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Original Research

Haptoglobin genotype is a risk factor for coronary artery disease in prediabetes: A case-control study

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HIGHLIGHTS

- Haptoglobin 2-2 protein increases oxidative stress and atherosclerosis potential.
- Haptoglobin 2-2 genotype increases CAD odds in prediabetes.
- Haptoglobin genotyping is a 1-time test, available for clinical use.

ARTICLE INFO

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Atherosclerosis
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ABSTRACT

Objective: Coronary artery disease (CAD) prediction remains inconsistent with many unappreciated risk factors. Haptoglobin genotype determines the haptoglobin protein's effectiveness to bind free hemoglobin and prevent oxidative stress, a contributor to atherosclerosis. The haptoglobin 2-2 genotype increases the prevalence of cardiovascular disease (CVD) approximately five times compared to the 1-1 genotype in individuals with diabetes. The risk is unknown in prediabetes. The purpose of this study was to determine an association between haptoglobin genotype and CAD in prediabetes.

Methods: The researchers used case-control convenience sampling from two cardiovascular disease prevention clinics in Memphis, TN, and Spokane, WA, from January 1, 2016 to March 31, 2020. Participants were ages 35–70, had prediabetes, and free of chronic inflammatory or infectious diseases. Cases had a history of subclinical or clinical CAD, while controls did not have a history of CAD. Differences between cases and controls and among haptoglobin genotypes were analyzed using *t*-tests and ANOVA for continuous variables and chi-square or Fisher's exact tests for categorical variables. Associations among Hp genotypes and CAD were estimated using logistic regression.

Results: The sample ($N = 178$; 72 cases and 106 controls) was 96 % white and 64 % male. Cases had lower total cholesterol ($p = 0.0001$) and high-sensitivity C-reactive protein ($p = 0.021$). Except for CAD, haptoglobin genotype was independent of any demographic or clinical variable. Haptoglobin 2-2 genotype had 4.0 times higher odds of CAD than haptoglobin 1-1 ($p = 0.01$).

Conclusion: Haptoglobin 2-2 genotype had approximately four times higher odds of having CAD compared to the haptoglobin 1-1 genotype. Cases had more desirable clinical profiles, likely attributable to more aggressive treatment of traditional risk factors than controls. Haptoglobin genotype is a potentially important CAD risk factor in prediabetes (88 million Americans). Further studies are needed for interventions to reduce the oxidative stress associated with the Hp 2-2 genotype and glycosylated hemoglobin and for CAD reduction.

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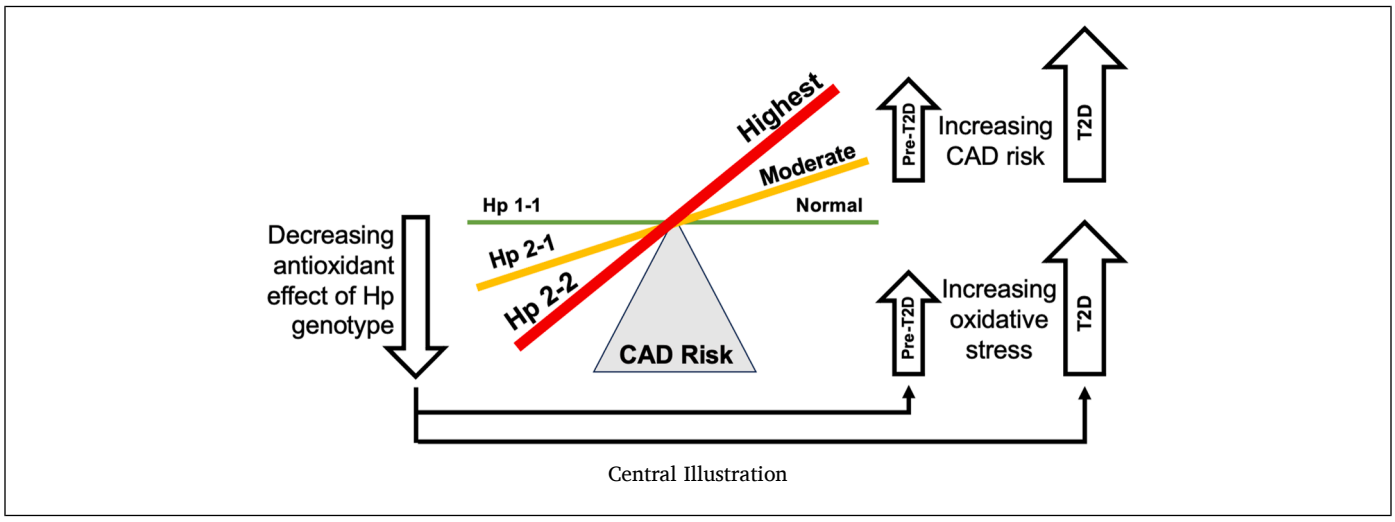


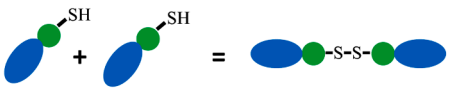
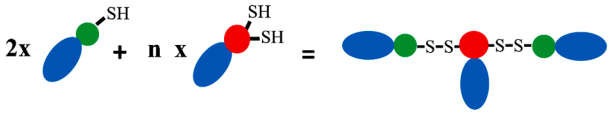
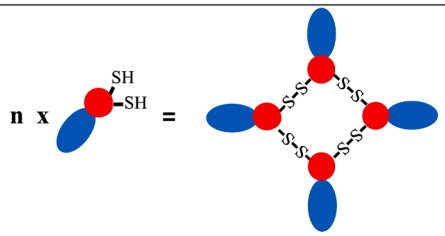
Central Illustration

1. Introduction

Despite national prevention campaigns and expanded treatment options, coronary artery disease (CAD) remains a leading cause of death in the United States [10], and the most common type of cardiovascular disease. Yet, current CAD-related risk estimation calculators lack the inclusion of comprehensive risk factors, including genetic information. Identification of CAD genetic risk factors, detectable at any time across the lifespan, could help explain the biological underpinnings of residual CAD risk or protection.

2. Background

Haptoglobin (Hp) genotype, which is 1 potential genetic risk factor, codes for the Hp protein. Hp, an α_2-glycoprotein produced by the liver, binds free hemoglobin released during physiologic or pathologic erythrocyte lysis [6,9,20]. Unbound hemoglobin leads to oxidative stress. Oxidative stress causes vascular smooth muscle cell (SMC)

Phenotype, Formula, & Shape	Structural Arrangement	Molecular Weight
Haptoglobin 1-1 $(a^1 b)_2$ Dimer		**86kd**
Haptoglobin 2-1 $(a^1b)_2 + (a^2b)_n$ Linear Polymer		**86-300kd**
Haptoglobin 2-2 $(a^2b)_n$ Cyclic Polymer		**170-900kd**

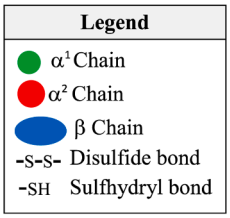


Fig. 1. Haptoglobin genotype structures and weights.
Abbreviations: kd, kilodaltons
Modified with permission from Bowman, B.H., Kurosky, A. Haptoglobin: The evolutionary product of duplication, unequal crossing over, and point mutation. In: Harris, H., Hirschhorn, K. (eds) *Advances in Human Genetics*, 1982, Springer Nature.

transformation into migratory secretory SMCs. These transformed SMCs secrete proteoglycans into the subendothelial space, which then trap apolipoprotein B. The lipids become oxidized, triggering the innate immune response and damage the arterial endothelium. This arterial insult leads to endothelial permeability, a critical step in CAD pathology. Therefore, Hp exerts its antioxidant property by clearing the free hemoglobin from circulation.

The Hp gene has 6 genotypes with 3 main phenotypes: 1-1, 2-1, and 2-2 [30]. The Hp 2 allele's protein is a heavier, larger cyclic polymer (compared to the smaller Hp 1-1 dimer and Hp 2-1 linear polymer) that enters tissues less efficiently to clear free hemoglobin [21] (Fig. 1). Therefore, Hp 2-2 is the least efficient antioxidant and most associated with oxidative stress; Hp 1-1 is the most efficient antioxidant; and Hp 2-1 is intermediate [18]. Hp 2-2 has been associated with more resistant hypertension (HTN), peripheral artery disease, myocardial infarction (MI), heart failure, and kidney disease [13,17,20].

Levy et al. [19] found that for patients with type 2 diabetes (T2D), the Hp 2-2 genotype increases the odds of developing cardiovascular disease by 5 times compared to the Hp 1-1 genotype and has 3 times higher odds than the Hp 2-1 genotype. Elevated glycosylated hemoglobin, as found in T2D and also in prediabetes, innately exudes more oxidative activity [5] and further increases the time the haptoglobin-hemoglobin (Hp-Hb) complexes remain in circulation, compounding the oxidative damage to HDL. Additionally, individuals with diabetes and the Hp 2-2 genotype exhibit decreased cholesterol efflux from converting the high-density lipoproteins (HDLs) from antiatherogenic to proatherogenic [4]. This adverse process occurs because the Hp-Hb complexes exist longer in circulation from reduced excretion in the liver. These complexes ultimately bind to HDLs' apolipoprotein A-1 receptor, oxidizing the HDL and reducing the HDLs' reverse cholesterol transport by approximately 30–40 % [4].

However, despite this information, the association of the Hp genotype and CAD has not yet been described in individuals with prediabetes, which occurs earlier on the continuum of T2D. Instead, most studies thus far have dichotomized glycemia into the presence or absence of T2D. This research design ignores the unique inflammatory pathophysiology in insulin resistance and prediabetes, which is present over a decade before T2D [14]. This study aims to determine the association between Hp genotype and coronary artery disease (CAD) in individuals with prediabetes in order to identify Hp genotype as a potential CAD risk factor.

3. Methods

This retrospective case-control study included electronic health record (EHR) review, performed by an experienced medical professional, of individuals from 2 cardiovascular disease prevention clinics in Memphis, TN, and Spokane, WA, seen in the office from 1/1/2016 to 3/31/2020. The Institutional Review Board of The University of Tennessee Health Science Center approved this study and waived the informed consent.

3.1. Inclusion and exclusion criteria

Inclusion criteria included adults ages 35–70 years with a history of prediabetes (defined by the American Diabetes Association [2] as having 1 or more of the following: a fasting glucose of 100–125 mg/dl, hemoglobin A1c level between 5.7 and 6.4 %, or a 2-hour oral glucose tolerance test (OGTT) result of 140–199 mg/dl); Hp genotype results; and carotid intima-media thickness (CIMT) results. Exclusion criteria included a history of familial hypercholesterolemia (which independently increases CAD risk); type 1 or T2D (where the risk of Hp genotype is already known); vasospastic angina (which is not a form of atherosclerotic CAD); rheumatoid arthritis; Sjogren's disease; systemic lupus erythematosus; or other chronic inflammatory arthritis, psoriasis, or inflammatory bowel disease. The latter inflammatory and infectious diseases were excluded because they could potentially confound CAD risk due to increased inflammation, as they are known risk enhancers of CAD [3].

3.2. Defining cases and controls

Cases were defined as individuals with CAD [16]. Categorization of CAD (i.e., subclinical or clinical) included a diagnosis of CAD, history of coronary stenting or revascularization, MI, acute coronary syndrome, coronary plaque on cardiac catheterization or coronary computed tomography angiography, or angina. Controls were defined as individuals with the absence of any documentation of CAD as previously described.

3.3. Data collection and variables

Demographic, anthropometric, lab, and imaging variables were collected. Clinical information collected included smoking status, medical history (e.g., HTN, hyperlipidemia, chronic kidney disease, periodontal pathogens, obstructive sleep apnea, and vitamin D deficiency), and family history of CAD or T2D. Lab results collected included Hp genotype, lipid results, inflammatory biomarkers, and 2-hour OGTT results. The Hp genotype was assessed previously by the clinic as part of patient care, using a blood specimen for a validated real time polymerase chain reaction test [8].

Imaging variables collected included CIMT measures (i.e., arterial age, mean common, mean maximum, and plaque burden measurements). CIMT uses noninvasive B-mode ultrasound technology to measure the thickness of the common carotid intimal and medial layers of the artery wall [32]. While different areas of the carotid artery (i.e., common, internal, external, bifurcation, and bulb of carotid artery) are used to identify atherosclerotic plaques, stenosis, or occlusion, IMT measurements specifically isolate a 10 mm section of the common carotid artery free of plaque [31]. Because CIMT can identify pathologic intima-media inflammation—which causes CIM thickening, 1 of the earliest signs of atherosclerosis—CIMT is a useful atherosclerotic cardiovascular disease surrogate [31,32]. Using CIMT allows for earlier, noninvasive, low-risk detection of subclinical-through-advanced atherosclerosis and can also be measured over time to assess treatment efficacy [15].

3.4. Statistical analysis

All statistical analyses were performed using SAS statistical software, Version 9.4 (SAS Institute Inc., Cary, North Carolina). Bivariate analyses were performed to examine differences between cases and controls and among Hp genotypes with respect to demographics, anthropometrics, medical history, lab, and CIMT measurements using chi-square or Fisher's exact tests for categorical variables and *t*-tests or ANOVA for continuous variables. Differences in the frequencies of the Hp genotypes between cases and controls were also analyzed. Smoking status was recoded from current, former, or never smokers and then combined current and former smokers, as there was only 1 current smoker (an Hp 1-1 control). The association between CAD and Hp genotypes was estimated using logistic regression, with age and sex as covariates, and the odds ratios, with 95 % confidence intervals being obtained. An alpha of 0.05 was chosen for statistical significance.

Table 1
Case-control and haptoglobin genotype frequencies.

	Case n (%)	Control n (%)	Total n (%)
Hp 1-1	6 (3.37)	23 (12.92)	**29 (16.29)**
Hp 2-1	40 (22.47)	58 (32.58)	**98 (55.06)**
Hp 2-2	26 (14.61)	25 (14.04)	**51 (28.65)**
Total	**72 (40.45)**	**106 (59.55)**	**178 (100)**

Hp, haptoglobin.

Table 2
Logistic regression of Hp genotype and CAD.

Effects	OR	95 % CI	p-value
Model 1:			
Hp 1-1 vs. Hp 2-1	2.644	(0.99–7.08)	0.05
Hp 1-1 vs. Hp 2-2	3.987	(1.39–11.43)	0.01
Model 2:			
Hp 1-1 vs. Hp 2-1	3.367	(1.20–9.49)	0.02
Hp 1-1 vs. Hp 2-2	5.261	(1.71-16.16)	0.004
Sex	7.223	(3.28–15.90)	<0.001
Model 3:			
Hp 1-1 vs. Hp 2-1	3.470	(1.23–9.82)	0.02
Hp 1-1 vs. Hp 2-2	5.480	(1.77–16.96)	0.003
Sex	7.483	(3.38–16.59)	<0.001
Age	1.022	(0.98–1.06)	0.29

Hp=haptoglobin, CAD=coronary artery disease, OR=odds ratio, CI=confidence interval.

4. Results

The sample ($N = 178$) was 96 % white and 64 % male with 72 (40.45 %) cases and 106 (59.55 %) controls. The prediabetes categorization included 100 individuals with a fasting glucose, 102 individuals with a hemoglobin A1c, and 32 individuals with a 2-hour OGTT result within prediabetic range. Again, individuals could have one or more of the glucose-related results within prediabetic range to be included in this study. Hp genotype frequencies were 29 (16.3 %) for Hp 1-1, 98 (55.1 %) for Hp 2-1, and 51 (28.7 %) for Hp 2-2 (Table 1), which met Hardy-Weinberg equilibrium ($p = 0.12$). Overall, the Hp 2-2 genotype had a significantly higher prevalence of CAD than the other genotypes ($p = 0.03$). The odds of CAD were 4.0 (1.39–11.43) times higher in the Hp 2-2 genotype compared to the Hp 1-1 genotype ($p = 0.01$) (Table 2). The Hp 2-1 genotype had 2.64 (1.0–7.08) higher odds of CAD than the Hp 1-1 genotype; however, this was marginally statistically significant ($p = 0.05$). When sex was added to the logistic regression model, male sex had 7.223 higher odds of CAD ($p = < 0.001$). Additionally, the Hp 2-2 genotype had 5.261 higher odds of CAD ($p = 0.004$) and the Hp 2-1 genotype had 3.367 higher odds of CAD ($p = 0.02$) than the Hp 1-1 genotype. When age and sex were added to the model, the Hp 2-1 and Hp 2-2 genotypes still had significantly higher odds of CAD than the Hp 1-1 genotype. However, age was not a statistically significant covariate ($p = 0.29$).

4.1. Differences between cases and controls

While 64 % of the sample was male, 62 of the 72 cases (86 %) were male ($p < 0.0001$). Overall, 27 (15.13 %) cases had history of an MI. No significant differences in race, smoking history, chronic kidney disease, periodontal pathogens, vitamin D deficiency, or family history of CAD or T2D between cases and controls were found (Table 3). Cases were taller ($p = 0.0002$), had lower body fat ($p = 0.01$), and more often had a history of HTN ($p = 0.03$) and obstructive sleep apnea ($p = 0.01$), with no difference in weight or systolic or diastolic blood pressure being observed when compared to controls. Cases also had significantly lower total cholesterol ($p = 0.0001$), low-density lipoprotein (LDL) cholesterol ($p = 0.01$), and non-high-density lipoprotein (non-HDL) cholesterol ($p = 0.04$) and ApoB ($p = 0.04$) when compared to controls. However, cases had significantly lower HDL cholesterol ($p = 0.0021$) than controls. No differences were found between cases and controls for inflammatory biomarkers, glucose, insulin, or CIMT results.

4.2. Differences among haptoglobin genotypes

The prevalence of CAD for the Hp 1-1, Hp 2-1, and Hp 2-2 genotypes was 20.69 %, 40.82 %, and 50.98 %, respectively. Individuals with the Hp 2-2 genotype had lower hemoglobin A1c ($p = 0.0084$), with A1c means of 5.53, 5.65, and 5.48 for the Hp 1-1, 2-1, and 2-2 genotypes, respectively, being reported (Table 4). No other differences in glucose tests were detected. Furthermore, no differences in demographics, cholesterol, inflammatory biomarkers, or CIMT were found among the Hp genotypes (Table 4).

5. Discussion

An estimated 88 million, or 34.5 %, US adults have prediabetes [25]. Therefore, detecting an association between Hp genotype and CAD could greatly impact treatment considerations for a significant segment of the US population and globally. This study found that Hp 2-2 was associated with significantly higher odds of having CAD than Hp 1-1 genotype for adults with prediabetes. Specifically, these results support an additive model of increasing risk with the Hp 2 allele, whereby the presence of 1 Hp 2 allele (i.e., Hp 2-1 genotype) carries increased CAD risk, and the presence of 2 Hp 2 alleles (i.e., Hp 2-2 genotype) carries a greater CAD risk. While CAD risk for the Hp 2-1 genotype compared to the Hp 1-1 genotype was not statistically significant ($p = 0.05$), this may be a consequence of a small sample for the detected effect size. These overall results are consistent with the previous findings of Levy et al. [19], particularly in the context of an additive model of CAD risk associated with the number of Hp 2 alleles. As mentioned previously, Levy et al. [19] discovered patients with T2D and the Hp 2-2 genotype had 5 times higher odds of CAD compared to those with the Hp 1-1 genotype and 3 times higher odds compared to the Hp 2-1 genotype; their estimates lie within the respective 95 % confidence interval obtained in the current study for adults with prediabetes. Costacou et al. [11] also found an upward trend of CAD incidence from the Hp 1-1 to Hp 2-1 to Hp 2-2 genotypes in type 1 diabetes. A study of 935 individuals with diabetes, 1 year after percutaneous transluminal coronary angioplasty, found significant upward trends in acute MI, repeat stenting, and major adverse cardiovascular events among the Hp genotypes [28]. Previous studies have shown no association between cardiovascular disease outcomes and Hp genotype in individuals with normal blood sugar [19,24,29]. However, it is known that elevated blood sugar and insulin resistance increase oxidative stress and CAD risk [22,27]. Our findings support the understanding that as glycosylated hemoglobin increases, Hp 2-2 genotype further increases CAD risk. Because all individuals in our sample had prediabetes, we removed the potential confounder of glycemia contributing to CAD risk, revealing the effect of Hp genotype on CAD risk.

In this study, cases often had “healthier” laboratory results compared to controls (total, LDL, and non-HDL cholesterol and ApoB), the opposite of what would typically be expected for individuals with CAD. However, patients with CAD in the cardiovascular disease prevention clinics are treated aggressively with lifestyle and medication management, often above the standard of care. Therefore, it is plausible the cases are more aggressively treated than controls, resulting in better labs and biometrics, which should be a goal in clinical practice to improve primary through tertiary CAD prevention. Additionally, potential survivor bias from individuals with more severe CAD dying before participating in these clinics could explain the cases’ “healthier” clinical profiles. Additionally, HDL cholesterol was lower in cases, which is expected since HDL cholesterol is “protective” against CAD and more difficult to improve than the other cholesterol subtypes. This lower HDL in cases could also be attributed to the greater proportion of men among the cases as compared with the controls, as men tend to have lower HDL compared to women [26]. Similarly, the cases being taller, leaner, and having a higher prevalence of HTN and OSA are all consistent with a larger proportion of males.

Hp 2-2 has previously been shown to be associated with higher CIMT [1,12]. However, this study may not have detected differences in CIMT measurements because patients were already treated for varying lengths of time with lifestyle and medicine regimens by the time the CIMTs were performed and collected. CIMT is a well-established surrogate measure for CAD adverse events [32]. Since these patients with CAD are currently

Table 3
Demographic and clinical variables for cases and controls ($N = 178$).[a]

Variable	Overall mean ± SD or%	Case n	Case mean ± SD or%	Control n	Control mean ± SD or%	p-value
Age (years)	59.3 (8.6)	72	59.6 (8.4)	106	59.0 (8.8)	0.6372
Age (years)	59.3 ± 8.6	72	59.6 ± 8.4	106	59.0 ± 8.8	0.6732
Sex	100	72	40.5	106	59.6	**<0.0001**
Female (%)	35.96	10	5.62	54	30.34	
Male (%)	64.04	62	34.8	52	29.2	
Race	100	72	40.5	106	59.6	0.1206
White (%)	96.07	67	37.6	104	58.4	
Other (%)	3.93	5	2.8	2	1.1	
Smoking	100	72	40.45	106	59.55	0.8918
Never (%)	80.49	56	34.15	76	46.34	
Former/Current (%)	19.51	14	8.54	18	10.98	
Medical History						
Myocardial Infarction (%)	15.17	27	15.17	0	0	**<0.0001**
Hypertension (%)	71.35	58	32.58	69	38.76	**0.0251**
Hyperlipidemia (%)	98.31	72	40.45	103	57.87	0.2733
Chronic Kidney Disease (%)	1.69	2	1.12	1	0.56	0.5664
Periodontal Pathogens (%)	91.59	41	38.32	57	53.27	1
Obstructive Sleep Apnea (%)	53.68	29	30.53	22	23.16	**0.0075**
Vitamin D Deficiency (%)	89.77	65	36.93	93	52.84	0.8540
Family History of CAD (%)	75.00	52	32.5	68	42.5	0.7119
Family History of T2D (%)	47.17	29	18.24	46	28.93	0.3234
Height (in)	68.3 ± 4.6	71	69.9 ± 3.9	103	67.3 ± 4.8	**0.0002**
Weight (lbs)	184.9 ± 39.1	71	190.9 ± 32.9	106	180.9 ± 42.5	0.0805
Waist Circumference (cm)	88.0 ± 12.4	65	90.1 ± 10.2	97	86.7 ± 13.6	0.0737
Body Fat (%)	27.4 ± 9.0	52	24.9 ± 8.3	85	29.0 ± 9.1	**0.0083**
Systolic Blood Pressure (mmHg)	118.7 ± 13.5	70	118.4 ± 14.3	104	118.9 ± 13.1	0.8245
Diastolic Blood Pressure (mmHg)	73.3 ± 8.6	70	72.8 ± 9.1	104	73.7 ± 8.3	0.5326
Total Cholesterol (mg/dl)	167.7 ± 41.9	72	153.3 ± 43.3	106	177.4 ± 38.2	**0.0001**
LDL Cholesterol (mg/dl)	83.7 ± 36.5	72	74.9 ± 34.6	105	89.7 ± 36.8	**0.0077**
HDL Cholesterol (mg/dl)	64.4 ± 21.6	72	58.5 ± 18.6	106	68.5 ± 22.6	**0.0021**
Non-HDL Cholesterol (mg/dl)	103.5 ± 42.0	72	95.5 ± 40.6	106	108.9 ± 42.2	**0.0363**
Triglycerides (mg/dl)	85.2 ± 2.2	72	87.1 ± 2.2	106	83.9 ± 2.2	0.6189
Apolipoprotein B (mg/dl)	75.2 ± 25.3	70	70.3 ± 23.3	104	78.5 ± 26.2	**0.0352**
Lipoprotein(a) (mg/dl)	43.1 ± 4.4	48	48.2 ± 4.6	59	39.3 ± 4.2	0.3675
Fibrinogen (mg/dl)	301.3 ± 64.7	65	293.2 ± 62.7	91	307.1 ± 65.7	0.1850
Hs-CRP (mg/dl)	3.5 ± 0.5	72	3.2 ± 0.5	106	3.6 ± 0.5	0.0682
LPPLA-2 activity (nmol/min/ml)	105.1 ± 56.7	70	105.1 ± 50.5	106	105.1 ± 60.7	0.9931
Myeloperoxidase (pmol/L)	249.0 ± 2.3	66	235.5 ± 2.4	103	258.0 ± 2.1	0.1573
Hemoglobin A1c (%)	5.6 ± 0.3	71	5.6 ± 0.3	104	5.6 ± 0.3	0.1105
Variable	Overall mean ± SD or%	Case n	Case mean ± SD or%	Control n	Control mean ± SD or%	p-value
Fasting Insulin (mIU/L)	7.4 ± 1.2	45	7.5 ± 1.2	71	7.4 ± 1.1	0.8333
Oral Glucose Tolerance Test						
Fasting Glucose (mg/dl)	99.4 ± 10.4	72	99 ± 11.2	106	99.7 ± 9.9	0.6600
1-Hour Glucose (mg/dl)	157.3 ± 46.1	38	161.8 ± 37.9	49	153.9 ± 51.7	0.4280
2-Hour Glucose (mg/dl)	118.5 ± 38.0	39	121.1 ± 39	51	116.5 ± 37.6	0.5719
White Blood Cells (K/uL)	5.4 ± 1.4	71	5.3 ± 1.5	99	5.4 ± 1.3	0.6038
Carotid Intima Media Thickness						
Arterial Age	60.7 ± 12.0	72	61.1 ± 13.3	106	60.4 ± 11.1	0.7072
Age at Scan	59.6 ± 8.7	72	60.0 ± 8.5	106	59.3 ± 8.8	0.5988
Mean Common	0.75 ± 0.16	72	0.78 ± 0.19	106	0.73 ± 0.12	0.0813
Mean Maximum	0.85 ± 0.15	68	0.87 ± 0.16	104	0.83 ± 0.14	0.1045
Plaque Burden	2.5 ± 1.0	71	2.6 ± 1.1	105	2.4 ± 0.9	0.5245

Note: Totals may not add up to 178 due to missingness of data. CAD=coronary artery disease, T2D=Type 2 diabetes, LDL=low-density lipoprotein, HDL=high-density lipoprotein, Hs-CRP=high-sensitivity C-reactive protein, LPPLA-2=lipoprotein-associated phospholipase-A2.

[a] Chi-square and Fisher's exact two tailed tests for categorical variables and *t*-tests for continuous variables.

treated, the lack of association between CIMT and Hp genotype could indicate their risk of adverse CAD events was less at the time of their scan.

While the Hp 2-2 genotype group had a lower A1c, the difference of 0.15–0.17 % is not clinically significant, and no other differences in glucose were present. Therefore, this result may be attributed to sampling. Notably, this sample had lower average glucose than many individuals who clinicians consider prediabetic. The average hemoglobin A1c and fasting glucose were 5.60 % and 99.38 mg/dl, respectively. These averages are within normal range, although barely, but patients were considered prediabetic if only 1 glucose value was out of range. Based on previous studies and the physiology of oxidative stress, we would expect a smaller effect of the Hp 2-2 genotype as the glucose decreases. Previous studies have only seen differences in cardiovascular risk based on Hp genotypes in diabetes [19,24,29]; however, these studies dichotomized glycemia, which may conceal differences earlier in the continuum of abnormal glucose. This is the first published study to show an association between CAD and the Hp 2-2 genotype with prediabetes even though this sample was barely prediabetic based on their blood sugar averages.

Based on these results, clinicians may wish to consider testing Hp genotype for all patients with suspected insulin resistance, prediabetes, or T2D. Hp genotyping is now available through laboratories (for approximately 50 USD), increasing ease and access to testing. Clinicians

Table 4
Demographic and clinical variables for haptoglobin genotype ($N = 178$).[a]

Variable	Overall mean ± SD or%	Hp 1-1 n	Hp 1-1 mean ± SD or%	Hp 2-1 n	Hp 2-1 mean ± SD or%	Hp 2-2 n	Hp 2-2 mean ± SD or%	p-value
Age (years)	59.3 ± 8.6	29	60.5 ± 8.2	98	59.2 ± 8.8	51	58.7 ± 8.6	0.6641
Sex	100	29	16.29	98	55.06	51	28.65	0.7975
Female (%)	35.96	9	5.06	37	20.79	18	10.11	
Male (%)	64.04	20	11.24	61	34.27	33	18.54	
Race[b]	100							
White (%)	96.07	29	16.29	94	52.81	48	26.97	
Other (%)	3.93	0	0	4	2.25	3	1.69	
Smoking	100							0.3681
Never (%)	80.49	18	10.98	76	46.34	38	23.17	
Former/Current (%)	19.51	6	3.66	14	8.54	12	7.32	
Medical History								
Myocardial Infarction (%)	15.17	4	2.25	13	7.3	10	5.62	0.5771
Hypertension (%)	71.35	19	10.67	70	39.33	38	21.35	0.6935
Hyperlipidemia (%)	98.31	29	16.29	96	53.93	50	28.09	1
Chronic Kidney Disease (%)[b]	1.69	1	0.56	1	0.56	1	0.56	0.5731
Periodontal Pathogens (%)	91.59	15	14.02	56	52.34	27	25.23	1
Obstructive Sleep Apnea (%)	53.68	9	9.47	28	29.47	14	14.74	0.6516
Vitamin D Deficiency (%)	89.77	26	14.77	89	50.57	43	24.43	0.5516
Family History of CAD (%)	75.00	20	12.50	65	40.63	35	21.88	0.5829
Family History of T2D (%)	47.17	9	5.66	45	28.30	21	13.21	0.5700
Height (in)	68.3 ± 4.6	28	68.6 ± 5.4	95	68.1 ± 4.7	51	68.7 ± 4.2	0.6669
Weight (lbs)	184.9 ± 39.1	29	184.7 ± 32.5	97	188.8 ± 41.7	51	177.6 ± 37.1	0.2522
Waist Circumference (cm)	88.0 ± 12.4	25	88.4 ± 10.5	90	89.1 ± 13.12	47	85.8 ± 12.0	0.3214
Body Fat (%)	27.4 ± 9.0	22	27.8 ± 8.4	75	28.1 ± 9.6	40	26.1 ± 8.0	0.3691
Systolic Blood Pressure (mmHg)	118.7 ± 13.5	28	117.3 ± 12.5	97	119.9 ± 12.9	49	117.2 ± 15.2	0.4240
Diastolic Blood Pressure (mmHg)	73.3 ± 8.6	28	71.6 ± 8.2	97	74.4 ± 9.2	49	72.2 ± 7.5	0.1690
Total Cholesterol (mg/dl)	167.7 ± 41.9	29	167.8 ± 43.8	98	166.6 ± 43.2	51	169.7 ± 39.1	0.9151
LDL Cholesterol (mg/dl)	83.7 ± 36.5	29	80.8 ± 41.8	97	84 ± 36.6	51	84.8 ± 33.8	0.8886
HDL Cholesterol (mg/dl)	64.4 ± 21.6	29	68.9 ± 24.3	98	62.5 ± 21.1	51	65.1 ± 20.7	0.2640
Non-HDL Cholesterol (mg/dl)	103.5 ± 42.0	29	97.9 ± 44.1	98	104.6 ± 43.4	51	104.6 ± 38.7	0.7358
Triglycerides (mg/dl)	85.2 ± 2.2	29	81.2 ± 2.0	98	84.3 ± 2.3	51	89.4 ± 2.1	0.6792
Apolipoprotein B (mg/dl)	75.2 ± 25.3	29	72.4 ± 26.6	95	74.8 ± 26.3	50	77.5 ± 22.7	0.6769
Lipoprotein(a) (mg/dl)	43.1 ± 4.4	16	57.6 ± 4.7	55	42.8 ± 4.3	36	38.2 ± 4.4	0.5059
Fibrinogen (mg/dl)	301.3 ± 64.7	25	292.7 ± 72	83	304 ± 60.5	48	301.3 ± 68.6	0.7517
Variable	**Overall mean ± SD or%**	**Hp 1-1 n**	**Hp 1-1 mean ± SD or%**	**Hp 2-1 n**	**Hp 2-1 mean ± SD or%**	**Hp 2-2 n**	**Hp 2-2 mean ± SD or%**	**p-value**
Hs-CRP (mg/dl)	3.5 ± 0.5	29	3.1 ± 0.3	98	3.6 ± 0.5	51	3.4 ± 0.6	0.2960
LPPLA-2 activity (nmol/min/ml)	105.1 ± 56.7	29	103.5 ± 60.9	96	102.4 ± 57.5	51	111.0 ± 53.3	0.6722
Myeloperoxidase (pmol/L)	249.0 ± 2.3	28	249.5 ± 1.7	93	256 ± 2.2	48	235.3 ± 2.6	0.5062
Hemoglobin A1c (%)	5.6 ± 0.3	29	5.6 ± 0.3	96	5.7 ± 0.3	50	5.5 ± 0.4	0.0084
Fasting Insulin (mIU/L)	7.4 ± 1.2	21	7.9 ± 1.2	63	7.5 ± 1.2	32	6.9 ± 1.1	0.7107
Oral Glucose Tolerance Test								
Fasting Glucose (mg/dl)	99.4 ± 10.4	29	98.5 ± 12.3	63	99.4 ± 9.6	51	99.8 ± 10.9	0.8745
1-Hour Glucose(mg/dl)	157.3 ± 46.1	11	159.5 ± 43.9	47	156.8 ± 49.7	29	157.4 ± 42.4	0.9843
2-Hour Glucose (mg/dl)	118.5 ± 38.0	11	126.2 ± 38.9	49	117.5 ± 36.2	30	117.2 ± 41.5	0.7760
White Blood Cells (K/uL)	5.4 ± 1.4	27	5.4 ± 1.5	92	5.4 ± 1.3	51	5.2 ± 1.5	0.7358
Carotid Intima Media Thickness								
Arterial Age	60.7 ± 12.0	29	61.6 ± 12.2	98	60.9 ± 11.9	51	59.7 ± 12.3	0.7741
Age at Scan	59.6 ± 8.7	29	61 ± 7.4	98	59.6 ± 9	51	58.7 ± 8.8	0.5238
Mean Common	0.75 ± 0.16	29	0.77 ± 0.17	98	0.75 ± 0.17	51	0.73 ± 0.12	0.6171
Mean Maximum	0.85 ± 0.15	29	0.86 ± 0.18	94	0.85 ± 0.14	49	0.84 ± 0.14	0.7504
Plaque Burden	2.5 ± 1.0	29	2.6 ± 1.0	97	2.5 ± 0.9	50	2.5 ± 1.0	0.9466

Note: totals may not add up to 178 due to missingness of data. CAD=coronary artery disease, T2D=Type 2 diabetes, LDL=low-density lipoprotein, HDL=high-density lipoprotein, Hs-CRP=high-sensitivity C-reactive protein, LPPLA-2=lipoprotein-associated phospholipase-A2.

[a] Chi-square and Fisher's exact two tailed tests for categorical variables and ANOVA for continuous variables.

[b] Data are too sparse to analyze.

should note that Hp genotyping is a 1-time test with potentially significant implications for predicting CAD risk.

5.1. Limitations

Limitations of this study included a small sample size and a completely prediabetic sample, which limits generalizability and could impact the effect size of the Hp genotype on CAD risk. Furthermore, many of these patients diagnosed with CAD have subclinical disease found on coronary imaging (e.g., coronary artery calcium score); therefore, this may produce a smaller effect size than using hard CAD outcomes as previous studies have done (e.g., MI or CAD death). Additionally, due to the design of retrospective data collection from established health records in this case control design, temporal relationships cannot be established between the variables and CAD. However, because the Hp genotype is present across the lifespan, this study is able to suggest an association between Hp genotype and CAD prevalence.

Patients who participate in cardiovascular disease prevention clinics may be inherently healthier than those who do not seek this service. Therefore, the sample may be healthier with lower CIMT measurements and inflammatory biomarkers than in the general population, which could lower the effects seen between the Hp genotypes. Conversely, patients who choose to seek out cardiovascular disease prevention

clinics may be at higher risk of CAD; therefore, the controls may not be representative of a disease-free population.

5.2. Future direction of studies

Future studies should be developed to assess interventions to reduce oxidative stress and CAD associated with the Hp 2-2 genotype in prediabetes. Specifically, supplementing daily vitamin E (which has shown to reduce CAD in T2D [7,23]) and reducing zonulin (a protein that increases endothelial permeability) with gluten-free nutrition could be explored in the context of this study. More extensive studies should be performed for the prediabetic population to understand better the Hp genotypes' effect on CAD risk and using surrogate measures that would allow earlier intervention and prevention. Clinically, feasibility studies with healthcare providers could investigate ways to incorporate Hp genotyping into mainstream patient care.

6. Conclusion

Hp genotyping is a 1-time, clinically available test that may significantly affect the CAD risk prediction of individuals. Cardiologists, primary care providers, and endocrinologists should consider using this test in routine clinical practice, especially for individuals with prediabetes or T2D. This study is the first to show that the Hp 2-2 genotype is associated with higher CAD odds in individuals with prediabetes. When obtained in clinical practice, these genotypic results could help guide interventions (e.g., strict glucose control, vitamin E, and gluten-free nutrition) at a much earlier age in life, time point on the diabetes pathophysiologic continuum, and place in the CAD process to hopefully delay CAD development and progression.

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CRediT authorship contribution statement

Emily Kate Mewborn: Conceptualization, Data curation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Elizabeth Ann Tolley:** Formal analysis, Writing – review & editing. **David Bruce Wright:** Methodology, Data curation, Writing – review & editing. **Amy Lynn Doneen:** Methodology, Data curation, Writing – review & editing. **Margaret Harvey:** Writing – review & editing. **Ansley Grimes Stanfill:** Formal analysis, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Supplementary materials

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