No evidence for an association between genetic variation at the *MMP2* and *MMP9* loci and aneurysmal subarachnoid haemorrhage

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Dear Sirs,
Subarachnoid haemorrhage (SAH) is a serious condition that has a young age onset and poor outcome [1]. Approximately 75% of the cases of SAH is due to the rupture of an intracranial aneurysm (IA) [1]. The most important risk factors are smoking, hypertension, and excessive alcohol intake [1]. In addition, genetic factors have been shown to play an important role [2].

The matrix metalloproteinases (MMP) can degrade extracellular matrix (ECM) components, and alteration of the ECM leads to changes of the vessel wall. There is evidence that matrix remodelling plays a role in the formation and rupture of IA. MMP9 levels has been found to be increased in the aneurysmal wall [3], and both MMP2 and MMP9 was found to be overexpressed in cerebral ruptured aneurysms compared with unruptured aneurysms [4].

To evaluate the role of genetic variants in *MMP2* and *MMP9* in SAH due to ruptured IA (aSAH), the genes were tagged using HapMap CEU data (Rel 23a) and Haploview (r^2=0.8 and MAF=0.1), resulting in 11 tagSNPs. SNPs in the promoter region of *MMP2* (rs243865) and in the 3'UTR of *MMP9* (rs20544) were also genotyped.

Consecutive patients (n=183) presenting with aSAH admitted to the Neurointensive Care Unit at the Sahlgrenska University Hospital in Gothenburg, Sweden and 366 matched controls were recruited. A detailed description of the inclusion criteria has been published [5, 6]. All participants gave written informed consent, and the Medical Ethics Committee at the University of Gothenburg approved the study. Genotyping was performed by TaqMan SNP Genotyping Assays. The assay for rs243865 failed and was replaced by an assay for rs243864. All genotype frequencies were in Hardy Weinberg equilibrium, and genotyping success rates were above 98%. Haplotype analyses were conducted using HelixTree 6.4.3 (Golden Helix). Association between single SNPs and case-control status was investigated with logistic regression using an additive model. The study has 80% power to detect an OR of 1.45 at the 5% level for SNPs with a MAF of 0.3 for the risk allele.

Mean age of patients and controls was 55 years and 74% were females. As expected, smokers were more frequent in cases compared to controls. Baseline

characteristics has been presented previously [6].

Genotype frequencies are presented in Table 1. No SNP showed association to aSAH, and haplotype analysis did not add any further information (data not shown). As SAH is more common in women [1] we conducted a sex-stratified subanalysis. Two strongly linked SNPs in *MMP9* (rs3918256 and rs20544, r^2=0.99 in our material) showed significant associations with aSAH in men, both with OR 1.6 (95% CI 1.00-2.60). These associations did not remain after adjustment for smoking and hypertension. No association was found in the female subgroup. However, the effect of the SNPs did not differ significantly between men and women.

In line with previous studies on aSAH, hemorrhagic stroke, and IA [7-10], we did not detect any association with genetic variants in *MMP9*, although conflicting results has been reported for rs20544 and IA [11]. Furthermore, in concordance with the present study, a lack of association between genetic variants in *MMP2* and IA has been reported [11].

From this study, together with previous published data, we conclude that the analysed genetic variants in *MMP2* and *MMP9* are not major contributors to aSAH.

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Conflict of interest statement
The authors declare that they have no conflict of interest.

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Table 1. Genotype frequencies in controls and patients with subarachnoid haemorrhage due to ruptured aneurysm (aSAH).

MMP2			**MMP9**		
SNP	control n=366	aSAH n=183	SNP	control n=366	aSAH n=183
rs243864			rs17576		
AA, n (%)	209 (57)	96 (54)	AA, n (%)	158 (43)	73 (40)
AC, n (%)	130 (36)	69 (39)	AG, n (%)	161 (44)	83 (45)
CC, n (%)	26 (7)	13 (7)	GG, n (%)	44 (12)	27 (15)
rs865094			rs2236416		
AA, n (%)	244(67)	137 (75)	AA, n (%)	285 (78)	137 (75)
AG, n (%)	114 (31)	40 (22)	AG, n (%)	75 (20)	42 (23)
GG, n (%)	6 (2)	5 (3)	GG, n (%)	4 (1)	3 (2)
rs12934241			rs20544		
GG, n (%)	14 (39)	73 (40)	AA, n (%)	134 (37)	57 (31)
GA, n (%)	178 (49)	89 (49)	AG, n (%)	159 (43)	88 (48)
AA, n (%)	45 (12)	19 (10)	GG, n (%)	72 (20)	38 (21)
rs243847			rs3918256		
AA, n (%)	124 (34)	61 (33)	AA, n (%)	133 (36)	57 (31)
AG, n (%)	180 (49)	88 (48)	AG, n (%)	159 (43)	88 (48)
GG, n (%)	61 (17)	34 (19)	GG, n (%)	73 (20)	38 (21)
rs2287074			rs3787268		
GG, n (%)	117 (32)	54 (30)	GG, n (%)	215 (59)	107 (59)
GA, n (%)	181 (49)	92 (50)	GA, n (%)	123 (34)	64 (35)
AA, n (%)	67 (18)	35 (19)	AA, n (%)	20 (6)	10 (6)
rs1163996					
AA, n (%)	151 (41)	77 (42)			
AG, n (%)	167 (46)	84 (46)			
GG, n (%)	47 (13)	21 (11)			
rs11541998					
GG, n (%)	285 (78)	141 (77)			
GC, n (%)	77 (21)	38 (21)			
CC, n (%)	3 (1)	3 (2)			
rs7201					
AA, n (%)	110 (30)	45 (25)			
AC, n (%)	175 (48)	99 (54)			
CC, n (%)	80 (22)	39 (21)			

SNP, Single-nucleotide polymorphism*; aSAH*, subarachnoid haemorrhage due to ruptured aneurysm