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Abdominal Aortic Aneurysm: A Review of the Genetic Basis

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Abstract

Background: Abdominal aortic aneurysm (AAA) is a complex disease with a largely unknown pathophysiological background and a strong genetic component. Various studies have tried to link specific genetic variants with AAA. **Methods:** Systematic review of the literature (1947-2009). **Results: A total of** 249 studies were identified, 89 of which were eventually deemed relevant to this review. Genetic variants (polymorphisms) in a wide variety of genes, most of which encode proteolytic enzymes and inflammatory molecules, have been associated with AAA development and progression. **Conclusion:** The genetic basis of AAA remains unknown, and most results from "candidate–gene" association studies are contradictory. Further analyses in appropriately powered studies in large, phenotypically well-characterized populations, including genome-wide association studies, are necessary to elucidate the exact genetic contribution to the pathophysiology of AAA.

Keywords

abdominal aortic aneurysm, polymorphism, whole genome scan, genetics

Introduction

Abdominal aortic aneurysm (AAA) is a multifactorial disease with a strong genetic component.[1] Various investigators have studied polymorphisms of specific genes encoding key molecules thought to be involved in AAA formation, in relation to both AAA presence and progress (natural history of the disease as well as postoperative progress). Most studies have primarily focused on genes encoding structural proteins of the vessel wall, degrading enzymes such as matrix metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs), and genes involved in the expression of molecules of the inflammatory, coagulation, and tissue repair cascades, such as several interleukins (ILs), C-reactive protein (CRP), tissue growth factor β (TGF-β), platelet-activating factor (PAF), plasminogen activator inhibitor 1 (PAI-1), osteopontin (OPN), and osteoprotegerin (OPG). Associations with the human leukocyte antigen (HLA) complex have also been studied, while genome-wide association (GWA) studies have started to appear.

In this review, we summarize the current literature regarding the thus far described genetic associations of AAA presence and progress.

Methods

MedLine (1950-2009) and Embase Databases (1947-2009) were searched using the following keywords: "abdominal aortic aneurysm" AND "gene"; "abdominal aortic aneurysm" AND "polymorphism" (articles in English and German). Searches were not limited by language or study design. A total of 249 studies were identified. Abstracts and citations were screened by A.S. and A.A. Reference lists of the identified papers were also screened to identify further relevant publications. Overall, a total of 89 observational and comparison studies (AAA group vs disease-free controls) were deemed relevant to this review and were summarized by A.S. and A.A. Abdominal aortic aneurysm was confirmed by means of imaging (abdominal ultrasound, computed tomography) in all studies included in this review.

Matrix metalloproteinases and TIMPs

Matrix metalloproteinases constitute a family of over 20 distinct enzymes that are capable of degrading extracellular proteins as well as clotting factors, lipoproteins, growth factors, chemotactic

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and cell-adhesion molecules. Matrix metalloproteinases are activated by certain zymogens and regulated by TIMPs.[2-4] They play a major role in the degradation of the extracellular matrix of the vessel wall, which gradually leads to AAA formation.[1,5] Most researchers have focused on the role of MMP-2 and MMP-9 in AAA formation, as they both possess significant proteolytic properties. Matrix metalloproteinase 2 is mainly produced by vascular smooth muscle cells (VSMCs) and fibroblasts.[6] Activated MMP-2 leads to significant elastolysis in the extracellular matrix of the aneurysmal wall[7]; several studies have shown increased levels and tissue activity of MMP-2 in AAA.[8-11] Interestingly, Freestone et al[12] demonstrated that AAAs smaller than 5.5 cm had greater MMP-2 activity than larger aneurysms, indicating that early AAA growth is directed by MMP-2. Matrix metalloproteinase 2 (and MMP-9) has also been shown to affect the Ca^{2+}-dependent mechanism of aortic VSMC contraction that may have an important role in the early development of AAA.[13,14] Price et al[15] have analyzed the complete coding region and the promoter sequence of the *MMP-2* gene in a healthy population. They demonstrated that a common C>T transition in the promoter at position g.−1586, which disrupts an Sp1-type promoter site (CCACC box), was functional and that the C allele was associated with higher promoter activity. They confirmed that these differences in allelic expression were attributed to abolition of Sp1 binding[15]; Sp1 is a cellular transcription factor involved in a wide variety of processes and has been shown to bind to different promoters to regulate transcription.[16] Eriksson et al[17] performed an observational study including 455 individuals with a small AAA (4.0-5.5 cm) to evaluate the effect of MMP polymorphisms on AAA expansion. Ogata et al[18] analyzed *MMP* gene polymorphisms in relation to AAA presence in 387 patients with AAA and 425 controls. Neither study demonstrated any association between the −1306 C>T and −955A>C polymorphisms of the *MMP-2* gene and AAA (expansion or presence). Hinterseher et al[19] analyzed the entire coding region of the *MMP-2* gene and regions of its promoter. In all, 18 polymorphisms were identified, 6 of which were newly described; 3 were located in the introns (c.IVS1 + 31C>G, c.IVS7 − 18G>A, c.IVS10 + 26C>T), and 3 were located within the coding region (c.124G>A, c.1368C>T, c.1860C>T) of the gene; however there were no statistically significant differences in genotype or allele frequencies between 51 patients with AAA and 48 controls (Table 1).

Matrix metalloproteinase 9 (gelatinase B or 92 kd type IV collagenase) is a type IV collagenase and an elastase-type enzyme. It is considered the primary elastolytic enzyme in the aneurysmal wall and has been associated with accelerated aneurysm progression.[20,21] It is a more potent inhibitor of vascular contraction than MMP-2 and constitutes the main MMP in the extracellular matrix of AAAs, produced primarily by the infiltrating macrophages.[22] Apart from increased MMP-9 expression in the aneurysmal wall, several studies have also documented raised levels of circulating MMP-9 in patients with AAA.[22-24] Targeted gene disruption of MMP-9 in mice has been shown to suppress the development of experimental AAA.[25]

Various polymorphisms in the MMP-9 gene have been identified so far.[26] Most functional analyses and genetic epidemiological studies of this gene have focused on the 1562 C>T and the (CA)n polymorphism, both found within the promoter region of the gene. The C>T substitution in position 1562 of the promoter is thought to associate with a 1.5-fold increase in promoter activity.[26,27]

A study of aortic tissues by Medley et al showed that MMP-9 messenger RNA (mRNA) levels, MMP-9 protein levels, and MMP9 activity were higher in T-1562 allele carriers than in noncarriers.[28] Blankenberg et al also showed higher plasma MMP-9 levels in T-1562 allele carriers than in noncarriers.[29] A study of the MMP-9 gene (CA)n repeat polymorphism showed that the frequency of the (CA)23 allele which has higher MMP-9 promoter activity was significantly elevated in patients with intracranial aneurysm than in matched controls[30]; however, this was not supported by the findings of another study.[31]

Jones et al[27] compared the frequency of C-1562T polymorphism in 414 patients with AAA, 172 patients with peripheral vascular disease (PVD), and 203 healthy controls: the T allele was more frequently present in the AAA group; however, a subsequent study by Ogata et al involving a total of 387 AAA cases and 425 controls failed to confirm this.[18] In the group of 455 patients with small AAAs by Eriksson et al,[17] there was no association between the T allele and expansion rate. A recent meta-analysis of studies investigating the C-1562T polymorphism suggested a marginally positive association with AAA presence.[32] The authors of the meta-analysis comment that this was probably due to the weighting (57·8%) of the study performed by Jones et al.[27] Lastly, Smallwood et al[33] genotyped the −562C>T and 1811A>T variants in 678 men with an AAA (at least 30 mm in diameter) and 659 control participants; no significant association was found.

Matrix metalloproteinase 8 (a neutrophil collagenase) degrades type I, II, and III collagens. The gene[34] is part of a cluster of *MMP* genes that localize to chromosome 11q22.3. A recent study has disclosed increased MMP-8 concentrations in aneurysmal aortas compared with normal aortas.[35] Our literature search failed to disclose a study investigating the relationship between MMP8 gene polymorphisms and AAA in humans.

Matrix metalloproteinase 3 (stromelysin 1 or progelatinase) is an enzyme that degrades fibronectin, laminin, collagens III, IV, IX, and X, and cartilage proteoglycans. It has previously been isolated from AAA tissue.[36] A study from Finland,[37] including 47 AAA patients, 57 intracranial aneurysm patients, and 174 controls, investigated the promoters of the genes encoding MMP-3, MMP-9, and PAI-1. There was a trend ($P = .0609$) for a higher frequency of the 5A MMP-3 allele (position 1171 of the promoter of MMP-3; a functional polymorphism previously associated with cancer[38]) in the AAA group.

Matrix metalloproteinase 10 is an enzyme that degrades proteoglycans and fibronectin. Ogata et al[18] have found a significant association between the polymorphism nt + 180 of the *MMP-10* gene and sporadical AAA, but this was no longer

Table 1. Summary of Polymorphisms in Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Matrix Metalloproteinases (TIMPs) Genes

Gene	SNP	Reference, Year	Type of Study (Cases/Controls)	**Results**
MMP1	−1607G>GG	Ogata et al, 2005[18]	Comparison (387/425)	NS
MMP2	−1306 C>T	Powell et al, 2006[39]	Observational - 455 AAAs	NS
MMP2	−1306 C>T	Eriksson et al, 2005[17]	Observational - 455 AAAs	NS
MMP2	−955A>C	Ogata et al, 2005[18]	Comparison (387/425)	NS
MMP2	−1586C>T,-1070T>G, −61G>C, 124G>A, IVS1+31C>G, 228G>A, 678G>C, 750C>T, IVS5+12C>T, IVS7-18GA, 1149C>T, 1380G>A, IVS10+26C>T, IVS12-4A>G, 1806C>T, 1842C>G, 1860C>T	Hinterseher et al, 2006[19]	Comparison (51/48)	NS
MMP3	−1171 5A>6A	Powell et al, 2006[39]	Observational - 455 AAAs	NS
MMP3	−1612 5A>6A	Ogata et al, 2005[18]	Comparison (387/425)	NS
MMP3	−1612 5A>6A	Yoon et al, 1999[37]	comparison (47/174)	NS
MMP3	−1171 5A>6A	Eriksson et al, 2005[17]	Observational - 455 AAAs	NS
MMP9	−1562C>T	Eriksson et al, 2005[17]	Observational - 455 AAAs	NS
MMP9	−1562C>T	Powell et al, 2006[39]	observational - 455 AAAs	NS
MMP9	−1562C>T	Jones et al, 2003[27]	Comparison (414/203)	$P = .03$
MMP9	−1562C>T	Ogata et al, 2005[18]	Comparison (387/425)	NS
MMP9	−562C > T	Smallwood et al, 2008[33]	Comparison (678/659)	NS
MMP9	1811A > T	Smallwood et al, 2008[33]	Comparison (678/659)	NS
MMP9	(CA)n	Yoon et al, 1999[37]	Comparison (47/174)	NS
MMP10	+180A>G	Ogata et al, 2005[18]	Comparison (387/425)	NS
MMP12	−82A>G	Powell et al, 2006[39]	Observational - 455 AAAs	NS
MMP12	−82A>G	Eriksson et al, 2005[17]	Observational - 455 AAAs	NS
MMP13	−77A>G	Ogata et al, 2005[18]	comparison (387/425)	NS
TIMP1	+434C>T	Ogata et al, 2005[18]	comparison (387/425)	$P = .0047$
TIMP1	rs2070584	Ogata et al (18), 2005[18]	comparison (387/425)	$P = .015$
TIMP1	−372C>T	Powell et al, 2006[39]	Observational - 455 AAAs	NS
TIMP1	323C>T	Wang et al, 1999[42]	Comparison (84/51)	NS
TIMP1	434C>T	Wang et al, 1999[42]	Comparison (84/51)	NS
TIMP1	434C>T	Ogata et al, 2005[18]	Comparison (387/425)	NS
TIMP1	rs2070584	Ogata et al, 2005[18]	Comparison (387/425)	NS
TIMP1	372T>C	Hinterseher et al, 2007[43]	Comparison (50/44 and 96/89)	NS, $P = .029$ in the 1st group
TIMP2	306C>T	Wang et al,[41] 1999	Comparison (84/51)	NS
TIMP2	573G>A	Wang et al (41), 1999	Comparison (84/51)	NS, $P = .0374$, for males with AAA
TIMP2	479C>T	Hinterseher et al (44), 2008	Comparison (50/41)	$P = .054$
TIMP2	rs2009196	Ogata et al (18), 2005	Comparison (387/425)	NS
TIMP3	−1296T>C	Ogata et al (18), 2005	Comparison (387/425)	NS

SNP: single nucleotide polymorphism
Abbreviations: AAA, abdominal aortic aneurysm; NS, not significant;

significant after gender was included in the analysis. The same study failed to disclose any significant interactions with polymorphisms in the genes of *MMP-1* (−1607G>GG), *MMP-12* (−82A>G), and *MMP-13* (-77A>G). Additionally, an observational study by Powell et al[39] failed to disclose an association between AAA growth rates and polymorphisms MMP-2 (−1306 C>T), MMP-3 (−1171 5A>6A), MMP-9 (−1562 C>T), MMP-12 (−82 A>G).

The activity of MMPs is largely modulated by TIMPs. Four TIMPs (TIMP-1, -2, -3, and -4) have been identified, and their expression is regulated during development and tissue remodeling.[40] Downregulation of TIMPs could theoretically lead to an increase in the activity of MMPs and therefore contribute to the pathogenesis of AAA, through increased proteolysis. Tilson et al in 1993[41] were the first to report a single nucleotide polymorphism (SNP) in the *TIMP-1* gene (C>T in the third nucleotide of codon 101) in 6 patients; however, they found that the amino acid for which it coded was actually preserved and the TIMP mRNA expressed in fibroblasts was not affected.

A study by Wang et al,[42] including 84 patients with an AAA and 51 healthy controls, showed no significant difference for 2 polymorphisms in the *TIMP-1* gene (+323C>T, +434C>T) and 2 polymorphisms of the *TIMP-2* gene (+306C>T, +573G>A). The association reached significance ($P = .0374$)

for male individuals with AAA, regarding the +573G>A polymorphism. Ogata et al[18] detected a significant association of 2 *TIMP-1* gene polymorphisms (nt+434, $P = .0047$; rs2070584, $P = .015$) in male participants with AAA without a relevant family history; however, the association was not significant outside this subgroup. Powell et al[39] investigated the effect of the SNP −372C>T in the promoter of TIMP-1 on the growth rate of small AAAs and found no significant associations in 455 patients with a small AAA (diameter < 4.0) followed-up over a period of 2.6 years. Hinterseher et al[43] recently investigated SNPs of the *TIMP-1* gene (the entire coding region and selected parts of the promoter of the gene) in a group of 50 patients with AAA and 44 controls. A second sample (96 patients and 89 controls) was subsequently investigated to confirm significant results. Three polymorphisms were identified, including a newly identified SNP at intron 4 (TIMP-1: 328 + 16C > T). A statistically significant difference in allele frequencies for SNP TIMP-1 372T>C was detected in the first arm of the study; however, this was not confirmed in the second sample. Another study by Hinterseher et al[44] investigated SNPs of the *TIMP-2* gene; 6 polymorphisms were studied, including 1 described for the first time (231 + 23C>T). The SNP −479C>T was more frequent in patients with AAA than in the control group ($P = .054$). Ogata et al[18] in their study did not find a significant interaction between the rs2009196 SNP in the *TIMP-2* gene and the −1296T>C SNP in the *TIMP-3* gene. The latter was associated with AAA when the country of origin was included in the analysis but did not prove an independent risk factor.

Table 1 summarizes the various polymorphisms in genes encoding MMPs and TIMPs and their association with AAA.

C-reactive protein

The relationship between CRP and outcomes from cardiovascular disease has been extensively investigated in several population and intervention studies.[45] Although the exact mechanisms remain unclear, there is some evidence that CRP may be directly implicated in various steps of the atherosclerotic process, including induction of nitric oxide, inhibition of fibrinolysis, and altered expression of adhesion molecules and complement function.[46,47] The potential association between CRP and AAA has not been addressed to the same extent; Powell et al were the first to suggest a possible relationship between CRP levels and AAA, when they compared 20 patients undergoing elective surgical repair of AAA to 20 controls with occlusive aortoiliac disease, demonstrating increased levels of CRP in patients with AAA.[48]

A more recent report has also shown that serum high-sensitivity CRP (hsCRP) levels are associated with aneurysmal size and that, although CRP is known to be produced mainly be hepatocytes, it may be manufactured by aneurysmal tissue as well. The authors conclude that CRP produced in vascular tissue might contribute to aneurysm formation.[49] Other reports have also shown that elevated hsCRP levels can be an independent marker of AAA disease.[50] Lastly, a recent meta-analysis of 6 studies that compared CRP concentration between patients with AAA and patients without AAA demonstrated significantly higher CRP concentration in the AAA group.[51]

Carlson et al performed a systematic survey of the common nucleotide variations across the CRP gene locus and identified 7 SNPs as functionally important (rs3093058, rs3091244, rs1417938, rs1800947, rs3093066, rs1205, and rs2808630). Haplotype analysis revealed that the most important single SNP was the triallelic rs3091244; the C allele being associated with a low level of CRP, and the A or T alleles being associated with a high level of CRP.[52] Badger et al[53] have studied the rs3091244 SNP in relation to AAA: CC was the most common SNP genotype but there was no statistical difference between cases and controls, and no difference in CRP levels was demonstrated for each genotype in the overall study cohort. The authors comment that their negative findings for the role of the rs3091244 in AAA may imply that the increased CRP production seen in AAA could represent a response to the disease process.[53]

Human Leukocyte Antigen System

The HLA system represents the major histocompatibility complex (MHC) in humans; it involves a large number of genes related to immune system function and resides on chromosome 6. It encodes cell surface molecules that present antigenic peptides to the T-cell receptor on T cells.

The most recent study investigating the role of HLA genes in relation to AAA involved 241 patients compared with 1000 healthy participants from the general population of Northern Ireland.[54] Human leukocyte antigens A, B, and DR were determined by polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes. The investigators failed to demonstrate a risk association between AAA and HLA alleles and no statistically significant difference was found in HLA alleles between those with small and those with large aneurysms (Table 2).

Rasmussen et al[63] in 1997 investigated the relation between inflammatory AAAs and HLA; 37 patients with an inflammatory AAA (identified macroscopically at operation) were genotyped for HLA-DR B1 and HLA-DQ B1 alleles and compared to 90 ethnically matched controls. The HLA-DR B1 alleles B1*15 and B1*0404 were associated with inflammatory AAA.[63] The same investigators assessed the role of HLA in both inflammatory and degenerative AAAs by genotyping 12 major alleles of the HLA-DR B1 locus in patients with degenerative (n = 102) and inflammatory (n = 40) AAA, compared with 118 healthy controls. They found that the distribution of the HLA-DR B1 alleles was similar in both AAA groups; the DR-B1*02 and DR-B1*04 alleles were associated with both degenerative and inflammatory AAAs.[59] A subsequent study by the same group showed that the HLA-DR B1*02 and B1*04 alleles were significantly associated with the disease, more than doubling the risk of AAA development. DR B1*01 was inversely associated with inflammation, suggesting it could have a protective role.[60] Moñux et al have also shown that HLA-DR B1*01 may possess

Table 2. Summary of the polymorphisms in major inflammatory genes (CRP, interleukins) and loci of the HLA System and Their Association With AAA

Gene	SNP/loci	Reference, year	Type of study (cases/controls)	Results
CRP	rs3091244	Badger et al, 2009[52]	comparison (248/400)	NS
IL-6	rs1800795	Smallwood et al, 2008[55]	comparison (677/656)	NS
IL-6	rs1800796	Smallwood et al, 2008[55]	comparison (677/656)	$P = .031$
IL-6	rs1800797	Smallwood et al, 2008[55]	comparison (677/656)	NS
IL-1β	IL-1β+3953	Bown et al, 2003[56]	comparison (100/100)	NS
IL-6	IL-6-174	Bown et al, 2003[56]	comparison (100/100)	NS
IL-10	IL-10-1082	Bown et al, 2003[56]	comparison (100/100)	$P = .03$ for the IL-10-1082 A allele
IL-10	L-10-592	Bown et al, 2003[56]	comparison (100/100)	NS
TNF-α	TNFα-308	Bown et al, 2003[56]	comparison (100/100)	NS
IL-1α	899C>T	Marculescu et al, 2005[57]	comparison (135/270)	NS
IL-1α	4845G>T	Marculescu et al, 2005[57]	comparison (135/270)	NS
IL-1β	−511C>T	Marculescu et al, 2005[57]	comparison (135/270)	NS
IL-1β	−31C>T	Marculescu et al[57], 2005	comparison (135/270)	NS
IL-1β	+3954C>T	Marculescu et al, 2005[57]	comparison (135/270)	NS
IL-RN	2018C>T	Marculescu et al, 2005[57]	comparison (135/270)	NS
HLA-A	HLA-A*02, *01, *11	Badger et al, 2007[54]	comparison (241/1000)	NS
HLA-A	HLA-A2	Sugimoto et al, 2003[58]	comparison (49/237)	$P = .036$
HLA-B	HLA-B61	Sugimoto et al, 2003[58]	comparison (49/237)	$P = .0018$
HLA-B	HLA-B*07, *08, *35, *40, *44	Badger et al, 2007[54]	comparison (241/1000)	NS
HLA-DR	HLA-DRB1*01, *03, *04, *07, *13, and *15	Badger et al, 2007[54]	comparison (241/1000)	NS
HLA-DR	HLA-DR B1*15, *0404	Rasmussen et al, 1997[63]	comparison (37/90)	$P < .05$ (inflammatory AAAs)
HLA-DR	HLA-DR B1*02, *04	Rasmussen et al, 2001[59]	comparison (142/118)	$P = .03$, $P = .08$ (inflammatory & degenerative AAAs)
HLA-DR	HLA-DR B1*02, *04	Rasmussen et al, 2002[60]	comparison (142/118)	$P = .007$
HLA-DR	HLA-DR B1*0401	Monux et al, 2003[61]	comparison (72/380)	$P = .02$
HLA-DR	HLA-DRB1, -DRB3-5	Ogata et al, 2006[62]	comparison (387/426)	NS
HLA-DQ	HLA-DQA1, -DQB1	Ogata et al, 2006[62]	comparison (387/426)	$P = .027$ for the HLA-DQA1*0102 allele

Abbreviations: SNP, single nucleotide polymorphism; AAA, abdominal aortic aneurysm; HLA, human leukocyte antigen; NS, not significant; IL, interleukin; CRP, C-reactive protein.

protective properties; however, their results did not reach statistical significance. They also detected a higher incidence of the allele subtype HLA-DR B1*0401 in patients with AAA (72 patients with AAA vs 380 controls).[61]

The *HLA-DRB1* gene has previously been associated with rheumatoid arthritis (RA), a disease with a strong autoimmune background and cardiovascular manifestations associated with intramural vascular inflammation.[64,65] More specifically, a strong genetic association of RA with particular HLA-DRB1 alleles (DRB1*0401, *0404, *0405, *0408, *0101, *1001, and *1402) has been observed by various investigators.[66,67] Increased cardiovascular mortality has been shown in RA patients carrying the HLA-DRB1*0404 allele.[67] Temporal arteritis, another type of vascular disease with a strong autoimmune component, has been linked with variants of HLA-DRB1*04.[68] These associations suggest that the *HLA-DRB1* gene may be linked to autoimmune mechanisms that accelerate intramural vascular inflammation, as seen in AAA, RA, and temporal arteritis.

Hirose et al performed HLA-DR typing in 46 Japanese patients with AAA and 50 healthy controls. The HLA-DR2[15] antigen was associated with AAA.[69] A more recent study including 49 Japanese patients with AAA, investigating 78 HLA genotypes of class I (HLA-A and -B) and class II (HLA-DR), showed that HLA-A2 and HLA-B61 genotypes were significantly more frequent in those with AAA.[58]

Ogata et al studied 387 AAA cases and 426 controls of Belgian and Canadian descent, analyzing HLA-DQA1, -DQB1, -DRB1, and -DRB3-5 alleles. They found a significant difference in the HLA-DQA1*0102 allele frequencies.[62] A marginally significant association was also found in haplotype analyses, regarding haplotype HLA-DQA1-DRB1 and AAA.[62] Table 2 provides a summary of the association of HLA loci and AAA.

Interleukins

Interleukins are a group of cytokines that regulate the immune response and have other pleiotropic functions. Cytokines

derived from the aneurysmal tissue are thought to be involved in the cycle of inflammation and proteolysis that is responsible for the formation of AAA. As a result, several studies have attempted to correlate the levels of circulating and tissue ILs with the development and progression of AAA. A recent study comparing the levels of inflammatory molecules in aneurysmal and atherosclerotic aortas has demonstrated enhanced expression and activation of proinflammatory transcription factors, accompanied by IL-6 and IL-8 hyperexpression in aneurysmal tissue.[70]

Interleukin 6 promotes systemic inflammation by inducing the acute phase response; IL-8 is also a major inflammatory cytokine, promoting neutrophil chemotaxis. Wallinder et al recently showed significantly higher levels of circulating IL-6 in patients with AAA compared to the general population. They also assessed the ratio of IL-6 to the anti-inflammatory cytokine IL-10 and showed that it was higher in patients with large compared to small AAA, indicating a proinflammatory response and a proinflammatory to antiinflammatory imbalance in patients with large AAAs.[71] Other investigators have also shown a correlation between aneurysm surface area and mean plasma IL-6 levels.[72] A significant imbalance between IL-6 and IL-10 levels has also been shown in a study measuring circulating cytokines in 99 patients with AAA and 100 patients that had undergone AAA repair in the past. There was a significant reduction in IL-10, and a nonsignificant trend toward reduction of IL-6 following surgical AAA repair.[73] Middleton et al[74] applied a 42-cytokine antibody-based protein array in 10 patients with AAA and 9 controls. Several proinflammatory cytokines were upregulated in AAA including IL-6, IL-1α, IL-1β, tumor necrosis factor α (TNF-α), TNF-β, and oncostatin M, as was also the anti-inflammatory cytokine IL-10.[74]

Various investigators have attempted to correlate the increase in proinflammatory cytokines seen in AAA with specific functional SNPs. Smallwood et al performed an association study involving 677 men with AAA and 656 age-matched controls, analyzing 3 variants in the IL-6 promoter region: IL-6-174G>C (rs1800795), IL-6-572G>C (rs1800796), and IL-6-597G>A (rs1800797). IL-6-572G>C polymorphism (frequency 1.5% in cases) was identified as an independent risk factor for AAA in the recessive model of the analysis; no association was seen in the additive or dominant models. The investigators concluded that the IL-6 572G>C polymorphism is too rare to be an important cause of AAA.[55]

Jones et al[75] investigated 466 patients with a small aneurysm to assess whether the −174 G>C polymorphism in the IL-6 promoter predicted survival; the frequency of the C allele was similar to that in a healthy population but patients of the GG genotype had lower plasma concentrations of IL-6 compared with the GC and CC genotypes. Cardiovascular and all-cause mortalities were lower for the GG genotype; however, there was no association between plasma IL-6 or IL-6 genotype and aneurysm growth.[75]

Bown et al determined plasma levels and genotypes for various cytokines (TNF-α, IL-1, IL-6, IL-10) in 135 consecutive patients undergoing open AAA repair. The presence of a G allele at the IL-6-174 locus (*IL-6* gene) was associated with a higher incidence of organ failure and an A allele at TNF-α-308 locus (*TNF*-αgene) with prolonged critical care stay.[76] The same investigators subsequently performed a case−control study including 100 AAA patients and 100 matched controls to assess the effect of polymorphisms in cytokine genes on AAA pathogenesis. The following loci were assessed in relation to AAA presence: IL-1β+3953, IL-6-174, IL-10-1082, IL-10-592, and TNF-α-308. The IL-10-1082 A allele was significantly commoner in the AAA group ($P = .03$).[56] A subsequent study included 389 patients with AAA and 404 healthy controls, investigating IL-10-1082 polymorphisms. The IL-10-1082 "A" allele was again commoner in the AAA group. However, the IL-10-1082 genotype was not independently associated with AAA if age, tobacco use, hypertension, and history of coronary or peripheral arterial disease were taken into account in regression analysis. The IL-10 A allele also did not have any effect on the growth of small AAA but IL-10 AA carriers had a trend toward lower plasma IL-10 levels.[77] These results suggest a potential role of the IL-10 -1082 A allele in AAA development.

Marculescu et al performed a matched case−control study investigating the association between 6 genetic variants in IL-1 and IL-1 receptor antagonist (IL-1 RA) with AAA. The distribution of 6 SNPs were compared between patients and controls by multivariable conditional logistic regression analysis: IL-1A -889C>T, IL-1A + 4845G>T, IL-1B-511C>T, IL-1B-31C>T, IL-1B + 3954C>T and IL-1RN + 2018 C>T; IL-1A-889C>T and IL-1B-31C>T were not eventually considered for further analyses. None of the remaining 4 polymorphisms showed a significant association with AAA (71). Table 2 summarizes the association between AAA and polymorphisms in genes coding the production of ILs.

β-Chemokine Receptors

Chemokines regulate every part of arterial remodeling. Chemokine functions vary considerably between native atherogenesis and nonatherogenic arterial remodeling. The largest family of cheokines is known as the CC chemokines because the first 2 of the 4 conserved cysteine residues lie adjacent to each other. CC chemokines are found at sites of chronic inflammation. Their main function is to attract mononuclear cells. Monocyte chemoattractant protein 1 (MCP-1), a CC chemokine, is a potent agonist for monocytes, memory T cells, and basophils and has been implicated in the recruitment of monocytes into early atherosclerotic lesions, the development of intimal hyperplasia after angioplasty, as well as in vasculogenesis and in aspects of thrombosis. Other members of the CC family include RANTES or CCL5 (Regulated on Activation Normal T-cell Expressed and Secreted), macrophage inflammatory protein 1 (MIP-1), and MIP-1β (CCL4).

CC chemokine receptors (or β-chemokine receptors) are membrane proteins that specifically bind to cytokines of the CC chemokine family; 10 members of the CC chemokine receptor subfamily have been described, named CCR1 to CCR10.

CCR2 has been identified on the surface of monocytes, activated memory T cells, B cells, and basophils.[78] CCR5 is also expressed on several cells involved in the immune response. The natural chemokine ligands that bind to this receptor are RANTES, MIP-1α, and MIP-1β.

The gene encoding CCR5 is associated with a 32-base pair deletion (Delta32 polymorphism) which has been shown to affect CCR5 expression.[79] A recent case−control study including 285 patients with AAA and 273 controls failed to disclose an association between this polymorphism and AAA development, even though the polymorphism was again found to affect the expression of CCR5. A previous study including 70 patients undergoing surgical repair of AAA (21 ruptured AAAs and 49 elective repairs), 76 patients with peripheral arterial disease, 62 patients with carotid artery stenosis, and 172 matched controls, concluded that the Delta 32 polymorphisms was an independent risk factor for AAA, and is commoner in patients with a ruptured AAA.[80] Further analyses are necessary to determine the exact role of the Delta 32 polymorphisms, as it has been shown to indeed affect the expression of the molecule but data regarding its role in AAA development are contradictory.

Transforming Growth Factor β

Transforming growth factor β (TGF-β) is a protein involved in a large number of diverse functions in various cell types. TGF-β exists in 3 known subtypes, TGF-β1, TGF-β2, and TGF-β3. These are upregulated in Marfan syndrome[81] and have a dominant role in tissue regeneration, cell differentiation, apoptosis, motility, and regulation of the immune system. TGF-β has deleterious effects on vascular smooth muscle development and the integrity of the extracellular matrix. In normal individuals, fibrillin 1 is known to bind with TGF-β; the mutated fibrillin in patients with Marfan syndrome cannot bind to TGF-β, therefore leading to increased levels of TGF-β, affecting the aortic tissue and leading to the dilatation of the aorta seen in Marfan patients.

Thompson et al[82] recently studied 4 geographically distinct cohorts, totaling 1890 AAA cases and 3785 controls to assess 26 SNPs in 5 genes related with the expression of TGF-β and latent TGF-β binding proteins (LTBPs), a family of proteins that regulate TGF-β bioavailability.[83] No associations between genotypes or haplotypes and the presence of AAA disease were confirmed. Meta-analysis of the results in the 4 groups for AAA size and growth rates in aneurysms ≥45 mm in diameter, demonstrated a significant association with the LTBP4 21011A>T genotype (a 2% decrease in AAA diameter, or a 0.53 mm/year reduction in AAA growth rate, per T allele; $P = .03$, $P = .01$).

Baas et al[84] recently assessed the role of TGF-β polymorphisms in a study which analyzed all the common genetic variants in TGF-β receptors 1 and 2 (TGF-βR1 and TGF-βR2), using a 2-stage genotyping approach. A total of 736 cases and 1024 controls were included; a significant association between AAA presence and the following polymorphisms was observed: TGF-βR1 rs1626340, TGF-βR2 rs1036095, and rs4522809. Golledge et al[85] also investigated the association between TGF-βR1 and TGF-βR2 SNPs and serum TGF-β1 levels as well as AAA; serum concentrations of TGF-β1 and 58 SNPs in the *TGF-β1* and *TGF-β2* genes were determined in 2 groups consisting of 1003 and 1711 men, all of whom had taken part in the Health in Men Study, a population-based randomized trial of screening for AAA conducted in Perth, Western Australia in 1996-1999. Validation of the SNPs was examined in a second cohort of 1043 individuals from New Zealand, of whom 654 had an AAA. Serum TGF-β1 levels and the polymorphisms in TGF-βRs were not associated with AAA; the TGF-βR2 g.42917C > T SNP (rs1078985CC) was associated with AAA presence but this was not confirmed in the New Zealand group.

Lucarini et al[86] have demonstrated an interaction between angiotensin-converting enzyme insertion/deletion (ACE I/D) and TGFbetaR1 9A6A polymorphisms in predisposing to AAA, but no relation was found between AAA presence and the TGFBR1 9A6A polymorphism in isolation. In conclusion, the fairly strong interest shown recently by various investigators in determining a causal relationship between polymorphisms of the TGF-β family genes and AAA has practically failed to demonstrate any significant interactions in well-designed large case control studies involving ethnically diverse populations.

Angiotensin-Converting Enzyme

Tissue and plasma ACE levels have been shown to be under genetic control.[87,88] Approximately 50% of ACE plasma levels is thought to associate with the *ACE* gene insertion/deletion (ACE I/D) polymorphism[87]—an insertion polymorphism of 287 base pairs within the *ACE* gene. ACE D allele carriers have higher ACE plasma[88] and tissue[89] levels compared with the ID and II genotypes; plasma concentrations of ACE in DD homozygotes are approximately twice as high as in II homozygotes.[87] The increased ACE plasma levels subsequently affect the angiotensin II levels, which may lead to the vascular tissue remodeling and increased susceptibility to atherosclerosis.[90] Cambien et al[91] were the first to report an association of the ACE I/D polymorphism with coronary artery disease and myocardial infarction. The ACE I/D polymorphism has also been studied in relation to AAA disease on various occasions.

Pola et al[92] reported a significant increase of the D allele in normotensive patients with AAA ($n = 56$) compared to matched controls ($n = 112$), in an Italian population. Interestingly, this association was not present between hypertensive AAA patients and normotensive controls. Hamano et al[93] reported no statistical difference in 125 patients with AAA and 153 controls matched for age, sex, and cardiovascular risk factors regarding the ACE deletion polymorphism. Fatini et al[94] demonstrated a strong association between the ACE DD genotype and AAA presence (250 AAAs compared with 250 healthy controls): multivariate analysis (adjusted for age, sex, and traditional vascular risk factors) indicated that the ACE DD genotype was independently related to AAA. Another Italian case−control study assessed the role of the TGFβR1

9A6A polymorphism as a risk factor for developing AAA in isolation and in conjuction with the presence of the ACE DD genotype (201 participants with AAA versus 252 healthy controls). The ACE DD allele was associated with AAA even after adjusting for age, gender, and traditional cardiovascular risk factors, whereas the synchronous presence of ACE DD genotype and TGFβR1 6A allele, significantly increased the predisposition to AAA.[86] More recently, Korcz et al[95] investigated 829 individuals divided into 4 distinct groups (AAA [n = 133], aortoiliac occlusive disease [n = 152], controls [n = 152], and a random group of Polish individuals [n = 392]). The *ACE* I/D gene polymorphism was susequently analyzed: genotype distribution and allele frequency were not significantly different between patients with AAA or aortoiliac occlusive disease and the control, or the population group. Significant differences were found between hypertensive patients with AAA and normotensive patients with AAA as well as hypertensive patients with AAA and the random population group. There was no significant association of the *ACE* gene polymorphism with hypertension in aortoiliac occlusive disease patients.

The currently available data regarding the relation between the ACE I/D polymorphism and AAA are largely contradictory. Thompson et al[32] conducted a meta-analysis of the studies by Pola, Hamano, and Fatini, showing a strong association between the ACE D allele and AAA; however, the studies included in the meta-analysis did not involve a large number of participants and a more recent report by Korcz et al[95] failed to disclose a significant association. Despite that, ACE I/D polymorphism does affect the expression of ACE and further analyses are warranted to identify the exact role it may possess in AAA development.

Platelet-Activating Factor

Platelet-activating factor is a phospholipid activator and mediator of various leukocyte functions, involved in platelet aggregation, and inflammation, produced by neutrophils, basophils, platelets, and endothelial cells.[96] Platelet-activating factor acetylhydrolase (PAF-AH) catalyzes PAF, inhibiting its proinflammatory functions.[97] Platelet-activating factor has been shown to increase the activity of MMPs and also act as an inflammatory mediator for the migration of macrophages and lymphocytes to the vascular wall.[97] As a result, investigators have attempted to examine the association between PAF and AAA, a disease where proteolysis and vascular inflammation, mediated by macrophages and lymphocytes, is crucial.

Unno et al[97] investigated a missense mutation (−994G >T) in exon 9 of the *PAF-AH* gene in a case−control study including 131 patients with AAA and 106 controls matched for age and sex; plasma PAF-AH activity was also measured. The T allele was significantly higher in the AAA group and the association between the polymorphism and AAA proved independent of other risk factors in the subsequent analysis. Plasma PAF-AH activity was strongly correlated to the plasma concentration of low-density lipoprotein cholesterol in homozygotes, whereas individuals bearing the T allele with co-existent hyperlipidemia were more prone to cardiovascular morbidity.[96]

Plasminogen Activator Inhibitor 1

Plasminogen activator inhibitor 1, a serine protease inhibitor protein (SERPINE1), is a potent inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA), which, in turn, act as activators of plasminogen and fibrinolysis.[98,99] Plasminogen activator inhibitor 1 has also been shown to act as an inhibitor of the activation of inactive MMPs into active proteases,[100] therefore it may lead to degradation of the vascular wall.

Sofi et al[101] studied 438 patients with AAA, and 438 healthy participants and measured levels of homocysteine, lipoprotein a, PAI-1, anticardiolipin antibodies, the prevalence of the MTHFR C677T polymorphism, the prothrombin gene G20210A variant, and factor V Leiden mutation. Plasminogen activator inhibitor 1 levels were significantly higher in the AAA group and were associated with AAA even after adjusting for cardiovascular risk factors ($P < .0001$). There is a common deletion/insertion polymorphism known as 4G/5G in the promoter region of the PAI-1 gene. The 5G allele is slightly less transcriptionally active than the 4G allele.[98,99]

Jones et al[100] attempted to identify whether the 4G/5G polymorphism influences the natural history of AAA in 400 patients diagnosed with small AAAs, undergoing serial measurements of AAA diameter. The frequency of the 3 genotypes (4G4G, 4G5G, and 5G5G) among the group of these patients was similar to that in a healthy population. They conclude that the 4G/5G variant does not correlate with AAA development but the 5G5G genotype is associated with faster AAA growth and appears protective following open AAA repair. Yoon et al[37] in their study involving 47 AAA patients from Finland, besides determining *MMP-3* and *MMP-9* genotypes also evaluated the prevalence of the PAI-1 4G/5G polymorphism. No significant association with AAA was demonstrated. Additionally, Eriksson et al[17] in their comprehensive study of the effect of various polymorphisms of the *MMP-2, MMP-3, MMP-9*, and *MMP-12* genes also evaluated the effect of the PAI-1 4G/5G variants. Patients with 4G4G, 4G5G, and 5G5G genotypes had growth rates of 3.18, 2.92, and 3.47 mm/year, respectively, for aneurysms with a baseline diameter of 45.1, 44.6, and 46.2 mm; however, the increased growth rate for patients with PAI-1 5G5G genotype was not statistically significant, but they did have the lowest plasma PAI-1 concentrations. Lastly, Powell et al[39] tested the effect of the PAI-1 −675 4G>5G and −847 A>G polymorphisms on AAA growth (455 patients with an AAA <4.0 cm in diameter, all from the UK small aneurysm trial), demonstrating a significantly higher annual growth rate for PAI-1 −675/847 5G5G/GG patients, only when the 2 polymorphisms were assessed together; none of the polymorphisms tested in this study had an effect on AAA growth rates in isolation. The 4G/5G polymorphism, even though it has been shown to affect the expression of PAI-1, does not correlate with AAA development in well-designed case−control studies.

Endothelial Nitric Oxide synthetase

Nitric oxide (NO) activates guanylate cyclase, which in turn induces smooth muscle relaxation; endothelial NO synthetase

(eNOS) generates NO in blood vessels and therefore regulates vasodilation. There is evidence to suggest that NO modulates vascular disease and maintains endothelial function and an antithrombotic intravascular environment. Nitric oxide generation is depressed in blood vessels affected by atherosclerosis as well as in blood vessels exposed to atherosclerotic risk factors.[102] An experimental study in a murine model[102] demonstrated that the eNOS deficiency is able to change the disease pattern of atherosclerosis, thus determining peripheral arterial disease (PAD), myocardial ischemia, and vascular complications, such as aortic dissection and AAA formation. A study in eNOS knockout mice showed that NO is a major regulator of vessel reorganization in response to a remodeling stimulus and suggests that a primary defect in NOS/NO pathway can promote abnormal remodeling and may facilitate pathologic changes in vessel wall morphology.[103]

Kotani et al[102] investigated the frequency of a polymorphism in intron 4 of the *eNOS* gene in 58 patients with AAA and 410 healthy controls. Two alleles of the *eNOS* gene, containing 4 (a-allele) and 5 (b-allele) repeats, were studied. The a-allele frequency predicted the need of surgery for AAA. Fatini et al[104] investigated the *eNOS* gene T-786C, G894T, and 4a/4b polymorphisms in relation to AAA. Their cohort consisted of 250 consecutive patients with AAA compared with 250 healthy participants, with no previous history of vascular disease. They documented a significant difference in genotype distribution and allele frequency for eNOS G894T polymorphism, even when patients with other atherosclerotic risk factors were excluded from the analysis. Atli et al[105] recently investigated the eNOS G894 homozygote T>T genotype polymorphism and 894T allele frequency in 61 patients with AAA and 62 controls and showed that the eNOS G894 T>T polymorphism and 894T allele frequency were significantly higher in the AAA group ($P = .01$ and $P = .03$, respectively) and were in fact associated with larger AAAs. There appears to be an association with the eNOS G894 T>T polymorphism and AAA; however, both of the 2 studies demonstrating this association included a small number of participants in each group. Further studies are needed to confirm this association.

Osteopontin and Osteoprotegerin

Osteopontin was originally identified in osteoblasts, acting as a mineralization-modulatory protein of the matrix. However, OPN has been shown to be upregulated in various acute and chronic inflammatory conditions, such as wound healing, fibrosis, autoimmune disease, and atherosclerosis. Its expression is interestingly enhanced in activated T-lymphocytes in response to certain infections.[106] Osteopontin is also expressed at atherosclerotic sites, thought to inhibit calcification and induce decalcification, constituting a proinflammatory and proatherogenic molecule[107]

Buemmer et al[108] studied aneurysm formation in OPN and apolipoprotein E (ApoE)-deficient mice and proved that angiotensin II-induced AAA formation in ApoE−/−OPN−/− mice was reduced and associated with decreased MMP-2 and MMP-9 activity compared with ApoE−/−OPN+/+, ApoE−/−OPN+/− mice, suggesting a possible role for leukocyte-derived OPN in mediating ang II-accelerated AAA formation (101).

Golledge et al examined OPN genotypes in 4227 participants in which aortic diameter and relevant clinical risk factors were documented. Serum OPN was measured in 2 groups of 665 participants. The concentration of serum OPN was independently associated with the presence of AAA, and initial serum OPN predicted AAA growth after adjustment for other risk factors in a subgroup of 198 patients with sufficient 3-year follow-up. The concentration of OPN in the aortic wall was greater in patients with AAAs measuring <50 mm in diameter than those with aortic occlusive disease alone. They also assessed 5 distinct SNPs of the *OPN* gene that had been previously investigated in relation to other autoimmune processes, such as lupus erythematosus (rs4754, location: exon 6; rs9138, location: 3-UTR; rs11226616, location: exon 7; rs1126772, location: 3-UTR; and rs11730582, location: promoter) but found no association between these SNPs and aortic diameter or AAA expansion.[109]

Osteoprotegerin is a glycoprotein that belongs to the TNF family of proteins. Apart from its role as inhibitor of bone resorption, OPG acts as a regulatory molecule in vascular disease and is expressed in coronary smooth muscle and endothelial cells, where it possesses anti-apoptotic properties on the endothelium.[110] Osteoprotegerin-deficient mice are known to develop medial arterial calcification of the renal arteries and the aorta.[111] Kim et al recently showed that OPG levels are correlated with inflammation and arterial stiffness.[112]

Moran et al[113] recently demonstrated high concentrations of OPG in biopsies of AAA and documented that ligation of the nuclear receptor peroxisome proliferator−activated receptor γ (PPARgamma) downregulates OPG in vitro and in a relevant mouse model. They subsequently studied a population of 4227 men, 699 of whom with AAA, to assess the associations between circulating concentrations of OPG, polymorphisms of the gene encoding PPARgamma (*PPARG*), AAA presence, and AAA growth. The PPARG c.1347C>T polymorphism was associated with higher plasma concentrations of OPG and increased AAA growth; the PPARG c.34G>C polymorphism was weakly associated with the presence of AAA.

Our literature search has not disclosed further studies investigating SNPs associated with OPG and AAA.

Methylene Tetra Hydro Folate Reductase

Methylene Tetra Hydro Folate Reductase (MTHFR) is an enzyme that irreversibly reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; 5-methyltetrahydrofolate converts homocysteine to methionine. Homocysteine is an amino acid involved in carbon metabolism and methylation reactions; high levels are toxic to vascular tissues. Hyperhomocysteinaemia has been reported to constitute a risk factor for vascular disease but a causal relationship between hyperhomocysteinaemia and AAA has not been documented in our literature search.[114] *MTHFR* is coded by a gene situated on chromosome 1 (p36.3). Various

polymorphisms have been associated within the *MTHFR* gene.[115,116] A polymorphism at the 677 locus involves substitution of a cytosine (677C) nucleotide for a thymidine (677T). The 677T allele (leading to a valine substitution at amino acid 222) leads to reduced enzyme activity and raised homocysteine levels.[117]

Strauss et al[118] used a group of 63 patients with AAA compared to 75 controls to demonstrate an increased 677T allele frequency among the AAA group, leading to a 4.4-fold greater risk of AAA development.[118] Jones et al[119] investigated 428 patients with AAA, 271 coronary artery disease patients, 226 vascular disease patients, and 282 controls who were genotyped for the C667T variants of *MTHFR* gene. They did not detect an association between the C677T polymorphism and AAA formation; however, they demonstrated significantly larger AAAs among homozygotes for the T allele compared with those bearing a C allele. A total of 3 studies from Italy[101,114,120] have also focused on the C667(C>T) variants, all demonstrating a higher frequency of the T alleles in AAA patients. Thompson et al[32] performed a meta-analysis of all 5 of the aforementioned studies, showing that the T allele does increase the risk of developing AAA (RR 1.14 [95% CI 1.08-1.21]). Giusti et al[121] recently studied 56 polymorphisms in 17 genes involved in methionine metabolism, using 423 AAA patients and 423 matched controls. When adjusted for cardiovascular risk factors and chronic obstructive pulmonary disease, rs8003379 MTHFD1 (odds ratio 0.41) and rs326118 MTRR (odds ratio 0.47) polymorphisms were independently associated with AAA. TT homozygotes for the 677C>T polymorphism had significantly ($P < .0001$) higher homocysteine levels compared to CT and CC participants; however, none of the haplotypes significantly associated with AAA led to increased homocysteine levels. The currently available data regarding the 677C/T polymorphism confirm that this is a functional polymorphism leading to higher homocysteine levels and may be related with AAA size and AAA development.

Whole-Genome Analyses

Whole-genome analysis has recently revealed various loci that may be related to AAA. Shibamura et al[122] identified 36 families from Northern America and Europe (of white ethnic origin) with at least 2 members with AAA and performed a whole-genome scan on 62 affected siblings pairs and 13 affected relatives pairs. They eventually identified 2 susceptibility loci for AAA on chromosomes 19q13 and 4q31. Another genome-wide study in families with AAA from the Netherlands,[123] also depicted a linkage to chromosome 19q, proximal to the region identified by Shibamura et al[122]; however, the 2 regions were not found to overlap. Ruigrok et al[124] in a systematic review of the 2 aforementioned studies state that the differences in methods used for analysis in the 2 studies (parametric vs nonparametric analysis and inclusion vs no inclusion of covariates) may have led to differences in the confidence intervals of maximum logarithm of the odds (LOD) scores, and it is likely that they point toward the same loci. A more recent study by Giusti et al[125] determined the gene expression profiles in pooled RNA in 10 patients with AAA and 10 matched controls, using arrays representing a total of 14 000 transcripts. Ninety-one (91) genes were differentially expressed in the AAA group. The results were confirmed by real-time PCR in another 36 patients with AAA and 36 controls. By applying gene ontology analysis, the investigators subsequently indicated an increased haemoglobin, monoglyceride lipase, and low-density lipoprotein receptor-related protein 5 gene expression, associated inversely with serum lipoprotein-a concentration. Choke et al[126] also applied a commercially available microarray to analyze rupture site and paired anterior sac biopsies of 10 ruptured AAAs. One hundred and thirty-nine (139) genes (123 upregulated and 16 downregulated) were differentially expressed at the AAA rupture site. Quantitative reverse transcriptase PCR (Q-RT-PCR) confirmed the findings in 21 of these, including IL-6 and IL-8, E-selectin, prostaglandin-endoperoxidase synthase 2 (COX2), and prokineticin 2 (PROK2). Another study[127] recently used microarray platforms, including 18 057 genes and Q-RT-PCR to eventually confirm overexpression of immune-function-related genes in AAA. The enriched categories included the Gene Ontology category Immune Response, and the Kyoto Encyclopedia of Genes and Genomes pathways natural killer cell-mediated cytotoxicity and leukocyte transendothelial migration categories.

Discussion

Abdominal aortic aneurysm is a multifactorial disease, and even though atherosclerosis and AAA appear to share similar risk factors, it is widely accepted that AAA is not the result of a strictly atherosclerotic process.[128] In fact, AAA represents a complex disease of the vasculature, with strong genetic susceptibility that leads to a phenotype strongly affected by various environmental factors. So far, the attempts to clarify the genetic basis of AAA have been on a small scale compared to the progress that has been made in other areas. There are a large number of limitations in most of the studies that have been published to date, which, subsequently, do not allow safe conclusions to be made, regarding the exact underlying genetic processes that lead to AAA formation. The majority of investigators have adopted a candidate gene approach, focusing mainly on genes encoding proteins of vital importance for the conservation of the structural integrity of the aortic wall (MMPs, TIMPs) and genes encoding products involved in the inflammatory cascade (CRP, HLA, ILs, ACE, MTHFR), as inflammation, proteolysis, and extracellular matrix degradation are the key processes in AAA formation. However, the variations in most of these genes have been largely underinvestigated, especially when one considers the amount of genetic-linkage research in fields not completely irrelevant to AAA, such as atherosclerosis and ischemic heart disease. The quality and size of the available studies to date is very variable and the populations that have been investigated are diverse, especially in terms of geographical distribution, making meta-analysis difficult.

A major drawback is the fact that AAA disease itself is problematic for candidate gene analysis; AAA genetic linkage studies are indeed susceptible to significant confounding influences as it is difficult to avoid the effect of common risk factors (age, hypertension, smoking, vascular disease), which may have genetic overlap with other disorders. This could be, partly, addressed by meticulous matching between the 2 different (cases and controls) arms of each study. Additionally, it is of vital importance to specifically address the effect of each confounding risk factor when attempting to make a causal relationship between a certain functional genetic variation and AAA in every study. Case matching in the studies included in this review has been variable; however, all investigators have assessed the traditional risk factors for atheromatosis in both arms of their studies. Other factors such as malignancy, infection, and co-existing inflammatory disorders have been omitted in a significant number of these studies.

A common limitation in the studies evaluating the effect of genetic variants on AAA is the way they addressed the issue of excluding AAA from control groups and families (familial aneurysm). Some investigators exclude a potential 5% falsely identified as not having AAA disease when imaging the aortas in their control groups, which may lead up to a 4.7% loss in sensitivity prior to any analysis.[32,129] Regarding the exclusion of familial cases of AAA, most investigators are based on history taking to assess whether a family member of an AAA patient has developed the disease. It would be logical to scan the immediate relatives using an easily available imaging technique; however, this would be costly and time-consuming.

Another important issue that needs to be addressed before making a conclusive assumption regarding the effect of a polymorphism on AAA is the so-called gene-dose effect. The majority of the available studies fail to assess the effect of a polymorphism on the actual product of the relevant gene. The human genome comprises a vast number of SNPs. In reality, a gene has multiple SNPs that regulate the expression of its product in a synergistic fashion; the impact of each SNP on isolation on the eventual product is, therefore, unlikely to be major. As a result, we should aim to assess the additive or subtractive effects of multiple SNPs, the effects of other genes, and, subsequently, environmental factors before making safe conclusions. This necessitates a large number of studies in different population groups, all of which will evaluate the effect of each SNP on translation and the eventual product.

Another commonly encountered issue is the more frequent publication of studies depicting positive associations. Publication should be encouraged regardless whether statistical significance has been reached or not, to allow for future meta-analysis.

In conclusion, genetic analysis in AAA has been dominated by candidate gene association studies, which have proven a significant association with a limited number of SNPs. The future remains open and significantly larger populations are necessary to validate the currently available results and assess alternative associations. Genome-wide association studies in a large number of individuals with a clear phenotype of AAA is the next level and it should, in conjunction with functional genomics, proteomics, and enhanced knowledge of the underlying pathogenetic processes of AAA, lead to the identification of very specific susceptibility genes. Until then, studies evaluating certain SNPs in well-characterized (phenotypically) participants and reporting results (either negative or positive) in a standardized manner constitute the main tool that can lead us to identifying the underlying genetic basis of AAA.

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