Title

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Mitral Annular Calcification and Cardiovascular Risk Factors: Results from CRIC Study

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Abstract

Background
A high degree of correlation between risk factors for mitral annular calcification (MAC) and cardiovascular disease (CVD) has been previously shown. It is still unknown whether these calcium deposits are associated with traditional risk factors in the CKD population. The aim of this paper is to determine whether there are independent relationships between MAC and gender, race, age, and traditional and novel CVD risk factors using cardiac CT in the Chronic Renal Insufficiency Cohort (CRIC) in a cross-sectional study.

Methods
The sample included 2070 subjects who underwent coronary calcium scanning as part of the multi-ethnic CRIC study. Medical history, anthropometric measurements, and laboratory data were obtained for each participant.
All participants underwent two CT scans at the same time for evaluation of CAC. CT scans were obtained using either an Imatron C-300 Electron Beam computed tomography scanner or multi-detector CT scanner. Subjects were dichotomized into the presence and absence of MAC. Differences in baseline demographic and transitional risk factor data were evaluated across MAC groups using t-test for continuous variables and the Chi-Square test or Fisher's exact test for categorical variables, as appropriate. The following covariates were used in the multivariable adjustment: age, gender, body mass index, HDL, LDL, lipid lowering medications, smoking status, family history of heart attack, hypertension, and diabetes mellitus.

Results
Out of the 2070 subjects in our study, 331 had MAC with a prevalence of 15.99%. Among the risk factors, age, Caucasian race, HDL <40, history of smoking and diabetes remained significantly associated with presence of MAC in multivariable adjusted analyses (all $p<0.05$). In multivariable adjusted analyses, the association of hypertension, increased BMI, family history of CAD, LDL and CRP were not significantly associated with presence of MAC.

Conclusion
In the CRIC population, presence of MAC was independently associated with age, Caucasian race, HDL <40, history of smoking and diabetes. These results are explained by the dysregulation of inflammation, hormones, and electrolytes in subjects with renal disease.

Key Words: Coronary atherosclerosis, Mitral annular calcification, Cardiac computed

tomographic angiography

Introduction:
A high degree of correlation between risk factors for mitral annular calcification (MAC) and cardiovascular disease (CVD) has been previously shown [1-3]; age, female gender, diabetes mellitus (DM), and obesity were found to be independently associated with MAC. In these studies, however, the patient population included few subjects with chronic kidney disease (CKD)[3, 4]. Among this group, cardiovascular disease is one of the most common causes of increased mortality [5, 6]. Patients with renal disease suffer from dysregulation of inflammation, hormones, and electrolytes resulting in significant relative increases in

calcium deposits of the coronary arteries and cardiac valves [6-11]. Additionally, an association exists between MAC and coronary artery calcification (CAC) and coronary artery disease (CAD) [12, 13]. However, not all CKD patients have MAC. It is still unknown whether these increased calcium deposits are associated with traditional risk factors in the CKD population. Prior studies either lacked sufficient subjects or used echocardiography, which has low specificity in distinguishing dense collagen from calcification and leads to wide variation in MAC prevalence when compared to computed tomography (CT) [1, 2]. No previous study has examined the relationship between multiple traditional risk factors and MAC using Cardiac CT in the Chronic Renal Insufficiency Cohort (CRIC). Therefore, the aim of this paper is to determine whether there are independent relationships between MAC and gender, race, age, and traditional and novel CVD risk factors using cardiac CT in CRIC in a cross-sectional study.

Methods:

This study was approved by the Institutional Review Board at all participating centers. The sample included 2070 subjects who underwent coronary calcium scanning as part of the multi-ethnic CRIC study [14]. This cohort was established to examine risk factors for progression of chronic renal insufficiency (CRI) and cardiovascular disease (CVD) among patients with CRI and identify high-risk groups, inform future treatment trials, and increase application of preventive therapies. The scientific and Data Coordinating Center (SDCC), located in Philadelphia, is in charge of seven clinical centers, each with up to 500 subjects. All creatinine levels are performed at a central laboratory and GFR is estimated based on the simplified modification of diet in renal disease (MDRD) equation (GFR [mL/min per 1.73 m^2] = 186 x [serum Cr (mg/dL] $^{-1.154}$ x [age] $^{-0.203}$ x [0.742 if female] x [1.212 if black]).

Medical history, anthropometric measurements, and laboratory data were obtained for each participant. Questionnaires supplied information about age, gender, ethnicity, and medical history. Current smoking was defined as having smoked a cigarette in the last 30 days. Diabetes was defined as a fasting glucose ≥ 126 mg/dl or on hypoglycemic medication. Use of antihypertensive and other medications were based on clinic staff entry of prescribed medications. Resting blood pressure was measured three times in the seated position using a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon, Tampa, Florida) and the average of the 2nd and 3rd readings was recorded. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of medication prescribed for hypertension. Body mass index was calculated from the equation weight (kg)/height (m^2). Total and HDL cholesterol were measured from blood samples obtained after a 12-hour fast. LDL cholesterol was calculated with the Friedewald equation. CRP was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL) at the University of Vermont Laboratory for Clinical Biochemistry Research. Analytical intra-assay CVs ranged from 2.3 – 4.4% and inter-assay CVs ranged from 2.1–5.7%. The presence and number of risk factors for each subject was calculated based on the National Cholesterol Education Program guidelines. Risk factors included: age (>45 years for men, >55 years for women), current cigarette smoking, diabetes mellitus, history of premature coronary artery disease in first-degree relatives (< 55 years in men, < 65 years in women), hypertension, and hypercholesterolemia. Hypercholesterolemia was defined as use of cholesterol lowering medications or, in the absence of use of cholesterol lowering medications, a total serum cholesterol >200 mg/dL.

After signing informed consent, all participants underwent two CT scans at the same time for evaluation of CAC. CT scans were obtained using either an Imatron C-300 Electron Beam computed tomography scanner or multi-detector CT scanner [15]. Thirty to forty contiguous tomographic slices were obtained at 3 mm intervals beginning one centimeter below the carina and progressing caudally to include the entire coronary tree [16] . Methods for CAC scanning have previously been published [17]. All scans were analyzed by Neo Imagery Technologies software package (City of Industry, California). Calcific lesions were identified

by an attenuation threshold of 130 Hounsfield units and a minimum of 3 contiguous pixels, then was scored using Agatston's algorithm. A density factor was assigned based on the following: 1 for lesions with peak attenuation of 130–199 Hu, 2 for lesions with peak attenuation of 200–299 Hu, 3 for lesions with peak attenuation of 300–399 Hu, and 4 for lesions with peak attenuation >400 Hu. The total CAC score was determined by summing individual lesion scores from each of four anatomical sites (left main, left anterior descending, left circumflex, and right coronary artery).[1] The average of the two scores was used in the analysis.

Initially, MAC was dichotomized into the presence and absence of MAC defined as those with MAC=0 versus those with score > 0. Differences in baseline demographic and transitional risk factor data were evaluated across MAC groups using t-test for continuous variables and the Chi-Square test or Fisher's exact test for categorical variables, as appropriate. We described differences in demographics and risk factors between those with positive MAC scores and those with a 0 score. Then, we looked at only those who have positive MAC scores and assessed whether there were differences in age, gender, race and other risk factors.

We used logistic regression models to assess the relationship between each risk factor and the presence of calcium and adjusted for all other risk factors in the model. The odds ratios we estimate approximate relative risks because our endpoint is rare. The following covariates were used in the multivariable adjustment: age, gender, body mass index, HDL, LDL, lipid lowering medications, smoking status, family history of heart attack, hypertension, and diabetes mellitus. Statistical analyses were performed with SAS version 9.3 (Cary, NC) and a p-value <0.05 was considered statistically significant.

RESULTS

Overall, the 2070 subject CRIC cohort was comprised of 1112 males (53.7%) and 958 females (46.3%). The average subject age was 58.0 years. Whites comprised the majority of the cohort (43%) versus blacks (35%), Hispanics (17%), and others (5%). The average BMI of the cohort was 31 years, with mean HDL of 49 mg/dL, LDL 103 mg/dL, and GFR 43.

Out of the 2070 subjects in our study, 331 had MAC with a prevalence of 15.99%. The prevalence of MAC was highest in Caucasians (19.80%), followed by African Americans (13.05%), and Hispanics (12.90%). Those classified as "other" demonstrated prevalence of 14.02%. Table 1 demonstrates the baseline characteristics of the study population according to absence and presence of MAC. Individuals with MAC were more likely to be older and women, as seen in figure 1 and 2. Characteristics associated with MAC included age ($p=0.004$), history of smoking ($p=0.030$), history of diabetes ($p=0.035$), and low HDL levels ($p=0.043$). Hispanics were less likely to have MAC ($p=0.009$)

The MAC group had a higher prevalence of hypertension and diabetes mellitus (DM) as well as higher average BMI (all $p<0.001$) when compared with the absence of MAC group. There were no statistically significant differences in hsCRP levels or mean GFR between those with and without MAC. However, lipid lowering medications were used more in the MAC group. In addition, the MAC group tended to have lower total cholesterol, LDL, and HDL levels ($p<0.002$ for all).

Table 2 demonstrates the univariate and multivariable adjusted association of risk factors with presence of MAC. Increasing age (per 10 year) was associated with up to a 6-fold increase in the odds of presence of MAC in unadjusted as well as multivariate adjusted analyses. Males had the same likelihood for MAC as females. As compared to Caucasians, all ethnic groups had a lower odds ratio for presence of MAC, however, a statistical significance was observed only among Hispanics.

Among the traditional risk factors, history of smoking and history of diabetes remained significantly associated with presence of MAC in multivariable adjusted analyses (all $p<0.05$). In multivariable adjusted analyses, the association of hypertension, increased BMI, and family history of CAD were no longer statistically significant. Additionally, LDL and CRP were also not significantly associated with presence of MAC after taking into account other risk factors. Interestingly, lower HDL levels were associated with MAC in both univariate and multivariate analysis ($p=0.043$).

Discussion

In brief, our study results show that there is an association between MAC and increasing age, DM, Caucasian race, and low HDL (<40 mg/dL) in patients with CKD who are not dependent on dialysis and without kidney transplant. This is important due to the association of MAC with CAC and CAD [12].

We found several notable differences when compared to previous MESA studies, which had few CKD subjects (of 6785, 10% had eGFR <60) [4]. Our study had nearly twice the prevalence of MAC (9% vs 15.99%) [3, 4]. Unlike MESA, we did not find an association with use of lipid lowering medications in MAC. Female and obese patients were more likely to have MAC, although neither reached statistical significance in CRIC like they did in MESA. Although increasing age was independently significant in both studies, MAC patients in the CRIC population were younger by about 8 years. In the Penn Diabetes Heart Study (PDHS) of subjects without renal dysfunction, MAC subjects were 8 years older on average than those without MAC [13]. Former smokers were associated with a decrease in MAC in the CRIC population, but no significant association was found with current smoking. We found that this segment of former smokers had lower LDL levels than never-smokers, which is likely because more of them took lipid-lowering medications (data not shown). Current smokers in the MESA population were independently associated with MAC as they were in the PDHS population [3, 13]. Studies of MESA and CRIC populations are in agreement that MAC was associated with increasing age, Caucasian race, and DM and neither found an association between MAC and hypertension, family history of heart attack, increase in LDL, and increase in CRP [3, 4]These are in agreement with a related study on the PDHS population where age, female gender, Caucasian race, and duration of diabetes were independently associated with MAC, while hypertension, hyperlipidemia, and increased CRP were not [13].

The difference between MESA and CRIC in MAC prevalence and age of onset is likely related to renal patient's dysregulation of inflammation, hormones, and electrolytes [18]. Secondary hyperparathyroidism, stimulated by decreased active vitamin D and increased phosphorus excretion in CKD, is associated with increased circulating calcium and increased risk of calcification. Hyperphosphatemia, a hallmark of CKD, contributes by depositing in the tunica media or intimal layer and acts as mediator to activate genes leading to transformation of vascular smooth muscle cells (VSMC) and pericytes to osteoblast-like cells. Calcium sensing receptors (Ca-SR) expression is decreased in uremic patients. Ca-SR suppresses PTH and is also present in endothelium, VSMC, and cardiomyocytes where they protect against calcification. Diets high in calcium and drugs designed to suppress Ca-SR have been shown to be cardioprotective [19]. Fibroblast growth factor 23 (FGF23), upregulated in CKD earlier than PTH, has been shown to stimulate vascular calcification and be a strong and independent predictor of the extent of coronary stenosis and number of stenotic vessels [20, 21]. A related protein called Kothlo, which is downregulated early in CKD, is cardioprotective. In Kothlo knockout mice, there is increased phosphate transport into VSMC and increased transcription of factors that stimulate local osteoblastic transformation [22]. CKD patients also suffer from vitamin D deficiency, which stimulates vascular calcification and is associated with poor survival in ESRD and CKD [18]. Finally, inflammation is known to accelerate atherosclerosis and vascular calcification. CKD patients show upregulation of pro-

inflammatory markers IL-1, IL-6, TNFα while decreased levels of anti-inflammatory proteins such as fetuin A, which prevents precipitation of calcium and phosphate in serum and whose concentration is inversely related to survival [23]. Fortunately, calcific progression, whether by these processes or others, is attenuated after kidney transplantation [24]. In CRIC, MAC subjects tended to have a lower GFR than those without MAC without reaching statistical significance (MAC 44.2±20.3 v. 37.5±12.8; p=0.0706). Even after excluding diabetics, the trend continued but did not reach statistical significance.

Obesity and body mass index (BMI) are important predictors of calcification both in the general population and in patients with CKD. For instance, TNFα can be synthesized in adipose tissue, while adipocytokines (leptin, adiponectin, visfatin, resistin) are specific to fatty tissue [18]. This would explain why our MAC population had a higher average BMI, although, it was not significantly associated as a risk factor for MAC.

Use of lipid lowering medications, but not HDL <40, was significantly associated with MAC in the MESA population, while the inverse was true in CRIC. We hypothesize that this is in part due to the decreasing benefits of lipid lowering medications with advancing kidney disease [25]. These patients rely on the reverse cholesterol transport properties of HDL through bile, as well as HDL's anti-inflammatory properties (inhibition monocyte chemotaxis, adhesion molecule expression, enhanced nitric oxide and prostacyclin production), promotion of anticoagulation, augmentation urokinase-dependent fibrinolysis, and inhibition of platelet activation and aggregation [26, 27]. We do not expect these benefits to persist in severe CKD or end stage renal disease (ESRD) on hemodialysis (HD) as this population suffers abnormal clearance of apoA-1, a major component of HDL, and nonfunctional HDL of different proteome that is rather proinflammatory (an effect linked to SAA1 in uremic HDL) [28-31].

Diabetes mellitus is known to increase both intra and extra-coronary calcification [32]. It is a regulated process in part by the receptor activator and nuclear factor kβ ligand (RANKL) - receptor activator and nuclear factor kβ (RANK) - osteoprotegerin (OPG) signaling pathway, which ultimately results in osteogenic differentiation of cells (probably smooth muscle cells) and deposition of mineralized matrix in the vessel walls [33]. The ratio of RANKL:OPG (a decoy receptor for RANKL) reflects the level of activation of this pathway [34]. Our study findings are explained by this and are in agreement with other clinical studies that find MAC or CAC independently associated with DM [13, 32].

It is not clear why female gender is associated with MAC, however, one possible mechanism is the increase in bone demineralization (especially in postmenopausal women). Bone loss may be the reason for increased calcium deposition on the mitral annulus [35, 36]. In our study, MAC females had an average age of 64.46 with standard deviation 8.02 years. Estrogen plays an important part in bone density regulation by inducing osteoclast apoptosis, inhibiting osteoclast formation and differentiation from monocytes, increases OPG production, decreasing RANKL production, decreasing IL-1, IL-6, TNFα formation, inhibiting osteoblast apoptosis, and increasing osteoblast lifespan [37-43]. In particular, estrogen is responsible for inducing estrogen receptor α (ERα) binding to a scaffolding protein (BCAR1). The ERα/BCAR1 complex then sequesters TNF receptor-associated factor 6 (TRAF6), which decreases activation of NF- B and impairs RANKL-induced osteoclastogenesis. Perhaps the reason that female gender did not reach statistical significance in the CRIC population is that this pathway is already upregulated and the loss of negative feedback may not play a significant role in the disease process. Further research is needed to prove this hypothesis.

We identified several strengths in our study. For one, the lack of exclusion criteria in the CRIC population other than polycystic kidney disease (PCKD) means that this study is generalizable to most patients with CKD not on dialysis or having received renal transplants. Additionally, the use of CT scanners to assess MAC diminishes false positive studies as

compared to echocardiography. Our study had several limitations. For one, it is unlikely that a former smokers should have a decreased risk of MAC. This may in part be due to this segment of former smokers having lower LDL levels than never-smokers or being more likely to take lipid-lowering medications (data not shown). As well, our absolute number of patients with MAC (even though prevalence was higher than in other studies) may have been too low to detect associations in subgroup analysis. Finally, it is difficult to prove causation in cross-sectional studies.

This study is clinically relevant because it illustrates the risk factors for MAC in patients with CKD who are not dependent on dialysis and without renal transplant. The presence of these risk factors could prompt earlier diagnostic investigations or modification of lifestyle or medicines. It is also possible that earlier screening could discover associated conditions such as CAC prior to symptom onset. By whichever avenue, downstream adverse events could be prevented or mitigated via intervention, closer observation, or modification of medical management. Future prospective studies are needed to show whether these would have any morbidity or mortality benefit.

References:

1. Adler, Y., et al., *Usefulness of helical computed tomography in detection of mitral annular calcification as a marker of coronary artery disease.* Int J Cardiol, 2005. **101**(3): p. 371-6.
2. Budoff, M.J., et al., *Effect of scanner type on the reproducibility of extracoronary measures of calcification: the multi-ethnic study of atherosclerosis.* Acad Radiol, 2007. **14**(9): p. 1043-9.
3. Kanjanauthai, S., et al., *Relationships of mitral annular calcification to cardiovascular risk factors: the Multi-Ethnic Study of Atherosclerosis (MESA).* Atherosclerosis, 2010. **213**(2): p. 558-62.
4. Ix, J.H., et al., *Kidney function and aortic valve and mitral annular calcification in the Multi-Ethnic Study of Atherosclerosis (MESA).* Am J Kidney Dis, 2007. **50**(3): p. 412-20.
5. Go, A.S., et al., *Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization.* N Engl J Med, 2004. **351**(13): p. 1296-305.
6. Fox, C.S., et al., *Cross-sectional association of kidney function with valvular and annular calcification: the Framingham heart study.* J Am Soc Nephrol, 2006. **17**(2): p. 521-7.
7. Goodman, W.G., et al., *Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis.* N Engl J Med, 2000. **342**(20): p. 1478-83.
8. Oh, J., et al., *Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure.* Circulation, 2002. **106**(1): p. 100-5.
9. Adeney, K.L., et al., *Association of serum phosphate with vascular and valvular calcification in moderate CKD.* J Am Soc Nephrol, 2009. **20**(2): p. 381-7.
10. Chung, A.W., et al., *Upregulation of matrix metalloproteinase-2 in the arterial vasculature contributes to stiffening and vasomotor dysfunction in patients with chronic kidney disease.* Circulation, 2009. **120**(9): p. 792-801.
11. Kestenbaum, B.R., et al., *Incidence and progression of coronary calcification in chronic kidney disease: the Multi-Ethnic Study of Atherosclerosis.* Kidney Int, 2009. **76**(9): p. 991-8.

12. Tenenbaum, A., et al., *Advanced mitral annular calcification is associated with severe coronary calcification on fast dual spiral computed tomography.* Invest Radiol, 2000. **35**(3): p. 193-8.
13. Qasim, A.N., et al., *Cardiovascular risk factors and mitral annular calcification in type 2 diabetes.* Atherosclerosis, 2013. **226**(2): p. 419-24.
14. Feldman, H.I., et al., *The Chronic Renal Insufficiency Cohort (CRIC) Study: Design and Methods.* J Am Soc Nephrol, 2003. **14**(7 Suppl 2): p. S148-53.
15. Mao, S.S., et al., *Comparison of coronary artery calcium scores between electron beam computed tomography and 64-multidetector computed tomographic scanner.* J Comput Assist Tomogr, 2009. **33**(2): p. 175-8.
16. Carr, J.J., et al., *Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study.* Radiology, 2005. **234**(1): p. 35-43.
17. Budoff, M.J., et al., *Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic Renal Insufficiency Cohort) Study.* Am J Kidney Dis, 2011. **58**(4): p. 519-26.
18. Stompor, T., *Coronary artery calcification in chronic kidney disease: An update.* World J Cardiol, 2014. **6**(4): p. 115-29.
19. Smajilovic, S., et al., *The calcium-sensing receptor and calcimimetics in blood pressure modulation.* Br J Pharmacol, 2011. **164**(3): p. 884-93.
20. Heine, G.H., S. Seiler, and D. Fliser, *FGF-23: the rise of a novel cardiovascular risk marker in CKD.* Nephrol Dial Transplant, 2012. **27**(8): p. 3072-81.
21. Xiao, Y., et al., *Circulating fibroblast growth factor 23 is associated with angiographic severity and extent of coronary artery disease.* PLoS One, 2013. **8**(8): p. e72545.
22. Hu, M.C., et al., *Klotho deficiency causes vascular calcification in chronic kidney disease.* J Am Soc Nephrol, 2011. **22**(1): p. 124-36.
23. Eraso, L.H., et al., *Association of lower plasma fetuin-a levels with peripheral arterial disease in type 2 diabetes.* Diabetes Care, 2010. **33**(2): p. 408-10.
24. Stompor, T., et al., *Trends in coronary artery calcification in peritoneal dialysis and transplant patients.* Nephrol Dial Transplant, 2004. **19**(12): p. 3205-6; author reply 3206.
25. Kon, V., T.A. Ikizler, and S. Fazio, *Importance of high-density lipoprotein quality: evidence from chronic kidney disease.* Curr Opin Nephrol Hypertens, 2013. **22**(3): p. 259-65.
26. Soran, H., et al., *HDL functionality.* Curr Opin Lipidol, 2012. **23**(4): p. 353-66.
27. Wang, X. and D.J. Rader, *Molecular regulation of macrophage reverse cholesterol transport.* Curr Opin Cardiol, 2007. **22**(4): p. 368-72.
28. Vaisar, T., et al., *Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL.* J Clin Invest, 2007. **117**(3): p. 746-56.
29. Kalantar-Zadeh, K., et al., *HDL-inflammatory index correlates with poor outcome in hemodialysis patients.* Kidney Int, 2007. **72**(9): p. 1149-56.
30. Kilpatrick, R.D., et al., *Association between serum lipids and survival in hemodialysis patients and impact of race.* J Am Soc Nephrol, 2007. **18**(1): p. 293-303.
31. Lamprea-Montealegre, J.A., et al., *CKD, plasma lipids, and common carotid intima-media thickness: results from the multi-ethnic study of atherosclerosis.* Clin J Am Soc Nephrol, 2012. **7**(11): p. 1777-85.

32. Farrag, A., et al., *The association between extracoronary calcification and coronary artery disease in patients with type 2 diabetes mellitus.* Heart Vessels, 2013. **28**(1): p. 12-8.
33. Ndip, A., et al., *RANKL-OPG and RAGE modulation in vascular calcification and diabetes: novel targets for therapy.* Diabetologia, 2014. **57**(11): p. 2251-60.
34. Rasmussen, L.M., et al., *Plasma osteoprotegerin levels are associated with glycaemic status, systolic blood pressure, kidney function and cardiovascular morbidity in type 1 diabetic patients.* Eur J Endocrinol, 2006. **154**(1): p. 75-81.
35. Tohno, S., et al., *Accumulation of calcium and phosphorus in the mitral valve in comparison with the abdominal aorta and the scaphoid bone.* Biol Trace Elem Res, 2000. **77**(1): p. 33-42.
36. Davutoglu, V., et al., *Mitral annular calcification is associated with osteoporosis in women.* Am Heart J, 2004. **147**(6): p. 1113-6.
37. Robinson, L.J., et al., *Estrogen inhibits RANKL-stimulated osteoclastic differentiation of human monocytes through estrogen and RANKL-regulated interaction of estrogen receptor-alpha with BCAR1 and Traf6.* Exp Cell Res, 2009. **315**(7): p. 1287-301.
38. Oursler, M.J., et al., *Avian osteoclasts as estrogen target cells.* Proc Natl Acad Sci U S A, 1991. **88**(15): p. 6613-7.
39. Kousteni, S., et al., *Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity.* Cell, 2001. **104**(5): p. 719-30.
40. Breuil, V., et al., *Immune changes in post-menopausal osteoporosis: the Immunos study.* Osteoporos Int, 2010. **21**(5): p. 805-14.
41. Hofbauer, L.C., et al., *Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells.* Endocrinology, 1999. **140**(9): p. 4367-70.
42. Eghbali-Fatourechi, G., et al., *Role of RANK ligand in mediating increased bone resorption in early postmenopausal women.* J Clin Invest, 2003. **111**(8): p. 1221-30.
43. Srivastava, S., et al., *Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-kappa B ligand (RANKL)-induced JNK activation.* J Biol Chem, 2001. **276**(12): p. 8836-40.

Figure 1: Prevalence of MAC according to gender

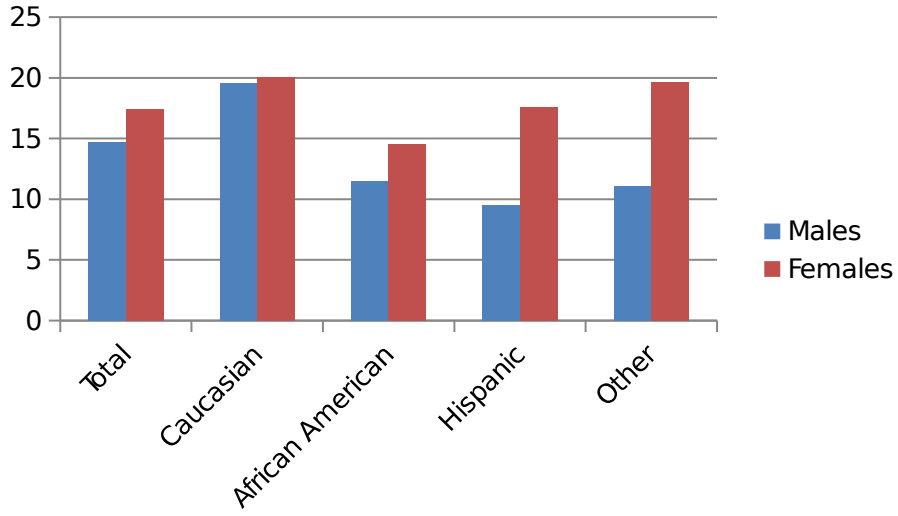

Figure 2: Prevalence of MAC according to increasing age

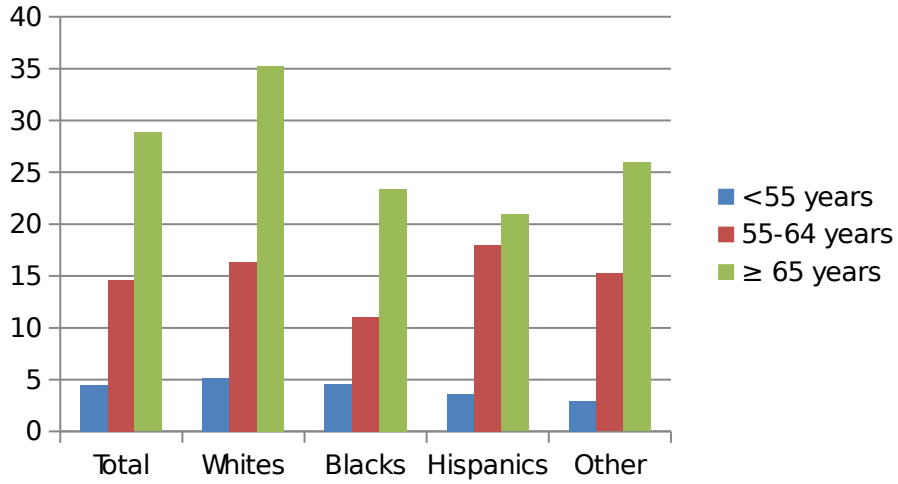

Table 1: Characteristics of CRIC absence/presence of MAC

	MAC = 0	**MAC >**	**p-value**

		0	
N	1739 (84)	331 (16)	
Age*	56.7±11.6	64.7±7.7	<0.0001
Males	948 (54.5)	164 (49.6)	0.0967
Race			
Caucasian	717 (41.2)	177 (53.5)	<0.0001
African American	633 (36.4)	95 (28.7)	0.0072
Hispanic	297 (17.1)	44 (13.29)	0.0888
Other	92 (5.3)	15 (4.5)	0.5677
Smoking			
Former	861 (49.5)	168 (50.76)	0.6782
Current	183 (10.5)	24 (7.25)	0.0689
BMI*	30.78±6.6	32.76±6.8	<0.0001
Systolic BP*	125.6±20.8	131.7±22.2	<0.0001
Hypertension	1481 (85.3)	319 (96.67)	<0.0001
Diabetes Mellitus	761 (43.8)	211 (63.75)	<0.0001
Family history of CAD	248 (14.3)	57 (17.2)	0.1638
Total cholesterol (mg/dl)*	187.9±44.4	178.3±41.1	0.0005
LDL (mg/dl)*	104.5±35.3	95.6±31.4	<0.0001
HDL (mg/dl)*	49.5±15.9	46.4±14.5	0.0016
Lipid lowering meds	1010 (58.35)	246 (74.77)	<0.0001
CRP*	4.4±6.9	5.7±13.8	0.3096
GFR*	44.2±20.3	37.5±12.8	0.0706

Numbers of patients are indicated for each group and numbers given in parentheses indicate relative percentages.
*are given in mean±SD.

Table 2: Association of Risk Factors with Presence of MAC in unadjusted and multivariate analyses using logistic regression

	Unadjusted OR (95% CI)	**Adjusted^ OR (95% CI)**
Age		
<55	Ref group	Ref group
55-64	3.62 (2.37-5.52)*	7.50 (2.22-25.35)*
≥65	8.58 (5.70-12.91)*	6.42 (1.81-22.78)*
Female Gender	1.22 (0.97-1.54)	1.64 (0.70-3.84)
Race		
Caucasian	Ref group	Ref group
African American	0.61 (0.46-0.80)*	0.10 (0.01-1.22)
Hispanic	0.60 (0.42-0.86)*	0.19 (0.05-0.66)*
Other	0.66 (0.37-1.17)	1.11 (0.06-20.63)
Smoking		
Former	1.05 (0.83-1.33)	0.36 (0.14-0.91)*
Current	0.67 (0.43-1.04)	1.02 (0.09-11.27)
BMI		
18.5-24.99 (Normal)	Ref group	Ref group
25-29.99 (Overweight)	1.66 (1.08-2.55)*	1.70 (0.29-9.87)
30-39.99 (Obese)	2.41 (1.60-3.63)*	1.66 (0.30-9.27)
≥40 (Morbidly obese)	3.01 (1.85-4.89)*	2.78 (0.38-20.26)
Hypertension	5.01 (2.71-9.28)*	0.79 (0.17-3.67)
Diabetes Mellitus	2.26 (1.77-2.88)*	2.80 (1.05-7.45)*
Family History of CAD	1.25 (0.91-1.72)	0.72 (0.21-2.47)
Lipid lowering meds	2.12 (1.62-2.76)*	0.67 (0.27-1.66)
LDL (mg/dl)		
<100	Ref group	Ref group
100-129	1.27 (0.96-1.68)	1.26 (0.51-3.07)
130-159	0.61 (0.38-0.97)	0.19 (0.02-1.61)
≥160	0.54 (0.28-1.04)	1.29 (0.27-6.09)
HDL (mg/dl)		
40-59 (average risk of CAD)	Ref group	Ref group
<40 (higher risk of	1.34 (1.02-1.75)*	2.46 (1.03-5.89)*

CAD)		
>60 (lower risk of CAD)	0.72 (0.51-1.002)	0.96 (0.20-4.68)
hsCRP (mg/L)		
1-2.9 (normal risk for CAD)	Ref group	Ref group
<1 (higher risk of CAD)	0.88 (0.37-2.13)	0.77 (0.26-2.26)
≥3 (lower risk of CAD)	1.38 (0.68-2.80)	1.12 (0.48-2.59)

^All variables adjusted simultaneously
*Indicates statistically significant