Published as: *Atherosclerosis*. 2010 February ; 208(2): 557–563.

Prospective study of matrix metalloproteinase-9 and risk of myocardial infarction and stroke in older men and women

Barbara J. Jefferis[a,*], **Peter Whincup**[b], **Paul Welsh**[c], **Goya Wannamethee**[a], **Ann Rumley**[c], **Lucy Lennon**[a], **Andy Thomson**[a], **Debbie Lawlor**[d], **Claire Carson**[e], **Shah Ebrahim**[e], and **Gordon Lowe**[c]

[a]Department of Primary Care & Population Health, UCL Medical School, London, United Kingdom

[b]Division of Community Health Sciences, St. George's, University of London, London, United Kingdom

[c]Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom

[d]MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, United Kingdom

[e]Non-Communicable Disease Epidemiology Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Objectives—The endopeptidase matrix metalloproteinase-9 (MMP-9) is implicated in atherosclerotic plaque rupture. We investigate prospective associations between MMP-9 and MI or stroke in an older general population cohort, accounting for established and novel cardiovascular risk factors.

Methods—Baseline serum MMP-9 was measured in incident MI (n = 368) and stroke (n = 299) cases and two controls per case, 'nested' in prospective studies of 4252 men and 4286 women aged 60–79 years, sampled from General Practices in Britain in 1998–2000, with 7-year follow-up for fatal and non-fatal MI and stroke.

Results—Geometric mean MMP-9 was 528 ng/mL (IQR 397, 743) in MI cases compared to 501 ng/mL (IQR 370, 743) in controls, p = 0.10. Participants in the top compared to bottom third of MMP-9 levels had an age-adjusted odds ratio for MI of 1.53 (95% CI 1.09, 2.13), which attenuated to 1.18 (95% CI 0.81, 1.70) after adjustment for established and novel cardiovascular risk factors. There was weak evidence that OR differed according to pre-existing CVD; the OR for MI in 187 participants with pre-existing CVD was 2.20 (1.04, 4.64) and 1.24 (0.84, 1.82) in 715 participants without (LR test for interaction p = 0.06). Geometric mean MMP-9 levels were higher in stroke cases than controls; 522 ng/mL (IQR 363, 673) vs 487 (IQR 393, 704), p = 0.045; however adjustments similarly attenuated the associations.

Conclusions—While serum MMP-9 is univariately associated with risk of MI and stroke, it is not a strong independent risk marker for either.

*Corresponding author. Tel.: +44 0207 830 2230; fax: +44 020 7794 1224. b.jefferis@pcps.ucl.ac.uk.

Abbreviations

MMP-9, matrix metalloproteinase-9; MI, myocardial infarction; CVD, cardiovascular disease; CHD, coronary heart disease; OR, odds ratio; IQR, inter-quartile range; CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin-6; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; t-PA, tissue plasminogen activator; LR, likelihood ratio

Keywords

Myocardial infarction; Stroke; Inflammation; Epidemiology; MMP-9; Prospective; Cohort

1 Introduction

Experimental evidence suggests that matrix metalloproteinase-9 (MMP-9) may play a causal role in new-onset cardiovascular disease (CVD). MMP-9 may contribute to weakening and rupture of atherosclerotic plaques. MMP-9 (also known as gelatinase B, 92 kDa collagenase) is one of a family of endopeptidase enzymes involved in the degradation and re-organisation of the extra-cellular matrix which is involved in vascular remodeling; inappropriate or absent remodeling can promote atherosclerosis or restenosis [1]. MMPs are expressed in cells including macrophages, endothelial cells and vascular smooth muscle cells and have been identified in the shoulder area of human plaques where MMPs may promote the fibrous cap to weaken, destabilizing the plaque [2].

Despite the experimental evidence, few epidemiologic studies have investigated associations between MMP-9 and myocardial infarction (MI) or stroke onset. Most have exclusively studied patients with existing CVD [3–6]. Among these, the Atherogene study reported elevated risk of CVD mortality (n = 97 deaths) which was completely attenuated by adjustment for classical risk factors and inflammatory markers [3]. Yet smaller studies did not report significant associations with CHD risk (n < 65 cases), although type 2 error due to the small sample size may account for this [4,5]. Another study of patients with carotid stenosis reported elevated risk of onset of ipsilateral stroke or cardiovascular death (n = 53 cases) but did not account for inflammatory markers [6]. Little evidence exists about MMP-9 and CVD onset from population studies; the only prospective study of healthy individuals is a study based on a different subset of the cohort of men investigated here when they were middle aged, and reported modest positive associations between serum MMP-9 levels and MI onset (n = 465 cases), but associations were mediated by factors associated with MMP-9; notably cigarette smoking and inflammatory markers CRP and IL-6 [7]. Evidence from genetic studies about the role of MMP-9 in CVD is mixed [3,8–11].

We have therefore examined associations between MMP-9 and MI and stroke in two prospective population-based studies of older men and women, taking account of a range of established and novel cardiovascular risk factors, particularly inflammatory markers CRP and IL-6. We investigate whether the associations differ between participants with evidence of pre-existing CVD (MI and stroke) and the healthy population, as previous studies have not addressed this question. We also investigate whether associations differ between smokers and non-smokers given the higher burden of atherosclerosis in non-smokers and previous findings that MMP-9 is higher in smokers [3,7].

2 Methods

In 1998–2000, 4252 men from a single General Practice in each of 24 British towns who were already participating in a prospective study of CVD ongoing since 1978–1980,

attended for follow-up measurements at the age of 60–79 years (response rate 77%) [12]. In 1999–2001, a parallel study of 4286 women of the same age and in the same Practices was established, omitting two study towns (Dewsbury and Maidstone) and adding one other (Bristol) [13]. Near identical study protocols were used. In both studies, nurses administered questionnaires, made physical measurements (weight, height and seated blood pressure), recorded an ECG and collected fasting venous blood samples, from which serum was stored at ≤−70 °C for subsequent analysis of lipids, hemostatic and inflammatory markers as described elsewhere [12–15]. MMP-9 (ng/mL) was measured in 2007 using a commercially available sandwich ELISA (R&D Systems, Oxon, UK) (intra-assay CV 4.4%, inter-assay CV 10.4%) by researchers blinded to case–control status of samples. In two study towns, MMP-9 was measured in 138 men in 1996 and again in 2000, to enable intra-individual comparisons of values over time [16]. Participants completed detailed questionnaires. Pre-existing CVD was defined from validated reports from General Practitioner, or self-report of MI or stroke at any questionnaire between 1978–1980 and 1998–2000 in men and in the baseline questionnaire (1999/2000) in women [12]. Data on cigarette smoking, alcohol consumption, physical activity, own longest-held occupation (or husband's occupation for non-married women, coded using the Registrar General's classification) were self-reported. Region of residence was recorded for women in 1998–2000 and for men at the start of their follow-up in 1978–1980. Participants were followed-up for 6.25–8.5 years for mortality and cardiovascular morbidity, with a follow up loss of <2%. Fatal cases were ascertained through National Health Service Central Registers from a death certificate: ICD-9 codes 410–414 for MI and ICD-9 codes 430–438 for stroke, indicating deaths with cerebrovascular disease as the underlying cause. Non-fatal MI or stroke was diagnosed using World Health Organisation diagnostic criteria based on validated reports from General Practitioners, supplemented by regular reviews of General Practice records [12,17]. Participants provided written informed consent to the investigation and ethical approval was provided by all relevant local research ethics committees.

We established a nested case–control study based on all 390 MI cases occurring between examination date (1998–2000) and June 2006 in men and between examination date (1999–2001) and September 2007 in women. 780 controls “frequency matched” to cases on town of residence, gender and age in 5-year bands were randomly selected from among subjects who survived to the end of the study free from incident CVD. Similar methods were used to establish a separate nested case–control study based on 324 cases of stroke and 648 (different) frequency matched controls.

2.1 Statistical analyses

Highly skewed variables were transformed using natural logarithms and baseline (age 60–79 years) characteristics of the MI case and control populations were calculated (mean and standard deviation, median and IQR for skewed variables, or *n* (%) for categorical variables). Continuous variables were adjusted for gender, region of residence and age. Tertiles of MMP-9 were defined separately in the MI and stroke control samples. Associations between tertiles of MMP-9 and covariables were examined using one-way ANOVA. Unmatched logistic regression analyses were used to examine associations between tertiles of MMP-9 and MI. Models were adjusted for cardiovascular risk factors selected *a priori*: (i) gender, age and region of residence; (ii) smoking status (current, ex, never); (iii) history of diabetes, or CHD, social class (manual or non-manual), alcohol use (1–2 drinks/day or other) physical activity (more or less than 3 h of moderate/vigorous activity per week), systolic and diastolic blood pressure (SBP and DBP), body mass index (BMI), total and HDL cholesterol as continuous variables; (iv) CRP and IL-6. Linear regression models of continuous MMP-9, log transformed to base 2 were used to assess the effect of a doubling of MMP-9 level on MI. Differences in the MMP-9-CVD associations

were tested using likelihood ratio (LR) tests for interactions by gender, age, pre-existing CHD and current smoking status. With 302 MI cases available for the multivariate analyses, we had 80% power to detect a relative risk of 1.66 in the top tertiles of MMP-9 compared to the bottom tertiles, at the 5% statistical significance level, assuming two controls per case. For the 237 stroke cases, the equivalent relative risk was 1.79. Rosner's method was used to calculate regression dilution ratios on a subset of men with repeated MMP-9 measurements, using a linear regression model of the repeat measurement predicted by the first measurement [18].

3 Results

3.1 MI

Baseline characteristics of MI cases and controls are shown in Table 1. MMP-9 levels were available for 368 of 390 MI cases (270 men and 98 women aged on average 70.8 years) and for 713 of 780 controls. Cases had higher prevalences of pre-existing CVD (MI or stroke) and diabetes than controls, were more likely to smoke cigarettes and less likely to be physically active or light drinkers (all $p < 0.02$) and had higher mean SBP, total cholesterol, triglycerides, insulin, glucose, CRP, IL-6, lower HDL cholesterol and forced expiratory volume (FEV_1) and similar SEP, BMI and DBP. Geometric mean serum MMP-9 was 5% higher (95% CI 4%, 6%) in the MI cases than the controls; 527 ng/mL (IQR 397, 743) compared to 501 ng/mL (IQR 3670, 743), p (no difference) = 0.096.

3.2 Stroke

MMP-9 data were available for 304 of 324 stroke cases (194 men and 110 women, mean age 71.4 years) and 596 of 648 controls. Characteristics of stroke cases and controls are shown in Table 1. Cases had higher prevalence of pre-existing CVD and current smokers than controls (both $p < 0.003$), but similar SEP, alcohol use, physical activity and prevalence of pre-existing diabetes. Cases had higher mean blood pressure, total cholesterol, IL-6 and white blood cell count, but lower FEV_1 and similar BMI, HDL, triglyceride, insulin, glucose, CRP and fibrinogen to controls. MMP-9 levels were 7% (95% CI 5%, 9%) higher among the stroke cases than the controls; 522 ng/mL (IQR 363, 673) compared to 487 ng/mL (IQR 393, 704), p (no difference) = 0.045.

3.3 Baseline correlates of MMP-9

Table 2 summarises the associations between MMP-9 and other markers of CVD risk across thirds of the MI control population (the larger control population). Associations in the stroke control population were similar unless specifically noted. MMP-9 levels were higher in men and in smokers, but did not vary with age, social class or other behavioural factors, BMI or BP. MMP-9 was inversely associated with FEV_1. Weak inverse associations with lipids did not reach conventional levels of statistical significance in the MI control population, although did in the stroke control population ($p < 0.02$ for total and HDL cholesterol). MMP-9 was positively associated with CRP and IL-6. Correlations between log MMP-9 and covariates were all $r < 0.3$ (e.g. $r = 0.27$ for CRP and $r = 0.28$ for IL-6, $p < 0.001$).

3.4 Association of MMP-9 with risk of MI

The odds ratio (OR) for MI associated with the highest compared to the lowest third of MMP-9 and adjusted for age, gender and region of residence (model 1) was 1.53 (95% CI 1.09, 2.13) with a linear trend across the tertiles ($p = 0.01$) (Table 3). Adjustment for cigarette smoking (model 2) attenuated the OR to 1.40 (95% CI 0.99, 1.97), p (trend) 0.049. Further adjustments for established risk factors and for IL-6 and CRP (models 3 and 4) completely attenuated the OR and linear trend. A doubling of MMP-9 level adjusted for age,

gender and region of residence (model 1) was associated with an OR for MI 1.20 (95% CI 0.97, 1.48), which was further reduced by adjustments.

There was no evidence that associations between MI and tertiles of MMP-9 or $\log_2$ MMP-9 varied by age, gender, or cigarette smoking status (LR tests $p > 0.12$). The OR for MI for a doubling of MMP-9 was 1.10 (95% CI 0.86, 1.40) in men and 1.54 (1.00, 2.37) in women. There was weak evidence that the association may differ according to pre-existing CVD status, LR test $p = 0.06$. In the 715 participants without CVD, the OR for MI in the top compared to bottom third of MMP-9 adjusted for age, gender and region was 1.24 (95% CI 0.84, 1.82), linear trend $p = 0.281$ (Table 3). The equivalent OR for MI in the 187 participants with pre-existing CVD was 2.20 (1.04, 4.64), linear trend, $p = 0.02$. The OR was little changed on full adjustment (Table 3).

3.5 Association of MMP-9 with risk of stroke

No significant associations between MMP-9 and stroke were observed in multivariate regression models (Table 4) in sample with or without pre-existing CVD and there was no evidence for interaction between by pre-existing CVD (Table 4), gender, age or cigarette smoking (LR tests all $p > 0.22$).

3.6 Impact of adjustment for regression dilution ratio

A regression dilution ratio of 0.46 (95% CI 0.32, 0.60) was calculated in a subset of 138 men with MMP-9 levels measured 4 years apart [16]. The OR of MI among men in the top third of MMP-9 compared with the lowest third and adjusted for age, gender and region, lipids, BMI and behavioural risk factors (model 3), corrected for regression dilution using Rosner's method for univariate models with RDR of 0.46 is 1.95 (95% CI 0.92, 4.22) in the full sample. The equivalent adjusted estimate for the full stroke sample using the RDR of 0.46 is 1.41 (95% CI 0.58, 3.33), further estimates are presented in Web Table 1.

4 Discussion

Serum MMP-9 levels were positively associated with MI and stroke onset over a 7-year period in older men and women, but the association was explained by established and novel risk factors, in particular cigarette smoking, and the inflammatory markers CRP and IL-6. In the subset with pre-existing CVD the associations of MMP-9 with onset of MI were somewhat stronger than in those without pre-existing CVD and robust to adjustments.

4.1 Comparison with previous studies

In line with other studies, MMP-9 levels did not vary with age [3,7,19], but were associated with smoking [3,7,20] and inflammatory markers (including CRP, IL-6, fibrinogen and white blood cell count) [3,7], but not consistently with other health behaviours [3,7,19], lipids, blood pressure or BMI [3,7,19]. The finding of a modest association between MMP-9 and MI which was attenuated by risk factor adjustment agrees closely with an earlier study of the men's cohort at age 40-59 years [7]. The Atherogene study, following patients with coronary stenosis over 4 years, reported an elevated HR for CVD (1.3 (95% CI 1.1, 1.6) per quartile increase of MMP-9 after adjusting for established risk factors. As in our study, the association was completely attenuated after adjustment for inflammatory markers [3]. The observed attenuation is line with expectation as MMP-9 production may be influenced by cardiovascular risk factors such as smoking, CRP and IL-6 (which are associated with serum MMP-9 in our current and previous studies [7] and with plasma MMP-9 in a cross sectional study [20]. Smaller studies have not reported significant associations between MMP-9 and CVD, although they may lack power [4,5]. Data from some [3,8,9] studies of the associations between polymorphisms associated with MMP-9 and CVD-related outcomes

lend weight to the potential for a causal association between MMP-9 and MI onset. However, the lack of consistency of the genetic studies caution against this interpretation [3,10,11]. Our finding that the associations between MMP-9 and MI or stroke did not vary by gender, smoking status (or indeed age) extends the literature as previous studies have not investigated such differences. We found some weak suggestive evidence of a stronger association between MMP-9 and MI in participants with pre-existing CVD. Although previous studies have not tested this, it fits with evidence from patient populations suggesting that MMP-9 is associated with CVD-risk, whilst the evidence from healthy population is weaker. MMP-9 may be a weaker marker of CHD risk in healthy sub-groups because MMP-9 may be more important in de-stabilizing established plaques rather than having a directly atherogenic effect. Speculatively, increased expression of MMP-9 (and decreased expression of tissue inhibitors of MMPs [TIMPS]) may be one way in which increased circulating inflammatory markers increase the risk of rupture in existing plaques [1,2] so that MMP-9 could be a biomarker of rupture-prone plaques. Our results however suggest that MMP-9 is probably too moderately associated with CVD to be clinically useful biomarker, although it may remain a potential therapeutic target, particularly in individuals with pre-existing disease.

In relation to stroke, a study of patients with carotid stenosis reported a combined HR for ipsilateral stroke or cardiovascular death of 1.9 (95% CI 1.1, 3.5) for MMP-9 above vs below median adjusted for risk factors including smoking [6]. We found no evidence that MMP-9 is independently associated with onset of stroke, in line with epidemiologic [7] and genetic data [11].

4.2 Strengths and weaknesses

This study adds to scant prior evidence about the relations of MMP-9 to CVD onset in generally healthy populations and is the first population-based prospective study of MMP-9 and CVD to investigate gender interactions. The study, which had sufficient size and statistical power to exclude a relative risk of 1.68 between top and bottom thirds of the MMP-9 distribution, benefits from validated data about MI and stroke events and comprehensive data on a range of established and novel cardiovascular risk factors. Few other studies have prospective data on MMP-9 and subsequent risk of either MI [3,7], or stroke [6]. The present study has a longer follow-up period and more events than previous studies in patient populations. One earlier population-based study reported similar results over a longer follow-up; it was based on a different nested case–control sample of men taken from the same cohort that provided the nested case–control data presented here, but the men's MMP-9 measures were from when they were 20 years younger, and the CVD cases occurred over an earlier period, so the studies have minimal overlap [7]. The results of stratified analyses suggested that positive associations of MMP-9 with MI may be particularly strong in subjects with pre-existing CHD. Whilst our study is large in comparison with previous studies, the wide confidence intervals indicate that we cannot exclude a modest but potentially important association. This study, like most others, is limited by having only a single measure of MMP-9. However, we estimated the intra-individual variability in serum MMP-9 levels. Estimates suggested lower stability of serum MMP-9 than several established risk factors [16] and after correction for within-person variability, the ORs for MI and stroke were less conservative and reached conventional statistical significance. The nested case–control design required that controls survived to the end of the study which could introduce some bias, however since observed adjusted associations were modest and not significant, bias is unlikely to be problematic. Response rates were reasonably high and it is not thought that attrition would affect our estimate of the association between MMP-9 and CVD, prior work on the men's cohort suggests that non-attenders did not differ substantially from attenders [21]. Finally, serum samples were used

in preference to plasma because the MMP-9 ELISA assay was not recommended for plasma samples due to interfering chelation of EDTA and citrate during the assay process. Some researchers have suggested that measurement of serum MMP-9 may be problematic for the interpretation of results, on the basis that levels of MMP-9 are higher in serum than plasma [22] suggesting platelet and leucocyte degranulation during clotting elevates levels. However, we like others [23] challenge the assertion that serum MMP-9 measurements are never physiologically useful. Serum or plasma levels of MMP-9 may not be truly reflective of MMP-9 concentrations within cellular rupture-prone plaques, which is mechanistically potentially the most important site of action for MMP-9. However, considering circulating MMP-9 as a biomarker, serum levels of MMP-9 correlate with both plasma MMP-9 and with the zymographically measured level of "active circulating" MMP-9 [23,24]. Additionally, in healthy populations, levels of detection of MMP-9 in plasma are very low, e.g. only 20% of participants free of CHD in Framingham studies had detectable plasma MMP-9 [19]. This may diminish the statistical power in subsequent data analysis. The validity of the serum MMP-9 levels, which were measured using standard laboratory protocols, is supported by the comparability of correlates of serum MMP-9, first with CHD risk factors [7,10], particularly smoking, and second, the moderate associations with CHD outcomes are consistent with earlier reports [7,10], finally, serum data are consistent with data on plasma MMP-9 [3]. Therefore, we see no reason to suppose that, as a biomarker, serum levels of MMP-9 in a large controlled study are any less relevant than plasma levels, despite differences in absolute levels detected.

4.3 Implications

MMP-9 was associated with modestly elevated risks of MI and stroke, in large part due to confounding, particularly by cigarette smoking and inflammation. The confidence limits of the estimates exclude effects of large magnitude, although any increase in risk of MI or stroke with increasing MMP-9 could still be modest, even after full adjustment. Our estimates of measurement imprecision suggest that associations between MMP-9 and CVD are conservatively estimated. Appreciably larger prospective studies, excluding prevalent disease and with full adjustments for confounding factors would be necessary to exclude effects of moderate magnitude. Further large studies could also investigate whether MMP-9 may be a better biomarker of risk among patients with a history of CVD than without, as we found only weak suggestive evidence and confidence intervals were wide. Our study suggests that MMP-9 provides little additional information to previously established risk factors and given inconsistency in findings about MMP-9 and MI, it is unlikely to be useful in identifying high-risk individuals.

Conflict of interest

None.

References

1. Galis Z.S. Khatri J.J. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res. 2002; 90:251–262. [PubMed: 11861412]
2. Galis Z.S. Sukhova G.K. Lark M.W. Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest. 1994; 94:2493–2503. [PubMed: 7989608]
3. Blankenberg S. Rupprecht H.J. Poirier O. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation. 2003; 107:1579–1585. [PubMed: 12668489]
4. Wu T.C. Leu H.B. Lin W.T. Plasma matrix metalloproteinase-3 level is an independent prognostic factor in stable coronary artery disease. Eur J Clin Invest. 2005; 35:537–545. [PubMed: 16128859]

5. Cavusoglu E. Ruwende C. Chopra V. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction. Am Heart J. 2006; 151:1101–1108. [PubMed: 16644343]
6. Eldrup N. Gronholdt M.L. Sillesen H. Nordestgaard B.G. Elevated matrix metalloproteinase-9 associated with stroke or cardiovascular death in patients with carotid stenosis. Circulation. 2006; 114:1847–1854. [PubMed: 17030690]
7. Welsh P. Whincup P.H. Papacosta O. Serum matrix metalloproteinase-9 and coronary heart disease: a prospective study in middle-aged men. QJM. 2008; 101:785–791. [PubMed: 18676684]
8. Ye S. Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. Cardiovasc Res. 2006; 69:636–645. [PubMed: 16122719]
9. Yasmin, McEniery C.M. O'Shaughnessy K.M. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. Arterioscler Thromb Vasc Biol. 2006; 26:1799–1805. [PubMed: 16709939]
10. Hlatky M.A. Ashley E. Quertermous T. Matrix metalloproteinase circulating levels, genetic polymorphisms, and susceptibility to acute myocardial infarction among patients with coronary artery disease. Am Heart J. 2007; 154:1043–1051. [PubMed: 18035073]
11. Kaplan R.C. Smith N.L. Zucker S. Matrix metalloproteinase-3 (MMP3) and MMP9 genes and risk of myocardial infarction, ischemic stroke, and hemorrhagic stroke. Atherosclerosis. 2008; 201:130–137. [PubMed: 18342317]
12. Walker M. Whincup P.H. Shaper A.G. The British Regional Heart Study 1975–2004. Int J Epidemiol. 2004; 33:1185–1192. [PubMed: 15319395]
13. Lawlor D.A. Bedford C. Taylor M. Ebrahim S. Geographical variation in cardiovascular disease, risk factors, and their control in older women: British Women's Heart and Health Study. J Epidemiol Community Health. 2003; 57:134–140. [PubMed: 12540690]
14. Wannamethee S.G. Lowe G.D. Whincup P.H. Physical activity and hemostatic and inflammatory variables in elderly men. Circulation. 2002; 105:1785–1790. [PubMed: 11956120]
15. Danesh J. Kaptoge S. Mann A.G. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med. 2008; 5:e78. [PubMed: 18399716]
16. Emberson J.R. Whincup P.H. Morris R.W. Extent of regression dilution for established and novel coronary risk factors: results from the British Regional Heart Study. Eur J Cardiovasc Prev Rehabil. 2004; 11:125–134. [PubMed: 15187816]
17. World Health Organization Expert Committee. Hypertension and coronary heart disease: classification and criteria for epidemiological studies. Geneva: World Health Organization; 1959. Report no.: 168.
18. Rosner B. Spiegelman D. Willett W.C. Correction of logistic regression relative risk estimates and confidence intervals for random within-person measurement error. Am J Epidemiol. 1992; 136:1400–1413. [PubMed: 1488967]
19. Sundstrom J. Evans J.C. Benjamin E.J. Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: the Framingham Heart Study. Circulation. 2004; 109:2850–2856. [PubMed: 15173025]
20. Garvin P. Nilsson L. Carstensen J. Circulating matrix metalloproteinase-9 is associated with cardiovascular risk factors in a middle-aged normal population. PLoS ONE. 2008; 3:e1774. [PubMed: 18335048]
21. Thomas M.C. Walker M. Lennon L.T. Non-attendance at re-examination 20 years after screening in the British Regional Heart Study. J Public Health Med. 2002; 24:285–291. [PubMed: 12546205]
22. Gerlach R.F. Demacq C. Jung K. Tanus-Santos J.E. Rapid separation of serum does not avoid artificially higher matrix metalloproteinase (MMP)-9 levels in serum versus plasma. Clin Biochem. 2007; 40:119–123. [PubMed: 17150202]
23. Thrailkill K. Cockrell G. Simpson P. Physiological matrix metalloproteinase (MMP) concentrations: comparison of serum and plasma specimens. Clin Chem Lab Med. 2006; 44:503–504. [PubMed: 16599849]

24. Wu C.Y. Wu M.S. Chiang E.P. Plasma matrix metalloproteinase-9 level is better than serum matrix metalloproteinase-9 level to predict gastric cancer evolution. Clin Cancer Res. 2007; 13:2054–2060. [PubMed: 17404086]

Appendix A Supplementary data

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The British Regional Heart Study is a British Heart Foundation (BHF) Research Group and is supported by BHF programme grant RG/04/003. The British Women's Heart and Health Study is joint funded by the UK Department of Health and BHF. MMP-9 analyses in participants aged 60–79 years were funded by BHF project grant PG/07/048. The views expressed in this publication are those of the authors and not necessarily those of the funding bodies.

Published as: *Atherosclerosis*. 2010 February ; 208(2): 557–563.

Table 1

Baseline characteristics (age 60–79) of the MI cases and controls and stroke cases and controls: mean (SD) or *n* (%)[a].

	MI cases (*n* = 368)	MI controls (*n* = 713)	Difference (*p*-value)	Stroke cases (*n* = 304)	Stroke controls (*n* = 596)	Difference (*p*-value)
Demographic/questionnaire						
Age (years)	70.83 (5.46)	70.80 (5.43)	Matched	71.36 (5.32)	71.39 (5.26)	Matched
Male, *n* (%)	270 (73.4)	516 (72.4)	Matched	194 (63.2)	377 (63.3)	Matched
Northern region of residence, *n* (%)[b]	156 (42.4)	298 (41.8)	Matched	111 (36.5)	227 (38.1)	Matched
Non-manual occupation, *n* (%)[b]	155 (44.5)	325 (47.8)	0.333	125 (46.6)	263 (47.2)	0.632
Prior evidence of CVD, *n* (%)	111 (30.2)	119 (16.7)	<0.001	86 (28.3)	90 (15.1)	<0.001
History of diabetes, *n* (%)	66 (17.9)	76 (10.7)	0.001	43 (14.1)	73 (12.3)	0.422
1–2 alcoholic drinks/day, *n* (%)	129 (38.2)	304 (46.2)	0.015	129 (38.2)	304 (46.2)	0.913
Current smoker, *n* (%)	77 (20.9)	94 (13.2)	0.002	53 (17.4)	58 (9.8)	0.002
Physical activity (inactive/occasional), *n* (%)	235 (66.4)	401 (58.8)	0.017	199 (71.7)	383 (66.8)	0.213
Physical measurements						
Body mass index (kg/m^2)[c,d]	27.06 (4.23)	27.02 (3.96)	0.934	27.06 (4.33)	27.06 (4.09)	0.828
Systolic blood pressure (mm/Hg)[c,d,e]	153.89 (27.29)	148.69 (24.03)	0.001	154.32 (24.96)	149.37 (24.42)	0.005
Diastolic blood pressure (mm/Hg)[c,d,e]	83.77 (12.09)	83.28 (11.50)	0.512	84.98 (12.61)	82.45 (11.29)	0.002
FEV_1 (L/min)[c,d,f]	2.15 (0.55)	2.23 (0.60)	0.028	2.09 (0.57)	2.23 (0.53)	<0.001
Lipids/metabolic markers						
Total cholesterol (mmol/L)[c]	6.24 (1.23)	6.09 (1.07)	0.032	6.13 (1.21)	6.32 (1.17)	0.023
HDL cholesterol (mmol/L)[c]	1.32 (0.33)	1.42 (0.36)	<0.001	1.43 (0.40)	1.43 (0.36)	0.924
Triglyceride (mmol/L)[c,e,g]	1.80 (1.31, 2.41)	1.58 (1.13,2.09)	<0.001	1.64 (1.11, 2.32)	1.69 (1.23, 2.29)	0.468
Inflammatory markers						
MMP-9 (ng/mL)[c,g]	527 (397, 743)	501 (370, 743)	0.096	522 (363, 673)	487 (393, 704)	0.045
C-reactive protein (mg/L)[c,g]	2.39 (1.05, 5.39)	1.77 (0.89, 3.62)	<0.001	2.12 (1.04, 4.35)	1.92 (0.91, 4.10)	0.173

	MI cases (*n* = 368)	MI controls (*n* = 713)	Difference (*p*-value)	Stroke cases (*n* = 304)	Stroke controls (*n* = 596)	Difference (*p*-value)
IL-6 (pg/mL)[c.e.g]	2.97 (1.92, 4.07)	2.43(1.61, 3.43)	<0.001	2.83 (1.87, 4.14)	2.56 (1.65, 3.55)	0.021

[a] Case–control sample is maximum available.

[b] Reported in 1978–1980 (age 40–59 years) for men and in 1998–2000 (age 60–79 years) for women.

[c] Adjusted for gender, age and region of residence (Scotland, North, Midlands and South).

[d] Adjusted for nurse number.

[e] Adjusted for time of day.

[f] Adjusted for height squared.

[g] Analysed as natural log transformed variable, geometric mean (IQR) reported on original scale.

Published as: *Atherosclerosis*. 2010 February ; 208(2): 557–563.

Table 2

Association between MMP-9 (tertiles) and cardiovascular risk factors in the MI control sample (*n* = 713).

	Low (10–410) *n* = 239	Medium (411–599) *n* = 237	High (600–4496) *n* = 237	Trend *p*-value[a]
Demographic/questionnaire				
Age (years)	70.81	70.29	71.31	0.310
Male, *n* (%)	162 (67.8)	173 (73.0)	181 (73.4)	0.037
Northern region, *n* (%)[b]	90 (37.7)	114 (48.1)	94 (39.7)	0.653
Non-manual occupation, *n* (%)[b]	105 (46.5)	116 (51.3)	104 (45.4)	0.819
Evidence of CVD, *n* (%)	40 (16.7)	38 (16.0)	41 (17.3)	0.870
History of diabetes, *n* (%)	16 (6.7)	34 (14.4)	26 (11.0)	0.131
1–2 alcoholic drinks/day, *n* (%)	102 (47.0)	104 (47.9)	98 (43.8)	0.490
Current smoker, *n* (%)	17 (7.11)	27 (11.4)	50 (21.1)	<0.001
Physical activity (inactive/occasional), *n* (%)	127 (54.7)	132 (57.9)	142 (64.0)	0.047
Physical measurements				
Body mass index (kg/m^2)[c,d]	26.81	26.99	27.26	0.222
Systolic blood pressure (mm/Hg)[c,e,d]	149.59	147.37	149.03	0.801
Diastolic blood pressure (mm/Hg)[c,e,d]	83.44	82.66	83.72	0.798
FEV_1 (L/min)[c,d,f]	2.34	2.20	2.14	<0.001
Lipids/metabolic markers				
Total cholesterol (mmol/L)[c]	6.13	6.09	6.05	0.411
HDL cholesterol (mmol/L)[c]	1.44	1.42	1.40	0.219
Triglyceride (mmol/L)[c,e,g]	1.55	1.60	1.59	0.562
Inflammatory markers				
C-reactive protein (mg/L)[c,g]	1.35	1.65	2.48	<0.001
IL-6 (pg/mL)[c,e,g]	2.09	2.29	2.86	<0.001

[a] Sample (*n* = 713) MI controls with MMP-9 value. Trend test adjusted for age, gender, region of residence.

[b] Reported in 1978–1980 (age 40–59 years) for men and in 1998–2000 (age 60–79 years) for women.

[c] Adjusted for gender, age and region of residence (Scotland, North, Midlands and South).

[d] Adjusted for nurse number.

[e] Adjusted for time of day.

[f] Adjusted for height squared.

[g] Geometric mean, *p*-value from linear regression with ln (variable).

Table 3

Odds ratio (95% CI) of MI in men and women with MMP-9 values in the higher tertiles compared to the lower tertile of the distribution (i) all participants; (ii) participants without CVD; (iii) participants with pre-existing CVD[a].

MMP-9	MI cases	MI controls	OR (95% CI) with adjustments			
Tertiles	***n***	***n***	**Model 1**	**Model 2**	**Model 3**	**Model 4**
All participants (*n* = 902)						
Range (ng/mL)						
600–4496	130	209	1.53 (1.09, 2.13)	1.40 (0.99, 1.97)	1.36 (0.96, 1.94)	1.18 (0.81, 1.70)
411–599	87	192	1.06 (0.74, 1.51)	1.06 (0.74, 1.52)	1.01 (0.70, 1.46)	0.99 (0.68, 1.43)
10–410	85	199	1	1	1	1
Total	302	600	$p = 0.010$	$p = 0.049$	$p = 0.123$	$p = 0.373$
Continuous: doubling of (MMP-9)[b]	302	600	1.20 (0.97, 1.48)	1.13 (0.91, 1.40)	1.11 (0.89, 1.39)	1.01 (0.81, 1.28)
Participants without pre-existing CVD (*n* = 715)						
Range (ng/mL)						
600–4496	83	176	1.24 (0.84, 1.82)	1.09 (0.71, 1.63)	1.01 (0.67, 1.54)	0.85 (0.55, 1.31)
411–599	64	158	1.06 (0.71, 1.60)	1.08 (0.73, 1.63)	1.04 (0.68, 1.60)	0.97 (0.63, 1.50)
10–410	65	176	1	1	1	1
Total	212	503	$p = 0.281$	$p = 0.671$	$p = 0.959$	$p = 0.451$
Continuous: doubling of (MMP-9)[b]	212	503	1.11 (0.87, 1.42)	1.03 (0.81, 1.32)	1.01 (0.78, 1.32)	0.89 (0.68, 1.17)
Participants with pre-existing CVD (*n* = 187)						
Range (ng/mL)						
600–4496	47	33	2.20 (1.04, 4.64)	2.17 (1.00, 4.71)	2.39 (1.05, 5.44)	2.52 (1.08, 5.87)
411–599	23	34	1.07 (0.48, 2.38)	1.06 (0.47, 2.38)	1.09 (0.47, 2.55)	1.11 (0.47, 2.46)
10–410	20	30	1	1	1	1
Total	90	97	$p = 0.023$	$p = 0.034$	$p = 0.026$	$p = 0.023$
Continuous: doubling of (MMP-9)[b]	90	97	1.28 (0.79, 2.05)	1.23 (0.75, 2.01)	1.20 (0.72, 2.00)	1.21 (0.72, 2.04)

Model 1 = age, gender and region. Model 2 = model 1 + smoking. Model 3 = model 2 + alcohol, physical activity, history of diabetes, BMI, SBP, DBP, TC, HDL. Model 4 = model 3 + IL-6 + CRP.

[a] Complete case analysis sample. Tertiles based on control group. *P*-value for test for trend over tertiles.

[b] OR of MI per 1 $\log_2$ increase in $\log_2$(MMP-9) i.e. doubling of MMP-9.

Table 4

Odds ratio (95% CI) of stroke in men and women with MMP-9 values in the highest compared to lowest tertile (i) all participants; (ii) participants without CVD; (iii) participants with pre-existing CVD[a].

MMP-9	Stroke cases	Stroke controls	OR (95% CI) with adjustments			
Tertiles	***n***	***n***	**Model 1**	**Model 2**	**Model 3**	**Model 4**
All participants (*n* = 734)						
Range (ng/mL)						
604–2296	89	165	1.36 (0.92, 2.00)	1.29 (0.87, 1.91)	1.17 (0.78, 1.74)	1.09 (0.72, 1.67)
401–603	82	170	1.20 (0.81, 1.77)	1.17 (0.79, 1.74)	1.11 (0.74, 1.67)	1.08 (0.72, 1.63)
10–400	66	162	1	1	1	1
Total	237	497	$p = 0.122$	$p = 0.204$	$p = 0.462$	$p = 0.683$
Continuous: doubling of MMP-9[b]	237	497	1.24 (0.98, 1.56)	1.20 (0.95, 1.51)	1.14 (0.90, 1.45)	1.10 (0.85, 1.41)
Participants without pre-existing CVD (*n* = 598)						
Range (ng/mL)						
604–2296	65	138	1.48 (0.95, 2.32)	1.42 (0.90, 2.23)	1.30 (0.81, 2.06)	1.22 (0.75, 1.99)
401–603	59	144	1.30 (0.83, 2.05)	1.28 (0.81, 2.01)	1.25 (0.79, 1.99)	1.22 (0.76, 1.95)
10–400	46	146	1	1	1	1
Total	170	497	$p = 0.087$	$p = 0.136$	$p = 0.283$	$p = 0.441$
Continuous: doubling of (MMP-9)[b]	170	497	1.32 (1.02, 1.72)	1.29 (0.99, 1.68)	1.24 (0.94, 1.63)	1.20 (0.90, 1.60)
Participants with pre-existing CVD (*n* = 136)						
Range (ng/mL)						
604–2296	24	24	1.00 (0.42, 2.39)	1.01 (0.42, 2.42)	0.86 (0.33, 2.25)	0.77 (0.28, 2.14)
401–603	23	26	0.89 (0.36, 2.17)	0.89 (0.36, 2.21)	0.87 (0.31, 2.46)	0.83 (0.29, 2.14)
10–400	20	19	1	1	1	1
Total	67	69	$p = 0.973$	$p = 0.959$	$p = 0.765$	$p = 0.625$
Continuous: doubling of (MMP-9)[b]	67	69	0.92 (0.52, 1.62)	0.89 (0.50, 1.58)	0.78 (0.41, 1.49)	0.72 (0.37, 1.42)

Model 1 = age, gender and region. Model 2 = model 1 + smoking. Model 3 = model 2 + SEP, alcohol, physical activity, history of diabetes, history of CHD, BMI, SBP, DBP, TC, HDL. Model 4 = model 3 + IL-6 + CRP.

[a] Complete case analysis sample. Tertiles based on control group. *p*-value for test for trend over tertiles.

[b] OR of MI per 1 $\log_2$ increase in $\log_2$(MMP-9) i.e. doubling of MMP-9.