may be that significant differences have been missed as a result of this loss of sensitivity.

Initial polymorphism association studies reported in high impact journals may not stand up to the test of time and almost always, the final size of the risk association is (considerably) smaller than first reported (the so called "winners cause"). It is therefore incumbent on workers in the field to follow several key principles in reporting studies. The first of these is that a clear biologically plausible hypothesis should be given. Secondly, that any observations are repeated in several independent sets and that where possible meta-analysis is performed. Study design should set out any secondary phenotypes and identify potential confounding factors. The strength of association and the gene-dose effect should be considered separately. Finally findings should be confirmed through experimentation.\textsuperscript{54}

Meta-analysis has been used here as a way of combining and giving an overview of the findings in candidate gene studies of AAA disease. This view should be balanced with an understanding of the limitations inherent to its methodology. Meta-analysis is the analysis of already analyzed data, and, should be distinguished from the re-analysis of primary data. A weakness of meta-analysis is in its inability to properly account for heterogeneity between studies. This is of particular importance in studies where geographical location or race may have a large influence. There are now complex models that can overcome problems of such heterogeneity, but they rely on large numbers of studies, which are usually unavailable to small fields of research.\textsuperscript{5} Meta-analysis is reliant largely on published data and as such has the potential to compound prior publication bias. Candidate gene analysis is particularly susceptible to this form of bias where thousands of candidate genes, each with multiple SNPs, may provide spurious associations attracting a cherry picking approach both to reporting and the publication of positive associations.\textsuperscript{55} Ways of combating this would include the use of much larger numbers within studies and encouraging publication regardless of the statistical significance of the result. Probably the most useful way forward would be the use of an online data base, providing scrutiny for all results and not just those published, as proposed by the Human Genome Epidemiology Network.

---

**Fig. 3.** Estimated sample size required to detect a relative risk of AAA disease with a particular statistical power for mutations of different frequency. Data calculated using the unmatched case control package in Epi program [Epi Info (version 6.02) Centres for Disease Control and Prevention, USA]. Taken from Humphreys et al.,\textsuperscript{52} with permission.
In its favor, meta-analysis combines studies, in a weighted manner (usually according to size), providing the power to both identify the presence of a difference and also to estimate the size of any difference. The importance of this ability to pick up small but significant effects can be seen in other diseases. An example is seen in the role of the Calpain-10 gene (CAPN10), SNP-44, in type 2 diabetes (T2DM). Out of 10 identified gene association studies, containing 3,303 subjects, only one study reported a weak association with T2DM (p = 0.05). However, despite the seemingly likelihood of any association, meta-analysis identified a moderate, but statistically robust increased risk of T2DM (OR 1.17 [p = 0.02]). This information was then used to adequately power a further gene association study with 4,213 subjects, which confirmed the association identified through meta-analysis (OR 1.18 [p = 0.01]). Another example within cardiovascular disease is that of three SNP’s in the endothelial Nitric Oxide Synthase gene (eNOS). These have been the subject of multiple association studies looking at coronary heart disease (CHD). Variation in the reported results of these studies made the interpretation, all but impossible without the use of meta-analysis. The resulting meta-analysis identified small but significant associations between two of the SNPs and CHD, which would not have been found, in the original case-control studies due to lack of power. Such small but significant differences have subsequently been used in combination to provide a prediction of CHD risk.

Although it is important that meta-analysis is not used to go beyond the claims of the original data, this gives a clear example of how meta-analysis can be used to identify small associations, not possible within the original studies, and of how these small associations can still be important tools in predicting disease. In this article, we have used the risk ratio, obtained through our meta-analysis of ACE I/D, to demonstrate how AAA gene association studies have been underpowered. Although the results of this meta-analysis can also be used to pass comment on gene associations they should then be confirmed by adequately-powered studies, free of the types of bias open to meta-analysis.

The results of our meta-analysis have demonstrated significant effects in ACE, MTHFR and MMP9. The ACE insertion polymorphism appears to have the greatest allele dose effect (RR 1.33 [1.20–1.48]). But, it should be noted that the reported control allele frequencies are different to those seen in larger population studies, which may have erroneously increased the size of any effect. In addition, the significance of hypertension, as a confounding factor, is difficult to reconcile in this group. Hypertension is a recognized risk factor for AAA. The role of the RAS in hypertensive AAA patients should now be the attention of biological studies in an attempt to confirm the first meaningful association made through candidate gene analysis and AAA. Hackman et al. reported an association between the use of ACE inhibitors and reduced rates of AAA rupture, in a group of Canadian AAA patients.

In conclusion, candidate gene studies, to date, have lacked the numbers to discover the small but significant associations likely to affect this complex multifactorial pathology. Subsequent attempts should involve collaborations to maximize the power of studies. The future of gene analysis in AAA disease remains wide open. New platform analysis techniques are likely to throw up many associations but there is a danger that much time will be wasted in following up biologically unsound leads.

Acknowledgments

ART, FD and SEH are supported by the British Heart Foundation (FS/04/012:RG2005/014), and HH by the Scott Research Unit, Chichester. We thank colleagues in CVG for their helpful comments in the development of this review.

References


Eur J Vasc Endovasc Surg Vol 35, January 2008
Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MCHCS), San Francisco City Cohort (SFCC), ALIVE Study. Science 1997;277(5328):959—965.


Accepted 4 July 2007
Available online 24 October 2007