Increased hepatocyte growth factor levels over 2 years are associated with coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA)

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Hepatocyte growth factor (HGF) is associated with subclinical and clinical atherosclerosis. However, the significance of change in HGF and development of atherosclerotic disease is unknown. In a large and diverse population-based cohort, we report that change in the biomarker HGF is an independent predictor of incident CHD. (Am Heart J 2019;213:30-4.)

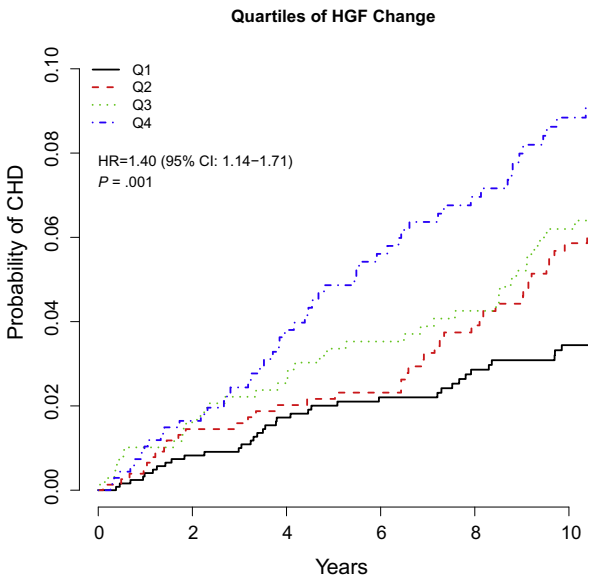
Hepatocyte growth factor (HGF) is a promising biomarker of coronary heart disease (CHD) given its release into circulation in response to endothelial damage. Higher circulating levels of HGF are associated with adverse cardiovascular risk profiles such as hypertension, diabetes and obesity.[1,2] Likewise, higher levels are associated with clinical (e.g., myocardial infarction) and subclinical (e.g., coronary atherosclerosis) heart disease.[3,4] In the Multi-Ethnic Study of Atherosclerosis (MESA), higher levels of HGF are associated with a greater burden of subclinical atherosclerosis and an increased risk of coronary heart disease.[5] These results provide preliminary evidence of the potential of HGF as a clinical biomarker of CHD. However, the clinical significance of changes in HGF levels over time is unknown. To more fully assess the potential clinical utility of HGF as a biomarker of CHD, we need to begin to understand the relevance of repeated measurements over time in a diverse population. Therefore, we hypothesize that change in HGF over a 2-year period will be associated with progression of subclinical atherosclerosis and greater increases in HGF will be associated with increased risk of CHD events.

Methods

Serum HGF was measured in 2379 subjects at exam 1 (2000-2002) and exam 2 (2002-2004) in the MESA.[6] All subjects were free of clinical cardiovascular disease at the time of enrollment. Traditional cardiovascular risk factor data were measured by standard protocols and laboratory methods. Hypertension was defined as systolic blood pressure of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or taking antihypertensive medication. Diabetes was defined as any participant who self-reported a physician diagnosis, used antidiabetic medication, or had a fasting glucose ≥126 mg/dL. Circulating levels of serum HGF protein were measured in serum by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using the Quantikine ELISA Human HGF Immunoassay Kit (R&D Systems, Minneapolis, MN) with a lower limit of detection of 40 pg/mL. The interassay laboratory coefficients of variation for the HGF method measure at exam 1 were 12.0%, 8.0%, and 7.4% at respective mean concentrations of 686.6, 2039.1, and 4079.5 pg/mL for lyophilized manufacturer's controls; and 10.4% at a mean concentration of 687.7 pg/mL for an in-house pooled serum control. The interassay laboratory coefficients of variation for the HGF method measure at exam 2 were 8.8%, 7.4%, and 6.9% at respective mean concentrations of 432.8, 1663.9, and 4689.9 pg/mL for lyophilized manufacturer's controls; and 6.2% at a mean concentration of 946.0 pg/mL for an in-house pooled serum control. Computed tomography of the coronary arteries was performed and Agatston coronary artery calcium (CAC) scores were quantified as has been previously described.[7] The cohort was followed for CHD events through 2015 using standard event ascertainment protocols.[8]

Ordinal logistic regression with a cumulative logit was used to assess the association of change in HGF from exam 1 to exam 2 with change in CAC from exam 1 to exam 2 (for the subset with CAC at exam 2). For these models, change in CAC was the dependent variable and change in HGF was the predictor with exam 1 CAC and exam 1 HGF included as covariates. For these models, changes in CAC and CAC at exam 1 were defined categorically as <0, 1-100, and >100. Additional exam 2 covariates included age, sex, current smoking, systolic blood pressure, hypertension, diabetes, total cholesterol, high-density lipoprotein (HDL), and race/ethnicity. Progression in CAC volume was assessed using the

Figure 1



Kaplan-Meier curves for incident coronary heart disease by quartile of change in hepatocyte growth factor. For each quartile the mean (SD) and range for change in HGF is: Q1: −178 (119); −1147, −58 Q2: 6 (34); −57, 63 Q3:120 (32); 63, 177 Q4: 305 (118); 177, 891. HR is from model adjusting for age, sex, exam 1 HGF and race/ethnicity.

square root transformation of calcium volume scores with a meaningful change in CAC volume from exam 1 to exam 2 defined as a difference of at least 2.5 mm^3.[9] The association of change in HGF with CAC volume progression was assessed using logistic regression. The association of change in HGF with clinical events was assessed using Cox proportional hazards regression. For these additional analyses, the exam 2 cardiovascular risk factors mentioned previously along with HGF at exam 1 were included as covariates. All time to event analyses were indexed at exam 2; subjects with events prior to exam 2 were excluded (n = 31). For all analyses, race/ethnic specific models were fit in addition to models combining all races including race and the race by protein interaction as covariates. For all analyses, change in HGF was scaled by the standard deviation (SD) of HGF at exam 1.

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Results

Exam 2 characteristics for the 2379 subjects with HGF measured at exams 1 and 2 are displayed in Table I overall and by quartile of change in HGF. Mean HGF at exam 1 was 919 ± 254 ng/mL and 982 ± 233 ng/mL at exam 2. Supplementary Figure 1 displays the change in HGF from exam 1 to exam 2 by HGF at exam 1. There were 183 incident CHD (MI, resuscitated cardiac arrest, definite angina, probable angina (if followed by revascularization), and CHD death) events during the follow-up period including 34 African, 42 Chinese, 52 Hispanic, and 55 non-Hispanic white Americans, respectively. CAC measurements were available at exam 2 on 1139 subjects. Table II summarizes the association of change in HGF with incident CHD and change in CAC. In an age and sex adjusted model, CHD risk was 40% higher per SD increase in change in HGF (P = .001). CHD risk was attenuated slightly to 35% per SD increase in change HGF (P = .005) when traditional risk factors were included in the model. Figure 1 displays the cumulative incidence of CHD by quartiles of change in HGF. Race stratified models were also assessed and the association with CHD risk was significant in Hispanic (HR = 1.56 (95% CI, 1.06-2.28); P = .024) and non-Hispanic white Americans (HR = 1.70 (95% CI, 1.20-2.43); P = .003) in fully adjusted models. The race by HGF interaction was not significant (P = .19). CAC increased at least 100 units for 9% of the subjects from exam 1 to exam 2. There was no association of change in HGF with change in coronary artery calcium (Table II).

Discussion

The main finding of this study is the association of change in HGF and clinical CHD. Specifically, increased change in HGF is associated with incident CHD independent of traditional cardiovascular risk factors and baseline HGF level. This extends our prior observation that higher levels of HGF measured at baseline are associated with risk of cardiovascular disease.[5] In contrast, change in HGF was not associated with change in subclinical atherosclerosis as measured by coronary artery calcium, albeit power was limited.

HGF is stimulated in response to tissue injury and its cardioprotective effects have been reported previously.[10] In contrast, HGF is implicated in atherosclerotic disease given its angiogenic properties and associations with adverse cardiovascular risk factor profiles. For example, previous work in MESA showed that HGF was positively associated with race/ethnicity, increasing age, higher body mass index (BMI) and systolic blood pressure, lower HDL levels, hypertensive and diabetic status, and current

Table I. Exam 2 characteristics, mean (standard deviation) or count (percentage)

	n	Q1	Q2	Q3	Q4
	2379	595	595	594	595
Exam 1 HGF, ng/mL	919 (254)	1118 (265)	899 (201)	849 (207)	810 (217)
Exam 2 HGF, ng/mL	982 (233)	941 (217)	905 (199)	969 (206)	1115 (249)
Change in HGF, ng/mL	63 (196)	−178 (119)	6 (34)	120 (32)	305 (118)
Race/Ethnicity					
African American	570 (24)	149 (25)	148 (25)	144 (24)	129 (22)
Chinese American	598 (25)	146 (25)	199 (33)	145 (24)	108 (18)
Hispanic American	597 (25)	170 (29)	122 (21)	137 (23)	168 (28)
Non-Hispanic white American	614 (26)	130 (22)	126 (21)	168 (28)	190 (32)
Age, years	63 (10)	63 (10)	62 (10)	63 (10)	63 (10)
Female	1257 (53)	304 (51)	301 (51)	321 (54)	331 (56)
Systolic blood pressure, mmHg	124 (21)	125 (21)	123 (20)	124 (21)	125 (21)
Hypertension	1029 (44)	269 (46)	244 (41)	255 (43)	261 (45)
Diabetes mellitus	366 (15)	119 (20)	85 (14)	79 (13)	83 (14)
Total cholesterol, mg/dL	193 (35)	192 (35)	191 (36)	194 (34)	192 (35)
HDL cholesterol, mg/dL	51 (15)	52 (14)	51 (14)	52 (15)	51 (15)
Smoking Status					
Never	1193 (51)	284 (48)	329 (56)	283 (48)	297 (50)
Former	888 (38)	235 (40)	190 (32)	240 (41)	223 (38)
Current	277 (12)	71 (12)	69 (12)	67 (11)	70 (12)
Exam 1 coronary artery calcium*					
0	622 (55)	171 (57)	171 (55)	148 (58)	132 (47)
1-100	283 (25)	67 (22)	72 (23)	58 (23)	86 (31)
>100	234 (21)	60 (20)	66 (21)	48 (19)	60 (22)
Exam 2 coronary artery calcium*					
0	568 (50)	158 (53)	153 (50)	135 (53)	122 (44)
1-100	307 (27)	73 (25)	81 (26)	63 (25)	90 (32)
>100	264 (23)	67 (23)	75 (24)	56 (22)	66 (24)
Change in coronary artery calcium*					
0	652 (57)	176 (59)	173 (56)	153 (60)	150 (54)
1-100	390 (34)	94 (32)	104 (34)	83 (33)	109 (39)
>100	97 (9)	28 (9)	32 (10)	18 (7)	19 (7)
Exam 1 coronary artery calcium, volume*	99 (284)	121 (348)	93 (268)	77 (208)	104 (283)
Exam 2 coronary artery calcium, volume*	121 (323)	143 (392)	115 (301)	99 (261)	125 (317)
Progression in coronary artery calcium, volume*	163 (14)	43 (14)	47 (15)	37 (15)	36 (13)
Coronary heart disease events	183 (8)	44 (7)	40 (7)	45 (8)	54 (9)

Progression in coronary artery calcium was assessed using the square root transformation of calcium volume scores with a meaningful change in CAC volume from exam 1 to exam 2 defined as a difference of at least 2.5 mm^3.
Values are n(%) unless indicated by mean ± SD.
HDL, high-density lipoprotein; *HGF,* hepatocyte growth factor.
* For the 1139 subjects with coronary artery calcium at exam 2.

smokers.[5] Furthermore, HGF levels were associated with CAC in non-Hispanic white and African Americans. Similarly, HGF was associated with CHD risk but the results were most compelling in the African Americans and non-Hispanic whites. In the current study, we extend these findings and demonstrate that a 2-year change in HGF levels is associated with risk of CHD. Low CHD event numbers diminished our ability to adequately assess race/ethnic heterogeneity.

We did not observe an association of change in HGF with change in CAC as assessed by Agatston score or by volume. Previous work using the full MESA cohort, reported that only a small subset of MESA participants had CAC progression and those with CAC = 0 at baseline, 84% were still at 0 at the follow-up scan.[11] The importance of CAC progression on CHD risk is a subject of debate. An editorial by Drs Khera and Greenland concluded that there is likely little benefit from repeated CAC measurements, with some specific caveats.[12]

The major limitation of the study is the relatively small number of events in each of the racial/ethnic strata that may have hindered our ability to detect heterogeneity. An additional limitation is that HGF and CAC were measured at the same time which could introduce reverse causation. Strengths of the study include longitudinal assessments of HGF in a large population. Further work is needed to demonstrate the clinical utility of measuring HGF longitudinally in CHD prediction.

Conclusions

Conventional risk factors for atherosclerotic diseases have been identified over the last 6 decades and have been incorporated into clinical risk scores. Ethnic-specific

Table II. Association of change in HGF from exam 1 to exam 2 and progression in coronary artery calcium and development of incident coronary heart disease.

	Overall*		African		Chinese		Hispanic		non-Hispanic white	
Change in coronary artery calcium,† Agatston	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Model 1	1.03 (0.83-1.28)	0.80	1.39 (0.89-2.17)	0.15	1.08 (0.63-1.85)	0.78	0.95 (0.64-1.40)	0.78	0.93 (0.62-1.40)	0.74
Model 2	0.98 (0.79-1.23)	0.87	1.70 (1.02-2.85)	0.04	1.09 (0.62-1.91)	0.78	0.82 (0.54-1.25)	0.37	0.90 (0.59-1.38)	0.63
Change in coronary artery calcium†, volume										
Model 1	1.06 (0.82-1.36)	0.67	1.27 (0.78-2.07)	0.34	1.35 (0.69-2.63)	0.38	1.09 (0.67-1.78)	0.72	0.81 (0.50-1.31)	0.39
Model 2	0.94 (0.72-1.24)	0.68	1.27 (0.75-2.16)	0.38	1.30 (0.60-2.79)	0.51	0.95 (0.55-1.64)	0.87	0.64 (0.37-1.11)	0.11
Coronary heart disease events	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Model 1	1.40 (1.14-1.71)	0.001	1.14 (0.71-1.82)	0.60	1.02 (0.62-1.68)	0.93	1.47 (1.03-2.12)	0.037	1.74 (1.25-2.42)	0.001
Model 2	1.35 (1.10-1.66)	0.005	1.09 (0.63-1.89)	0.76	0.89 (0.53-1.50)	0.66	1.56 (1.06-2.28)	0.024	1.70 (1.20-2.43)	0.003

For all analyses, change in HGF was scaled by the standard deviation (SD = 254) of HGF at exam 1.
Model 1 adjusts for age, sex, and exam 1 HGF (*race/ethnicity).
Model 2 adjusts for age, sex, exam 1 HGF, smoking, systolic blood pressure, hypertension, diabetes, total cholesterol, and HDL (*race/ethnicity).
†For the change in coronary artery calcium (Agatston) analysis, the exam 1 measure of coronary artery calcium was included as a covariate.
CI, confidence interval; *HGF*, hepatocyte growth factor.
For the change in coronary artery calcium analyses the number of subjects included for model 1 was 1139, 274, 281, 292, and 292 for overall, African, Chinese, and Hispanic, respectively; for model 2 the numbers were 1120, 266, 280, 289, and 285, respectively.
For the coronary heart disease analyses the number of subjects included for model 1 was 2379, 570, 598, 597, and 614 for overall, African, Chinese, and Hispanic, respectively; for model 2 the numbers were 2335, 554, 594, 589, and 598, respectively.

expression and regulation patterns of circulating biomarkers associated with CHD may contribute to differences in disease rates and provide insight into pathological mechanisms of atherosclerosis. In a large and diverse population-based cohort, we report that change in HGF is an independent predictor of incident CHD.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ahj.2019.03.018.

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Contributors

SJB, PAD, and NBL designed the analysis and PAD conducted the analysis. PAD and SJB collected the data and drafted the manuscript. All authors reviewed and approved the final manuscript.

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Declarations of interest

None.

References

1. Bancks MP, Bielinski SJ, Decker PA, et al. Circulating level of hepatocyte growth factor predicts incidence of type 2 diabetes mellitus: the Multi-Ethnic Study of Atherosclerosis (MESA). Metabolism 2016;65(3):64-72.
2. Lieb W, Safa R, Benjamin EJ, et al. Vascular endothelial growth factor, its soluble receptor, and hepatocyte growth factor: clinical and genetic

correlates and association with vascular function. Eur Heart J 2009;30(9):1121-7.
3. Sato T, Yoshinouchi T, Sakamoto T, et al. Hepatocyte growth factor (HGF): a new biochemical marker for acute myocardial infarction. Heart Vessel 1997;12(5):241-6.
4. Lamblin N, Susen S, Dagorn J, et al. Prognostic significance of circulating levels of angiogenic cytokines in patients with congestive heart failure. Am Heart J 2005;150(1):137-43.
5. Bielinski SJ, Berardi C, Decker PA, et al. Hepatocyte growth factor demonstrates racial heterogeneity as a biomarker for coronary heart disease. Heart 2017;103(15):1185-93.
6. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol 2002;156(9):871-81.
7. Carr JJ, Nelson JC, Wong ND, 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):35-43.
8. Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the Multi-Ethnic Study of Atherosclerosis. Circulation 2009;120(6):502-9.
9. Hokanson JE, MacKenzie T, Kinney G, et al. Evaluating changes in coronary artery calcium: an analytic method that accounts for interscan variability. AJR Am J Roentgenol 2004;182(5):1327-32.
10. Gallo S, Sala V, Gatti S, et al. Cellular and molecular mechanisms of HGF/Met in the cardiovascular system. Clin Sci 2015;129(12):1173-93.
11. Budoff MJ, Young R, Lopez VA, et al. Progression of coronary calcium and incident coronary heart disease events: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol 2013;61(12):1231-9.
12. Khera A, Greenland P. Coronary artery calcium: if measuring once is good, is twice better? Circulation 2018;137(7):680-3.