Association of functional MMP-2 gene variant with Intracranial Aneurysms: case-control genetic association study and meta-analysis

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Author Contributions

VSA, HH and DJW conceived and designed the study. VSA and XK performed statistical analysis. JG, SB, DCW, DB, NK helped with revision of the manuscript as well as recruiting patients for the study through their respective centres. VSA wrote the manuscript with support from all authors. HH and DJW were principal supervisors of the study.

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Abstract

Introduction: Abnormalities in Matrix Metalloproteinase (MMP) genes, which are important in extracellular matrix (ECM) maintenance and therefore arterial wall integrity are a plausible underlying mechanism of intracranial aneurysm (IA) formation, growth and subsequent rupture. We investigated whether the rs243865 C>T SNP (single nucleotide polymorphism) within the MMP-2 gene (which influences gene transcription) is associated with IA compared to matched controls.

Materials and Methods: We conducted a case-control genetic association study, adjusted for known IA risk factors (smoking and hypertension), in a UK Caucasian population of 1,409 patients with intracranial aneurysms (IA), and 1,290 matched controls, to determine the association of the rs243865 C>T functional MMP-2 gene SNP with IA (overall, and classified as ruptured and unruptured). We also undertook a meta-analysis of two previous studies examining this SNP.

Results: The rs243865 T allele was associated with IA presence in univariate (OR 1.18 [95% CI 1.04-1.33], p=0.01) and in multi-variable analyses adjusted for smoking and hypertension status (OR 1.16 [95% CI 1.01-1.35], p=0.042). Subgroup analysis demonstrated an association of the rs243865 SNP with ruptured IA (OR 1.18 [95% CI 1.03-1.34] p=0.017), but, not unruptured IA (OR 1.17 [95% CI 0.97-1.42], p=0.11).

Conclusions: Our study demonstrated an association between the functional MMP-2 rs243865 variant and IAs. Our findings suggest a genetic role for altered extracellular matrix integrity in the pathogenesis of IA development and rupture.

Keywords: Extracellular matrix, Genetics, Intracranial aneurysms, MMP-2, meta-analysis.

Introduction

Despite modern neurosurgical care, aneurysmal subarachnoid haemorrhage (SAH) remains a debilitating condition with a 50% mortality rate and significant morbidity in survivors.[1] However, factors which determine intracranial aneurysm (IA) formation, growth, and rupture require ongoing investigation.

Evidence suggests a genetic contribution to both the formation and rupture of sporadic IA through effects on arterial wall integrity or response to haemodynamic stress.[2-5] Evidence exists for variants related to genetic pathways regulating vascular endothelium integrity.[6] The family of matrix metalloproteinases (MMPs) are responsible for breakdown of extracellular matrix proteins (e.g. elastin, collagen and laminin) which are important in vascular remodelling in human aneurysmal and animal model tissue samples, so might contribute to IA pathogenesis.[7 8-12] MMPs are released by vascular endothelial, smooth muscle and inflammatory cells,[13] while increased expression of MMP-2 and 9 activity have been consistently shown within the walls of IAs,[7, 9-12, 14] with upregulation of MMP-2 in ruptured IA.[11, 12, 15, 16] The matrix metalloproteinase (MMP) - 2 rs243865 C>T single nucleotide polymorphism (SNP) is of interest because it can influence transcription factor Sp1 (Specificity protein), thereby regulating the specificity and level of MMP-2 gene transcription.[17] Previous studies on this SNP in different populations have given inconclusive results [13, 18]. We therefore undertook a genetic association study of the rs243865 SNP in patients with proven IA (including both ruptured and unruptured) in a large, prospective, multi-centre UK study. We also undertook a meta-analysis with all available published data on this SNP[13, 18] including our results, with stratification for population ethnicity.

Methods

We included participants as part of the Genetic and Observational Subarachnoid Haemorrhage (GOSH) study, a prospective, multicentre UK-wide study in 20 neurosurgical centres. We included patients with proven IA, including angiographically-proven ruptured (SAH) IA (irrespective of severity), and unruptured IA. Consent from subject(s) or nominated consultee(s) was obtained for all participants. We excluded patients with known inherited connective tissue disorders, such as; Marfan's, Ehlers-Danlos syndrome and adult polycystic kidney disease (ADPKD), and those with non-aneurysmal SAH (e.g. from arterio-venous malformations, trauma, mycotic aneurysms and peri-mesencephalic SAH where no aneurysm was detected). The GOSH study was ethically approved by the Central London REC 3 committee and funded by the Stroke Association.

We conducted our genetic association study according to the STREGA[19] (STrengthening the REporting of Genetic Association studies) framework. We obtained control subject DNA from the 1958 Wellcome cohort,[20] and 297 participants from the Queen Square Brain Bank, confirmed to have no IA on post-mortem reports. To minimise population stratification, we included only patients of European Caucasian ethnicity. DNA samples were extracted from participant blood samples. Polymerase Chain Reaction (PCR) was undertaken using KASP (KBioscience Competitive Allele-Specific) genotyping assays at LGC Genomics bio-laboratories. The KASP primer mix, which is assay-specific for the MMP-2 (rs243865) SNP, was mixed with a universal KASP master mix, then added to DNA samples. Following PCR thermal cycling, an end-point fluorescent read was conducted using labelled allele-specific forward primer dyes, to perform bi-allelic discrimination.

Statistical analysis was conducted using R and PLINK software. Chi-squared tests, with odds ratio calculations, were performed under an additive model. We investigated whether this SNP was differentially associated with IA according to rupture status. The effect of smoking and hypertension status on overall genetic association were examined in multi-variable regression analysis.

We searched all public databases including, PubMed, EMBASE and Google Scholar for articles in English and non-English (Figure 1), examining the genetic association between the MMP-2 (rs243865) SNP and IA using search terms including: intracranial aneurysms, MMP, Extracellular matrix, SNP, genes and genetics . We then performed a meta-analysis of all previously published studies[13, 18] on the MMP-2 (rs243865) SNP and our data, in accordance with the Human Genome Epidemiology (HuGE) Network guidelines[19] and following recommendations to improve the quality of meta-analyses of genetic association studies.[21] We included studies reporting either odds ratios with confidence intervals under an additive genetic model, or those with genotype counts. Pooled odds ratios (ORs) with confidence intervals were calculated under a fixed effects (FE) model. Inter-study heterogeneity was assessed using the Higgins I^2 statistic. Publication bias was evaluated by using the Egger regression asymmetry test and visualisation of funnel plots. Comprehensive Meta-Analysis (2.2) software from Biostat (Tampa, FL) was used to create the forest plots, perform sensitivity and publication bias analysis. We also performed a meta-analysis on sub-group data in Caucasian populations only. Controls were checked for Hardy-Weinberg Equilibrium (HWE) in all studies.

Results

In the GOSH study, 1609 cases and 1299 controls were genotyped. Quality control for genotyping errors was less than 1%. After exclusion of non-Caucasian samples, we included 1409 cases and 1290 controls in our genetic association statistical analysis. Controls were in HWE (p=0.5004). Basic demographic data for cases and controls are presented in Table 1. Allele frequencies and genetic associations for the rs243865 C>T SNP are shown in Table 2. Comparing the whole IA population (n= 1409) to controls (n= 1290), the rs243865 C>T SNP was associated with IA status (OR 1.18 [1.04-1.33], p=0.01) under an additive genetic model (see Table 2). Sub-group analyses demonstrated a statistically significant (1.18 [1.03-1.34), p=0.017) association of the MMP-2 SNP with ruptured IA (n= 1074), but not with unruptured IA (n= 335) compared with controls (see Table 2).

In multi-variable logistic regression, including hypertension and smoking, the rs243865 C>T SNP remained associated with IA (see Table 3). Moreover, there was no statistical interaction between hypertension (p=0.5), smoking (p=0.2) and genetic association with the MMP-2 SNP. Gender did not have a modifying effect on genetic association with IAs, as expected, (OR 1.15 [0.98-1.37], p=0.084).

A meta-analysis (Figure 2) of our data with all previously published studies[13, 18] (3,584 cases and 3,359 controls) on the rs243865 C>T SNP confirmed a statistically significant association with IA (Fixed effects OR 1.12 [95% CI 1.01-1.23], p=0.03), in a univariate model. There was no significant publication bias (Egger p-value=0.76) nor statistical heterogeneity (p=0.32, I^2=13%), but only our study showed a statistically significant association between this SNP and IA presence. Sub-group analysis including only Caucasian participants (Figure 3) with IA[13] confirmed an

association of the rs243865 C>T SNP with IA (OR 1.174 [95% CI 1.042-1.323], p=0.008). There was no significant publication bias (Egger p-value=0.78) nor statistical heterogeneity (p=0.768, I^2=0%).

Discussion

Our results demonstrate an association of IA with T allele carriers of the MMP-2 rs243865 SNP under an additive genetic model (OR 1.18 [1.04-1.33], p=0.01). We found only two previous studies examining this SNP with presence of IA, which provided inconclusive results. The first study analysed 125 cases against 234 controls in a US Caucasian population.[13] The second study was conducted in 2050 cases against 1835 controls in a Japanese population.[18] Our study had a larger sample of participants with IA, so had greater statistical power to detect an association.

Our results are consistent with the hypothesis that the MMP-2 rs243865 SNP affects MMP-2 activity, thus influencing vascular remodelling and IA formation, growth or rupture. Evidence by Bruno[7] *et al.* demonstrated increased activity of MMP-2 in unruptured aneurysms when compared to control arteries, with focal areas of remodelling within the aneurysm wall. These findings may imply dysregulation of MMP-2 activity in aneurysmal disease as part of vascular remodelling.
This genetic variant is functional, leading to altered MMP-2 gene promoter activity, through its effect on transcription factor Sp1 (Specificity protein) which is ubiquitously expressed regulating several genes in an inducible or constitutive manner by binding to GC/GT-rich elements. Sp1 is multi-functional and can bend DNA thereby inhibiting the action of other promoter sites within the MMP-2 gene or affecting tissue-specific expression. The MMP-2 polymorphism (C>T) can abolish Sp1 binding, affecting specificity and level of gene transcription. Sp1 can also act as a

transcriptional activator by recruiting and combining with other transcription factors, but can also serve as a repressor, depending upon which promoter site it binds to.[17] By affecting promoter activity in this way, deleterious effects on MMP-2 expression could lead to an imbalance of ECM regulatory elements, potentially leading to development or rupture of intracranial aneurysms. Interestingly, overexpression of Sp1 has been shown to induce apoptosis in tumour cells,[22] which is also a key pre-terminal event in IA rupture.[23]

The C>T polymorphism has been shown to reduce MMP-2 transcription activity as a result of abolishing Sp1 binding,[24] which might disrupt homeostatic mechanisms involved in vessel wall integrity and remodelling in response to chronic haemodynamic stress.

The finding of an association between the rs243865 SNP and ruptured but not unruptured IA is interesting but remains hypothesis-generating; the OR in each group was similar, and the lack of statistical association might simply reflect the smaller number of patients with unruptured IA included in our study (335 unruptured IA v 1079 ruptured IA). Further studies including larger number of participants with unruptured IA will be helpful in assessing whether the association of the rs243865 SNP is stronger for ruptured than unruptured IA. Nevertheless, questions remain as to whether unregulated MMP-2 activation is responsible for rupture of IAs. This has been supported by Jin[14] *et al.* who demonstrated increased MMP-2 expression in the walls of ruptured compared to unruptured aneurysms.

Caution needs to be taken when interpreting the meta-analysis, as this was dominated by data from our study, which was much larger than the only previous report from a Caucasian population.[13] Ours was the only individual study to show a significant association with IA, which may reflect

differing frequencies of this SNP in different ethnicities. Our study was restricted to European Caucasian patients and may not be generalizable to other populations.

A limitation of our study is that we did not sequence the entire MMP-2 gene including the promoter region. It is not clear whether the rs243865 SNP is in strong linkage disequilibrium (LD) with other as yet unknown variants within the MMP-2 gene that could increase the risk of IA development.

We also note that the rs243865 SNP was not detected in previous GWA (genome-wide association) studies on IAs.[2-4, 25, 26] However, it is unclear if this was a limitation of the technology or the number of SNPs examined using the genotyping arrays available at that time. It may be possible this SNP was examined and may have reached statistical significance, but not GWAS level of significance ($p<5x10^{8}$). This can only be known if authors of the previous GWA studies reveal genotype results for this SNP or SNPs in linkage disequilibrium within the region. Despite this drawback, candidate-gene association studies and sequencing of entire gene(s) are still required to define which SNPs in a GWAS peak are the causal variants for the disease.

Conclusion

In our large, prospective multi-centre UK study, we demonstrated association of the MMP-2 rs243865 C>T variant with IA in a UK Caucasian population and in a meta-analysis with other available data. Our findings provide genetic evidence for the involvement of MMP-related ECM regulatory pathways in IA. Future large studies investigating MMP genes are warranted in different ethnicities, stratified by IA rupture status, to definitively determine their contribution to IA pathogenesis.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest.

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Table 1. Characteristics of cases and controls

	Cases (IA)	**Controls**
Number	1409	1290
Female (%)	962 (68%)	636 (49%)
Mean Age (years)	54.1	57.7
Current smokers (%)	621 (44%)	258 (20%)
Hypertensive (%)	448 (32%)	191 (15%)
Ruptured IA (%)	1074 (76%)	-

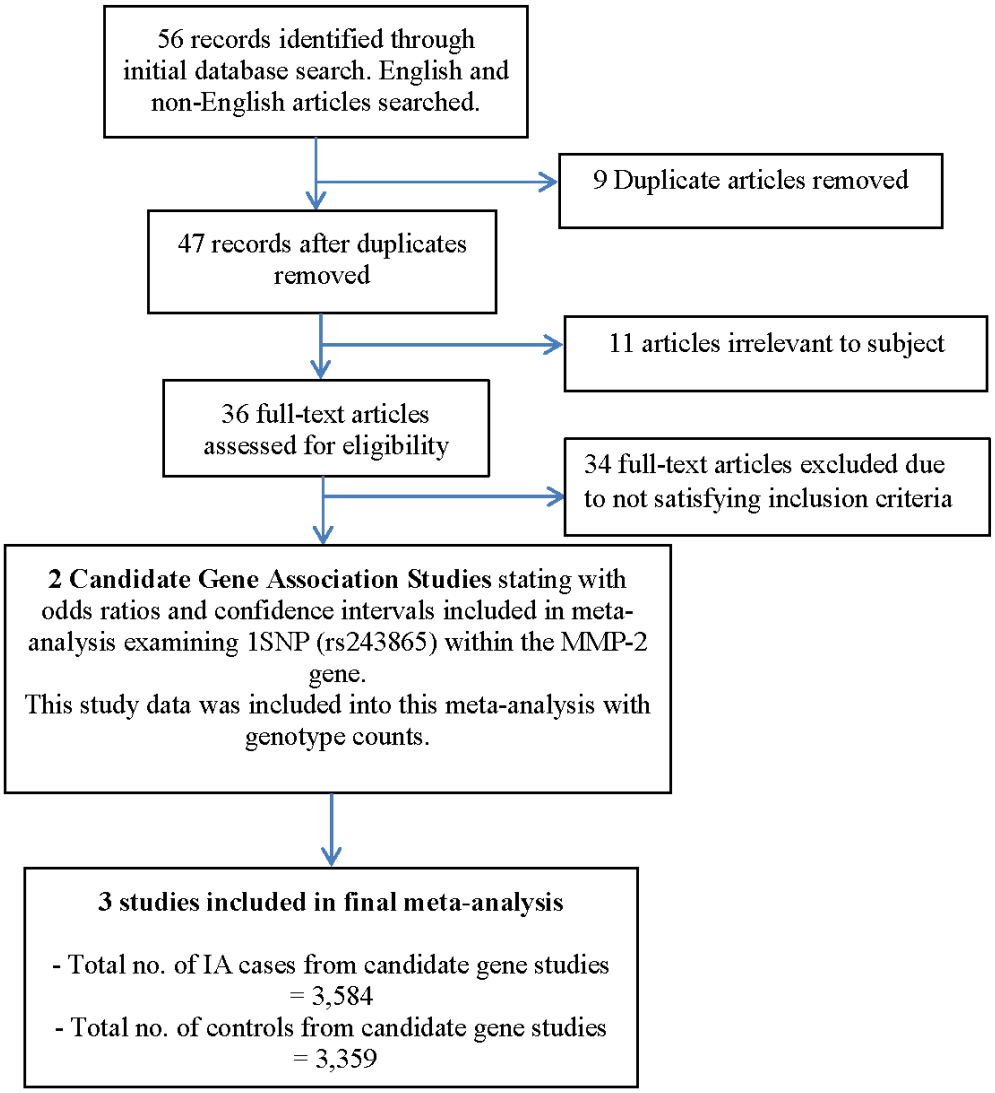
Table 2. Allele frequencies and associations with intracranial aneurysms (IA) for the rs243865 C>T SNP

	Cases (IA)	**Controls**
CC	748	758
CT	573	456
TT	88	76
C Allele	2069 (0.73)	1972 (0.76)
T Allele	749 (0.27)	608 (0.24)
	Association tests (Additive model)	
	OR [95%CI]	**p-value**
All IA (n=1409)	1.18 [1.04-1.33]	0.01
Ruptured IA (n=1074)	1.18 [1.03-1.34]	0.017
Unruptured IA (n=335)	1.17 [0.97-1.43]	0.106

Table 3. Associations of known IA risk factors and the rs243865 C>T SNP in all IA

Variable	**OR [CI]**	**p-value**	**Interaction p-value with MMP-2**
Hypertension	1.74 [1.41-2.15]	$3.44x10^{-07}$	0.50
Smoking status	2.76 [2.29-3.32]	$1.77x10^{-26}$	0.2
MMP-2 SNP	1.16 [1.01-1.35]	$4.20x10^{-02}$	-

Figure 1


56 records identified through initial database search. English and non-English articles searched.
9 Duplicate articles removed
47 records after duplicates removed
11 articles irrelevant to subject
36 full-text articles assessed for eligibility
34 full-text articles excluded due to not satisfying inclusion criteria
2 Candidate Gene Association Studies stating with odds ratios and confidence intervals included in meta-analysis examining 1SNP (rs243865) within the MMP-2 gene.
This study data was included into this meta-analysis with genotype counts.
3 studies included in final meta-analysis
- Total no. of IA cases from candidate gene studies = 3,584
- Total no. of controls from candidate gene studies = 3,359

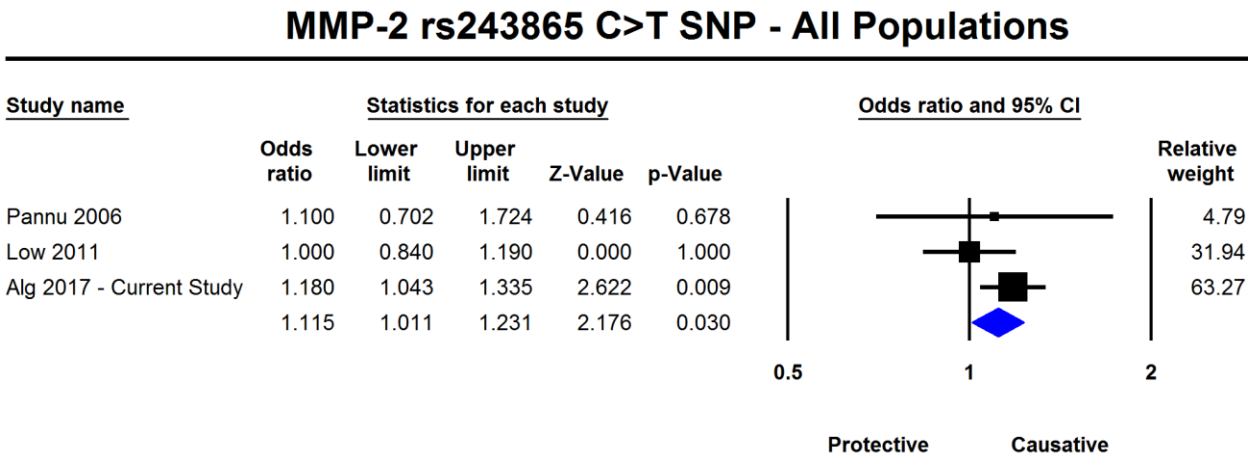


Figure 2

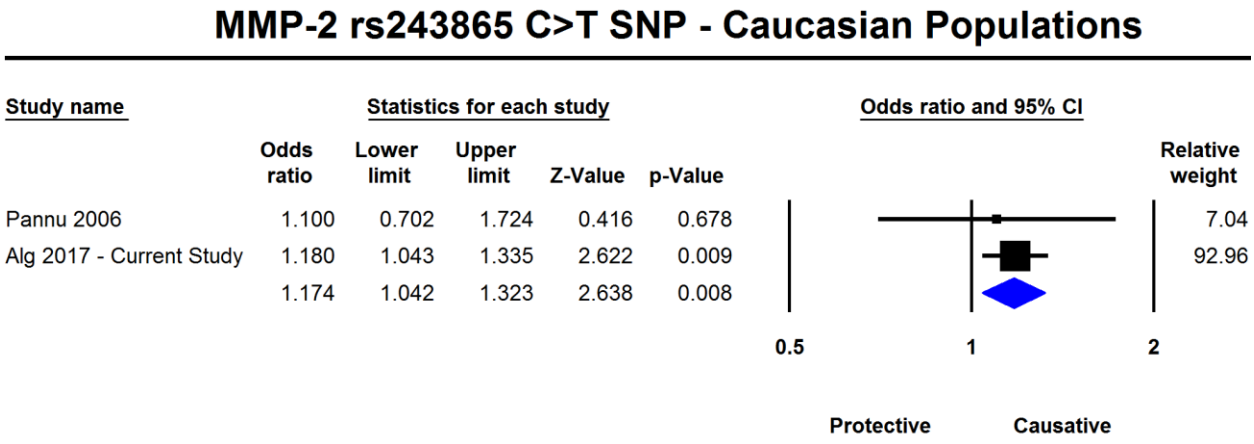


Figure 3

Figure Legends

Figure 1 – Flow chart demonstrating search strategy for meta-analysis.

Figure 2 – Meta-analysis of all populations testing for the MMP-2 rs243865 SNP.

Figure 3 - Meta-analysis of Caucasian studies on the MMP-2 rs243865 SNP.