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Replication and meta-analysis of the gene-environment interaction between body mass index and the interleukin-6 promoter polymorphism with higher insulin resistance

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ABSTRACT

Insulin resistance (IR) is a complex disorder caused by an interplay of both genetic and environmental factors. Recent studies identified a significant interaction between body mass index (BMI) and the rs1800795 polymorphism of the interleukin-6 gene that influences both IR and onset of type 2 diabetes mellitus, with obese individuals homozygous for the C allele demonstrating the highest level of IR and greatest risk for type 2 diabetes mellitus. Replication of a gene-environment interaction is important to confirm the validity of the initial finding and extend the generalizability of the results to other populations. Thus, the objective of this study was to replicate this gene-environment interaction on IR in a hypertensive population and perform a meta-analysis with prior published results. The replication analysis was performed using white individuals with hypertension from the Hypertensive Pathotype cohort (N = 311), genotyped for rs1800795. Phenotype studies were conducted after participants consumed 2 diets—high sodium (200 mmol/d) and low sodium (10 mmol/d)—for 7 days each. Measurements for plasma glucose, insulin, and interleukin-6 were obtained after 8 hours of fasting. Insulin resistance was characterized by the homeostatic model assessment (HOMA-IR). In Hypertensive Pathotype, BMI was a significant effect modifier of the relationship between rs1800795 and HOMA-IR; higher BMI was associated with higher HOMA-IR among homozygote CC individuals when compared with major allele G carriers ($P = .003$). Furthermore, the meta-analysis in 1028 individuals confirmed the result, demonstrating the same significant interaction between rs1800795 and BMI on HOMA-IR ($P = 1.05 \times 10^{-6}$). This rare replication of a gene-environment interaction extends the generalizability of the results to hypertension while highlighting this polymorphism as a marker of IR in obese individuals.

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1. Introduction

Chronic inflammation plays a role in the development of many cardiometabolic diseases including type 2 diabetes mellitus (T2DM), insulin resistance (IR), and hypertension (HTN) [1-3]. The pleiotropic cytokine, interleukin-6 (IL-6), is a major player in the pathophysiology of chronic inflammation [4]; and systemic levels of IL-6 are positively associated with T2DM, IR, and HTN [2,5]. It is evident that the interplay of genetic and environmental factors contributes to the proinflammatory process present in cardiometabolic diseases; however; data on the role of the IL-6 gene in these processes conflict [6-10]. Recent studies clarify these conflicting results by demonstrating that the association between rs1800795- a guanine (G) to cytosine (C) nucleotide change in the promoter region of the IL-6 gene- and IR is modified by body mass index (BMI), with the C allele associated with higher IR and T2DM in individuals with obesity [11-13].

Although the coaggregation of IR and HTN is genetically linked and their association increased in obese individuals [14], the association of rs1800795 with IR has not been demonstrated in HTN. Identifying this relationship in HTN would reaffirm the connection between IL-6, obesity, and HTN in humans and extend the generalizability of this gene-environment interaction on IR. Thus, the objective of this study was to (1) examine the association of rs1800795 with IR in HTN, (2) determine whether BMI modifies this association, and (3) perform a meta-analysis of the Hypertensive Pathotype (HyperPATH) data and prior published results [11] to confirm that the rs1800795-BMI interaction on IR exists in a larger population.

2. Methods and procedures

2.1. *HyperPATH participants*

The 311 participants studied were part of the HyperPATH Protocol. All participants were white with data available for IL-6 genotype and homeostatic model assessment of insulin resistance (HOMA-IR)[15]. Population characteristics are listed in Table 1. Serum IL-6 was available in a subset (n = 130 high sodium [HS]; n = 144 low sodium [LS]). Although results from the HyperPATH have been reported previously [14,16-19], the present analyses are original.

The protocol was approved by the institutional review boards of each site, and informed consent was obtained before enrollment.

2.2. *HyperPATH Protocol*

Details of this protocol have been described previously [14,17]. In brief, participants completed 2 diets for 7 days each: HS (200 mmol/d) and LS (10 mmol/d), with each diet also containing 100 mmol/d potassium and 20 mmol/d calcium. Antihypertensive medications were stopped at least 3 weeks before study evaluation. Exclusion criteria included participants with known secondary HTN, T2DM, coronary artery disease, stroke, current tobacco or illicit drug use, or alcohol intake more than 12 oz/wk [16]. On the final day of each diet, participants were admitted to the Human Research Center. Insulin, glucose, and blood pressure (BP) were measured between 8:00 AM and 10:00 AM as previously described after participants remained fasting and supine overnight [14]. Interleukin-6 levels were also collected concurrently and measured using a quantitative enzyme-linked immunoassay (R&D Systems, Minneapolis, MN).

Table 1 – Clinical characteristics of the HyperPATH cohort on both HS and LS diets

HyperPATH cohort baseline characteristics			
	HS	LS	*P* value
Age (y)	49.0±8.1		–
BMI (kg/m^2)	28.07 ±3.8		–
Female (%)	127 (41%)		–
Fasting glucose (mg/dL)	90.0±15	94.0±16	.006
Fasting insulin (mg/dL)	7.9±7.2	9.6±8.0	<.0001
HOMA-IR	1.82±1.73	2.27±2.12	.0005
Systolic BP (mm HG)	146.1±19.3	131.2±17.2	<.0001
Diastolic BP (mm Hg)	86.93±11.14	78.9±9.99	<.0001
IL-6 (pg/mL)	2.13±1.33	1.9±1.67	.5
IL-6 G>C (rs1800795) genotype			
GG	110 (35%)		–
GC	142 (46%)		–
CC	59 (19%)		–

Mean ± SD for normally distributed continuous variables; median ± interquartile range for nonnormal continuous variables (glucose, insulin, HOMA-IR); percentages for categorical variables.

2.3. *Genotyping in the HyperPATH cohort*

DNA was extracted and genotyped as previously described [18]. We analyzed rs1800795 to replicate previous findings related to this variant [11,12]. Genotyping for this single nucleotide polymorphism (SNP) had a completion rate of 97%. Repeat genotyping for 10% of the SNPs on this platform demonstrated concordance with the original genotype call.

2.4. *Phenotypes examined*

The continuous variable, HOMA-IR, was analyzed as the primary outcome for both the replication analysis and meta-analysis.

2.5. *Statistical analyses*

Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC). A χ^2 test evaluated Hardy-Weinberg equilibrium for the SNP. The HyperPATH population characteristics measured after HS and LS diets were compared using a paired *t* test. Nonnormally distributed variables (insulin, glucose, HOMA-IR index) are shown as median values with the interquartile range and compared using the nonparametric Wilcoxon ranks test. All linear regression models were performed with the continuous independent variable HOMA-IR as the outcome and accounted for age, sex, BMI, sibling relatedness, and study site (PROC MIXED). The natural-log of HOMA-IR and IL-6 levels was used to meet normality assumptions. To enable direct comparisons with prior reports,

we assumed a dominant genetic model (GG/GC = 0, CC = 1) [11,12]. All statistical models conducted as a replication used a 1-sided test. All other statistical tests were 2-sided. Significance is indicated for $P < .05$.

2.6. Meta-analysis

The meta-analysis was conducted using data collected from (1) HyperPATH and (2) previously published data from the Framingham Heart Study (FHS) [11]. The following information was extracted from both: (1) sample size and (2) *P* value of the interaction between rs1800795 and BMI on HOMA-IR using a multivariate model. In the FHS, the multivariate model was conducted in men only and accounted for age, BMI, smoking status, physical activity, and alcohol use. The multivariate model for HyperPATH is described above.

The meta-analysis was conducted using a weighted z score method using the METAL software package (http//www.sph.umich.edu/csg/abecasis/metal/). METAL accounts for both the direction of association relative to the reference allele and the sample size of each population. The *P* values from each study are converted to z scores, and a sum of z scores is calculated and weighted by the square root of each study's sample size. The resulting sum is divided by the square root of the total sample size to obtain an overall z statistic [20].

3. Results

3.1. Genetic association with HOMA-IR in HyperPATH: primary phenotype

In the HyperPATH population, rs1800795 was in Hardy-Weinberg equilibrium and had a minor allele (C allele) frequency of 42%. Baseline characteristics demonstrated that sodium intake significantly affected HOMA-IR and BP values, but had no effect on circulating IL-6 levels (Table 1). Multivariate linear regression demonstrated that rs1800795 was independently associated with HOMA-IR on both diets, with higher HOMA-IR observed in the CC genotype (HS, $P = .01$; LS, $P = .01$). In a subset of individuals with available serum IL-6 levels, the multivariate model was repeated to include IL-6 levels as a predictor of HOMA-IR. Serum IL-6 levels did not significantly contribute to the variance of HOMA-IR on either diet (HS, $P = .7$; LS, $P = .9$). Furthermore, when the association of rs1800796 with IL-6 levels was tested, IL-6 levels did not differ significantly by genotype (data not shown).

3.2. Genetic association with HOMA-IR in HyperPATH: replication of SNP and BMI interaction

To replicate previous findings, we examined whether an interaction between rs1800795 and BMI existed in the HyperPATH cohort. A significant interaction between rs1800795 and BMI existed on both HS ($P = .003$) and LS ($P = .004$) diets (Fig. 1). CC individuals had greater HOMA-IR values than G allele carriers at higher BMI levels (Fig. 1). When dichotomized by sex, the HyperPATH population demonstrated similar trends for the SNP-BMI interaction, suggesting that sex did not affect the outcome in our population.

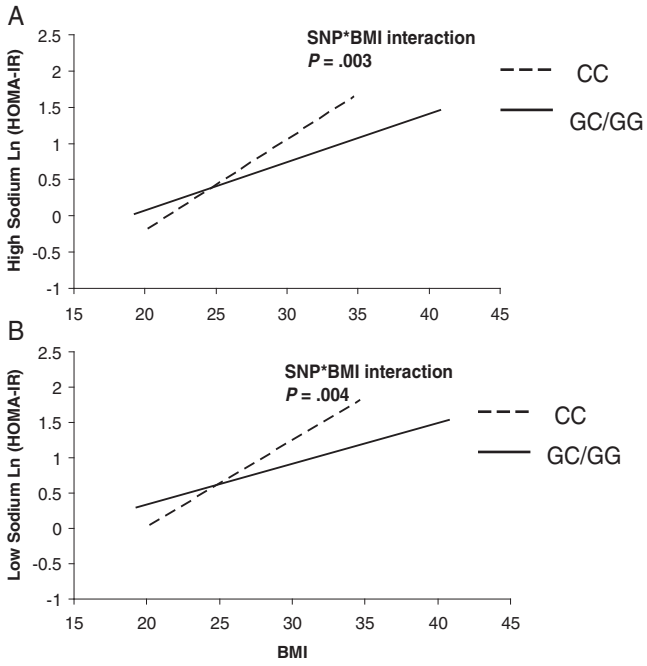

Fig. 1 – The interaction between rs1800795 and BMI on HOMA-IR in the HyperPATH cohort on HS and LS diet. The graph depicts the unadjusted data against the predicted lines for rs1800795 using log HOMA-IR as the dependent variable. The *P* value depicts the interaction for rs1800795 and BMI accounting for age, sex, BMI, sibling relatedness, and study site on (A) HS and (B) LS dietary intake.

Because the renin-angiotensin-aldosterone system is highly implicated in the pathophysiology of HTN and IR and prior investigations demonstrate that LS diet raises HOMA-IR [17], we examined whether dietary sodium influenced the SNP-BMI interaction; however, we observed no difference between diets (Fig. 1).

3.3. Meta-analysis

A meta-analysis of the HyperPATH and previously reported FHS cohorts was performed. As expected, a significant interaction between rs1800795 and BMI existed and contributed the variance of HOMA-IR. CC individuals had greater

Table 2 – Results of the meta-analysis

Results of meta-analysis of rs1800795*BMI interaction on HOMA-IR

	SNP	n	SNP*BMI interaction *P* value	Meta *P* value
HyperPATH	rs1800795	311	0.003[a]	$P = 1.05 \times 10^{-6}$
FHS	rs1800795	717	0.0001[b]	

[a] *P* value for interaction of rs1800795 and BMI on HOMA-IR accounting for age, sex, BMI, sibling relatedness, and study site and controlling for smoking status, physical activity, and alcohol use.
[b] *P* value for interaction of rs1800795 and BMI on HOMA-IR in unrelated men accounting for age, BMI, smoking status, physical activity, and alcohol use.

HOMA-IR values than G allele carriers at higher BMI levels ($P = 1.05 \times 10^{-6}$) (Table 2).

4. Discussion

Our study demonstrates a significant association between rs1800796 and HOMA-IR in a hypertensive cohort and indicates that this association is modified by BMI. This gene-environment interaction is consistent with findings from prior studies and confirmed via meta-analysis. This study furthers the generalizability of the rs1800796-BMI interaction on the association of IR and implicates inflammation and obesity as intertwined contributors to IR.

Many studies report a significant association between rs1800796 and IR; however, the allele associated with increased IR differs by population. For example, the G allele has been associated with T2DM in lean male subjects [8] and fasting glucose in a meta-analysis [21]; however, an SNP-BMI interaction was never examined. Interestingly, when the study is conducted in a heavier population or when a BMI-SNP interaction is considered, the C allele is associated with IR [11,12]. We confirm this latter finding in our hypertensive population demonstrating that the BMI-SNP interaction influences IR. Our findings also support previous reports that the C allele is associated with the metabolic syndrome [22,23], a population that exhibits both IR and HTN. Furthermore, we demonstrate that, similar to other studies, IL-6 levels did not appear to influence this association [11,12], albeit we were likely underpowered to detect this effect because very few circulating IL-6 levels were available in our study.

Because of the associative nature of this study, we are unable to determine the direct mechanism underlying the results. However, it is likely that the known relationship between inflammation, obesity, and IR in humans is involved. Obesity and HTN are associated with a proinflammatory state [1,2], and it has been suggested that increased adiposity may lead to higher IL-6 levels and worse IR [24]. However, this study and others found no relationship between rs1800795 and circulating IL-6 levels [11,12]. As described previously, it is unknown whether average, peak, or inflammatory levels of IL-6 are contributing to IR; and fasting IL-6 measurements may not be appropriate for examining the identified genotype-phenotype relationship [11]. Furthermore, because IL-6 is involved in a multitude of inflammatory processes, it is possible that this SNP is directly related to other genes and/or other inflammatory pathways contributing to IR.

This study has several limitations. First, the sample size is relatively small; however, our findings are consistent with prior reports, and the meta-analysis adds power and validity to the finding. The cross-sectional nature of this analysis limits us from drawing conclusions of causality or directionality. Further studies are necessary to determine the functionality of this genetic variant in HTN and whether haplotype analyses demonstrate different results. Strengths of the analysis include (1) use of the HyperPATH cohort representing a distinct hypertensive population with extensive phenotyping including antihypertensive medication washout and (2) use of a meta-analysis approach to validate prior findings.

In summary, this study demonstrates that rs1800795 of the IL-6 gene promoter is associated with IR in a white hypertensive cohort, this association is modified by BMI, and this gene-environment interaction was confirmed via meta-analysis. These findings highlight the roles of adiposity and inflammation in the process of IR. Furthermore, this study implicates this polymorphism as a marker of IR in obese individuals with and without HTN and may potentially identify individuals in whom IR may improve with exercise and/or weight reduction [25]. Using genomic markers to identify and design targeted prevention and treatment strategies for individuals most at risk for IR is essential to implement personalized medicine and hopefully improve clinical outcomes. The identification of this SNP as a marker for IR in obese individuals from multiple cohorts is the first step toward initiating such targeted care.

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Conflict of Interest

The authors have no disclosures.

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