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Common polymorphisms of Fibulin-5 and the risk of abdominal aortic aneurysm development

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Abstract

Fibulin-5 is a crucial protein in the connective tissue structure of the aortic wall. The purpose of this study was to determine if genetic variation within the Fibulin-5 gene was associated with abdominal aortic aneurysms (AAA). AAA patients, with disease-free controls, were recruited and a past medical history questionnaire completed. Three single nucleotide polymorphisms (SNPs) in the *FBLN5* gene (rs2498834, rs2430366 and rs2254320) were genotyped. The two cohorts were compared and haplotype analysis performed. A total of 230 AAA cases and 278 controls were successfully genotyped. The mean age was 71.9 years (± 6.8). No difference between cases and controls was found in the distribution of alleles of *FBLN5* SNPs rs2498834 ($p = 0.47$), rs2430366 ($p = 0.45$) or rs2254320 ($p = 0.46$). Haplotype analysis did not reveal any significant difference. In conclusion, genetic variation within *FBLN5* is unlikely to play any role in the development of AAA.

Keywords

abdominal aortic aneurysm; Fibulin-5; genetic polymorphism

Introduction

The mechanical strength of the aortic wall lies in the elastic fibres, fibrillar collagens and associated proteins. These molecules form the integral scaffolding responsible for the viscoelastic properties of the aorta. The media is composed of elastin, collagen, smooth muscle cells and proteoglycans, which are targeted for enzymatic degradation over time.[1] Early in the formation of an abdominal aortic aneurysm (AAA) there is loss of elastic fibres, with fragmentation and decreased concentration of elastin; this is also found in subsequent growth and at the time of rupture.[2,3] This medial attenuation is crucial in the development of an aneurysm, but collagen degradation, with weakening of the adventitia, is ultimately responsible for rupture.[2] Serum elastin peptides have been noted to be elevated in patients who go on to rupture.[4] The latter is an imbalance of the dynamic ongoing process of synthesis and breakdown of collagen, with gradual loss demonstrated in AAA patients.[5]

Fibulin-5 is involved in normal elastogenesis, by inducing elastic fibre assembly and organizing tropoelastin.[6,7] It is part of a family of five extracellular matrix glycoproteins, where Fibulin-1 and 2 are much larger than the remaining three.[8] It is a 56 kDa modular calcium-binding protein that is predominately expressed in developing arteries.[9,10] It is minimally expressed in adult arteries, but production is enhanced in the presence of vascular injury or atherosclerosis.[10,11] Other roles of this protein include acting as a bridging peptide between blood vessel wall cell surface integrins and elastin fibres, regulating superoxide dismutase in blood vessels and also vascular cell signalling and migration.[6,7,12] Since Fibulin-5 expression has been shown to be enhanced in developing arteries it is important in angiogenesis.[13] However, angiogenesis is also an ongoing process in the aortic wall and, hence, may be vital in the pathogenesis of AAA.[14] Mice deficient of the Fibulin-5 gene display a dramatic reduction in mature elastic fibres.[15] Additional features of these mice are elastinopathy manifestations such as loose skin, severe emphysema and loss of compliance in blood vessels.[6,7] In humans with the gene mutations, cutis laxa and age-related macular degeneration are well recognized clinical manifestations.[16–18]

The importance of the sister-protein, Fibulin-4, in arterial disease has been demonstrated.[19] Reduced expression can lead to dilatation, tortuosity and stiffening of the ascending

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aorta. As a consequence of the disorganized elastic fibre networks, dissection of the aortic wall may be seen together with thickened aortic valvular leaflets. Further analysis suggests that genetic aberration in Fibulin-4 may be related to multiple aneurysm phenotypes.[19] Wang et al., in 2005, studied the effect of decreased expression of Fibulin-5 in vascular patients and found that patients with thoracic aortic dissection have lower Fibulin-5 mRNA and elastin content, relative to controls.[20] Similar to other reports, a strong correlation was found between decreased Fibulin-5 expression and decreased or disorganized elastin in intra-operative samples of the aortic wall, which is similar to other reports.[21] Thus, this decreased expression may lead to aortic dissection by impairing elastic fibre assembly.

The aim of this study was to determine if genetic variation within the *FBLN5* gene (which encodes Fibulin-5), found on the long arm of chromosome 14, is associated with the formation of AAA.

Patients and methods

Patient recruitment

The project received ethical approval from the Northern Ireland Regional Ethical Committee, while the Belfast City Hospital provided clinical indemnity and local sponsorship. All study participants gave written informed consent and completed a past medical history questionnaire. Patients known to have AAA (aortic diameter > 30 mm) were recruited from vascular outpatient clinics and the local screening programme. Participants who screened negative for the disease were recruited as controls. Neither cases nor controls were assessed for thoracic aortic aneurysmal development, since this is much less common and also a computerized tomography scan would be required for this screening. Such radiation exposure could not be justified. All cases and controls included in this study were of Caucasian origin, with no mixed ethnicity in the whole cohort.

Genetic analysis

FBLN5 extends over 79 kb, with 96% of the gene from the promoter to the latter part of intron 10 falling within a region of high linkage disequilibrium (LD). The selected single nucleotide polymorphisms (SNPs) (rs2498834, rs2430366 and rs2254320) within intron 4 were in strong LD with each other, and together allowed discrimination of the four common haplotypes in their region. These haplotypes showed strong LD ($D' = 0.8$) to the promoter region and lower LD ($D' = 0.6$) to the haplotype block starting at the distal end of intron 10. The haplotypes assessed therefore can be expected to reflect well any genetic variation from the promoter to intron 10, and less perfectly, variation in the small final exon and 3′ untranslated region of the gene. The SNPs were chosen to act as tagged markers of the haplotypes, rather than as functional polymorphisms that may alter expression levels, which was not assessed in this study. If a common coding mutation within the gene was resulting in AAA development, this type of haplotype tagging with the three SNPs would reveal the culprit, even if the representative SNPs were not the direct cause.

A 10-ml sample of whole blood was obtained from each AAA patient and control. DNA was extracted, using the 8LX Magtration Genomic DNA system (Precision System Science Co. Ltd, Mainz-Hechtshein, Germany) and stored at –80°C until analysis.

All DNA samples were diluted to a uniform polymerase chain reaction (PCR) concentration of 25 ng/µl. SNPs rs2498834, rs2430366 and rs2254320 were genotyped in the AAA cases and controls. Multiplex PCR was then carried out on the DNA to amplify the target regions according to standard protocols. The PCR product, containing the amplified genetic material, was then cleaned of excess multiplex reagents using ExoSapIT (GE Healthcare, Chalfont St Giles, UK). The next step was the SNaPshot reaction, which involved adding the SNP extension primers to the PCR product which then underwent the standard cycle according to the standard protocol, with the exception that the concentration of primers for extension was increased threefold. The SNaPshot reaction solution was then cleaned of excess reagents by the SAP (shrimp alkaline phosphatase) reaction and 1 µl of product was added to 12 µl formamide and 0.2 µl of LIZ120 size standard before analysis using an AB13100 DNA analyser. Data were analysed and genotypes called using the Genemarker software.

Statistical analysis

Age and aortic diameter were expressed as mean (± standard deviation). The proportion of patients in the cohorts of cases and controls for each risk factor was expressed as a percentage and compared using the chi-squared test. The cohort proportion for each genotype was also expressed as a percentage and compared using the chi-squared test. Our study had 85% power to detect a common allele conferring a genotype relative risk of 1.5 in heterozygous state and 2.25 in homozygous state, fully associated with any haplotype, at a significance value of $p = 0.05$. Statistical analysis was performed using SPSS (Version 12, SPSS Inc, Chicago, IL, USA).

When alleles at adjacent polymorphic sites are inherited in haplotypes rather than in a random fashion, LD gives a statistical measurement of their non-random association. LD between SNPs and haplotype calculations were performed using Haploview software.

Results

Demographics and past medical history

The overall mean age was 71.9 years (± 6.8) in AAA cases and 69.1 years (± 4.3) in the control cohort. There was a history, either formerly or presently, of smoking in 70% of the cases, compared to 57% in the controls. There were some differences between cases and controls in their risk

Table 1. Risk factors of AAA cases and controls

Risk factor	Case (%)	Control (%)	*p*-value
Ex-smoker	48.5	45.5	0.51
Current smoker	22.4	11.5	0.0007
Diabetes mellitus	10	14	0.14
Ischaemic heart disease (IHD)	46	43	0.0001
Myocardial infarction	26.8	21.3	0.07
Cerebrovascular accident (CVA)	6.4	7	0.90
Peripheral arterial disease (PAD)	27.2	23.8	0.29
Hypertension	45.6	43.8	0.49
Hypercholesterolaemia	32.8	30	0.32
Chronic obstructive airways disease	8.4	5.3	0.15
Family history of IHD	31.2	44	0.008
Family history of CVA	20.4	19.8	0.88
Family history of abdominal aortic aneurysms	18.4	7.5	< 0.0001
Family history of PAD	9.2	13.3	0.16

factors, with a greater number reporting either ischaemic heart disease or a family history thereof (Table 1). The mean size of AAA in the cases was 51 mm (± 16). The controls were all screened negative with an aortic diameter of < 30 mm, although an exact measurement was not recorded.

Three highly polymorphic SNPs, rs2498834, rs2430366 and rs2254320, within *FBLN5* were genotyped successfully in 230 cases (222 male) and 278 controls (268 male). The allele frequencies of each SNP were in Hardy–Weinberg equilibrium (rs2498834 $p = 0.47$; rs2430366 $p = 0.31$; rs2254320 $p = 0.99$). No significant difference was demonstrated between the genotype distribution in AAA cases and controls in rs2948834 ($p = 0.47$, Figure 1), rs2430366 ($p = 0.45$, Figure 2) or rs2254320 ($p = 0.46$, Figure 3). The genotypes obtained for each SNP were in Hardy–Weinberg equilibrium.

Haplotype analysis for FBLN5

As expected, the three SNPs studied showed a high degree of LD (91% between rs2498834 and rs2430366; 96% between rs2430366 and rs2254320; and 96% between rs2498834 and rs2254320). Seven haplotypes were identified in the combined population, as shown in Table 2. There was no significant difference between the AAA and control cohorts in their distribution of haplotypes (Table 3).

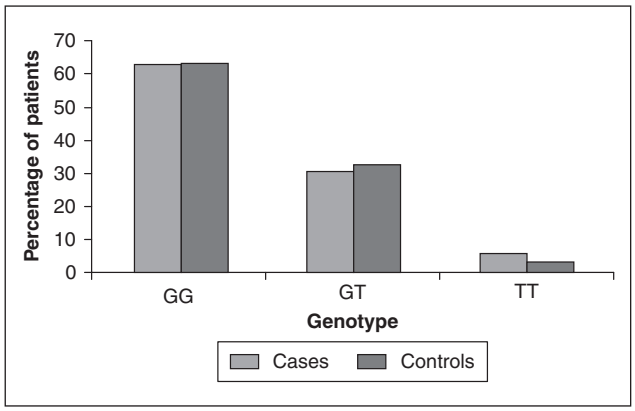

Figure 1. Genotype distribution for rs2948834 ($p = 0.47$).

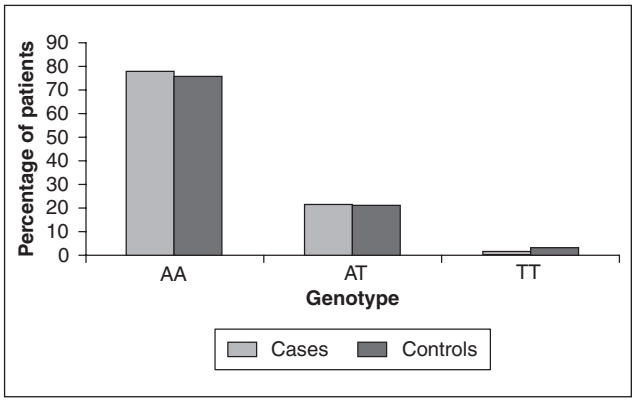

Figure 2. Genotype distribution for rs2430366 ($p = 0.45$).

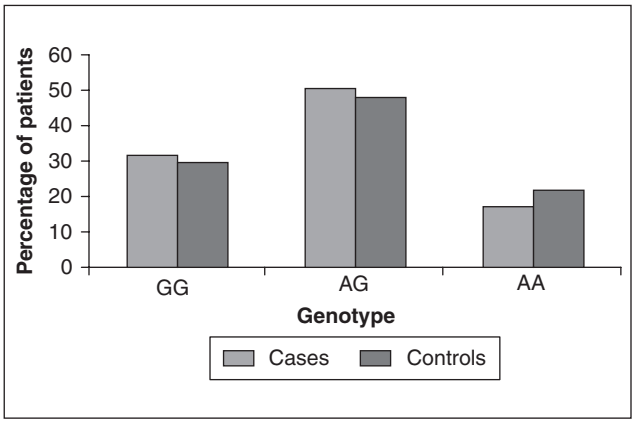

Figure 3. Genotype distribution for rs2254320 ($p = 0.46$).

Discussion

The stiffness of the aortic wall is a crucial factor in the development of vascular disease and is higher in men with an increase with age.[22] It is an independent predictor of cardiovascular mortality in hypertensive patients.[23,24] Aortic stiffness is determined by the extracellular matrix, elastin, collagen and fibrillin, with additional contribution from associated genetic factors. Matrix metalloproteinases (MMP) and other proteolytic enzymes promote the destruction of the elastic and collagen fibres and are elevated in patients with AAA.[3,25–27] Mouse models with targeted gene disruption of MMP-9 have been demonstrated to suppress the development of aneurysms.[28] The tissue inhibitors of matrix metalloproteinases (TIMP) are increased in the aortic wall, but the balance is in favour of proteolysis.[26,29,30]

Table 2. Distribution of *FBLN5* haplotypes in the overall population

Haplotype	Frequency
1,3,5	34.5
1,3,6	31.0
1,4,5	21.0
1,4,6	12.8
2,3,5	0.3
2,3,6	0.1
2,4,5	0.1

Table 3. Distribution of *FBLN5* haplotypes in the cases and controls

Haplotype	Case (%)	Control (%)	Chi-square	*p*-value
1,3,5	34.6	34.4	0.003	0.96
1,3,6	31.0	31.0	0.0	0.99
1,4,5	22.4	20.0	0.87	0.35
1,4,6	11.6	13.9	0.91	0.34
2,3,5	0.1	0.5	0.91	0.34
2,3,6	0.3	0.0	0.86	0.35
2,4,5	0.0	0.2	0.64	0.42

The elastic architecture of the aortic media is an essential component of the strength and stability of the aorta. The main protein for this is elastin, which is very stable, and has a half-life of about 70 years. It is largely produced in early childhood, with little synthesis in later life.[31,32] In the normal state, elastin exists in a continuous framework structure, made of elastin laminae and interlaminar fibres that interconnect the laminae.[33,34] Elastin degeneration characterizes aneurysmal formation with elastase and MMPs contributing towards elastolysis.[33,35–37] Fibulin-5 knockout mice have been demonstrated to have tortuosity and elongation of the aorta, particularly in the ascending aorta, resulting in the brachiocephalic trunk and left common carotid artery being juxtaposed.[6,7] In addition, homozygous mutant mice have disorganized elastic laminae, but no dissection or aneurysmal dilatation of the medial aortic layer.

The gene encoding Fibrillin-1 is abnormal in Marfan syndrome, with elevated pulse pressure and aortic dilatation.[38] The relationship between blood pressure and *FBN1* genotype has been demonstrated in terms of pulse pressure and diastolic pressure.[39,40] Fibulin-5 interacts with Fibrillin-1 in the pericellular space during elastic fibre assembly. The exact mechanism is unclear, but it may regulate the initial deposition of tropoelastin, the precursor of elastin, on microfibrils before cross-linking to Fibrillin-1.[41] It has been shown to bind to tropoelastin and ternary complexes of Fibulin-5; Fibrillin-1 and tropoelastin may bind microfibrils in the process of elastic fibre formation.[17,42]

This is the first study to consider the possible influence genetic variation within the gene encoding Fibulin-5 has on AAA development. Although there is increasing evidence emerging of its important role in elastogenesis, the results of this study indicate that the contribution of polymorphic variation may be negligible. There is no confirmed SNP within the gene, which is known to cause the sequence of the protein to be altered. Our study using haplotype-tagging SNPs has failed to find, by association, support for a common variant within the gene that affects risk of development of AAA. It is possible, however, that rare or sporadic mutations could be identified in a more rigorous assessment of the gene, or that expression may be affected by genetic variation at a distance from the gene itself. Although Fibulin-5 clearly plays an important role in maintaining the elasticity and strength of the aortic wall, the negative results of our study would suggest that it is not subject to common genetic variation. There is no evidence to suggest that the processes involved in atherosclerosis and aneurysmal formation are linked to Fibulin-5, even though they may share many of the other risk factors.

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Presentations

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