Research: Genetics

Association between salivary amylase (*AMY1*) gene copy numbers and insulin resistance in asymptomatic Korean men

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Abstract

Aims Salivary amylase gene (*AMY1*) copy number variations (CNVs) correlate directly with salivary amylase activity and serum amylase levels. Previously, individuals with high *AMY1* CNVs exhibited low postprandial glucose levels and postprandial early insulin surge, suggesting that high *AMY1* gene copy numbers may play a role in lowering the risk of insulin resistance.

Methods We verified the relationship between *AMY1* CNVs and homeostatic model assessment–insulin resistance (HOMA-IR) in a cohort of 1257 Korean men aged 20–65 years who visited two medical centres for regular health check-ups, and in subgroups of current smokers and regular alcohol drinkers. Individuals with fasting plasma glucose levels > 10.0 mmol/l, $HbA_{1c} \geq 64$ mmol/mol (8.0%) or who used oral hypoglycaemic agents or insulin were excluded.

Results *AMY1* CNVs correlated negatively with HOMA-IR even after adjusting for covariates (e.g. BMI, systolic blood pressure, triacylglycerol, alcohol consumption, smoking and physical activity). When the participants were divided according to current smoking and alcohol consumption habits, negative correlations between *AMY1* CNVs and HOMA-IR were more evident among non-smokers and regular drinkers and were non-significant among smokers and non-regular drinkers.

Conclusions Low *AMY1* CNVs correlated with high insulin resistance in asymptomatic Korean men, and such a relationship presented differently according to the status of smoking and alcohol consumption.

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Introduction

Type 2 diabetes mellitus is a serious global health burden. In 2013, ~ 382 million people in 130 countries worldwide had diabetes, and the number is expected to increase to 592 million by 2035 [1]. The underlying mechanisms and risk factors of diabetes are crucial to the development of better prevention and management regimens. Among the many aetiological aspects associated with Type 2 diabetes, insulin resistance has elicited the most attention.

In recent years, a few researchers have focused on salivary amylase in the context of diabetes and metabolic syndrome. Although amylase is a well-known key enzyme in carbohydrate digestion, its physiological role in digestion remains to be elucidated; in particular, its relationship with insulin resistance and diabetes remains largely unknown [2]. Salivary and serum amylase levels are known to correlate positively with copy numbers of the *AMY1* gene, which encodes salivary amylase [3–5]. *AMY1* copy numbers were high in starch-consuming communities (agricultural societies), whereas they were low in hunter–gatherer societies [5], which suggests that *AMY1* gene copy number variations (CNVs) are naturally selected to support starch digestion. A previous study has shown that individuals with high *AMY1* CNVs had high salivary amylase activity, and exhibited lower postprandial blood glucose and high insulin levels shortly after starch consumption, compared with individuals with low *AMY1* CNVs [4]. Chinese and Japanese studies have shown associations between a low serum amylase activity and an increased risk of diabetes, high insulin resistance and obesity [6,7]. Among modifiable lifestyle

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What's new?

- Our study is the first to show the direct relationship between *AMY1* copy numbers and homeostatic model assessment–insulin resistance (HOMA-IR) in healthy Korean men.

- The negative correlation was consistent and significant in the subgroup of non-smokers and regular alcohol drinkers, but not significant in the subgroup of smokers and non-regular drinkers.

- This provides an additional clue suggesting some kind of mechanism through which salivary amylase acts on insulin sensitivity, although its role has been somewhat underestimated so far.

factors, cigarette smoking is known to increase insulin resistance [8–11] and the risk of Type 2 diabetes [12–14]. By contrast, alcohol consumption is known to have a protective effect on insulin sensitivity [15–19].

Although salivary amylase has become known as a factor that might influence insulin resistance and diabetes, neither the direct relationship between *AMY1* CNVs and insulin resistance nor the interactions between the *AMY1* gene and smoking and alcohol drinking have been specifically studied. To the best of our knowledge, in this study we verified for the first time a relationship between *AMY1* CNVs and insulin resistance and this relationship differed according to the participants' cigarette smoking and alcohol consumption statuses.

Methods

Study population

Participants included adult men aged 20–65 years who visited the Center for Health Promotion and Disease Prevention at Seoul National University Hospital or Gangnam Center for Health Promotion, affiliated with Seoul National University Hospital, for regular health check-ups from December 2009 to November 2011. This study was originally designed to investigate the associations between abdominal obesity, metabolic syndrome and copy numbers of various genes. Hence, factors that might influence body weight were excluded. The exclusion criteria targeted individuals who had: (1) been diagnosed with hyperthyroidism or hypothyroidism; (2) taken medication or undergone a procedure or operative treatment for obesity within three months of the time of enrolment; (3) experienced weight loss exceeding 10% of their original body weight within the three months; (4) a medical history of stroke, cardiovascular diseases, diabetes mellitus treated with medication, cancer or abdominal surgery; (5) been taking medication, including thyroid drugs, antipsychotics, obesity treatment drugs

(including herbal medicine), oral hypoglycaemic agents and insulin, and steroids, within the previous month; (6) a fasting plasma glucose level > 10.0 mmol/l and HbA$_{1c}$ ≥ 64 mmol/mol (8.0%); and/or (7) any condition considered inappropriate for study enrolment. Application of these exclusion criteria yielded 1454 participants, from among whom the final analysis included 1257 participants without missing data. The study was approved by the Institutional Review Board at Seoul National University Hospital (IRB No. H-0911-010-299).

Measurements

Anthropometric values such as height, weight and waist circumference were measured by trained medical personnel and BMI (kg/cm^2) was subsequently computed. Blood pressure (BP) was measured using a standardized method. Blood tests were conducted after ≥ 8 h of fasting and included: fasting plasma glucose (mg/dl, converted to mmol/l), HbA$_{1c}$ (%, converted to mmol/mol), serum insulin (μIU/ml) and a lipid panel comprising total cholesterol (mg/dl, converted to mmol/l), HDL-C (mg/dl, converted to mmol/l), LDL-C (mg/dl, converted to mmol/l) and triacylglycerol (mg/dl, converted to mmol/l). The homeostasis model assessment of insulin resistance (HOMA-IR) was computed as glucose (mg/dl) × insulin (μIU/ml)/405, as described previously [20].

Participants filled out a standardized questionnaire routinely used for health check-ups at the Center for Health Promotion and Disease Prevention at Seoul National University Hospital; the questionnaire included items regarding smoking, alcohol intake, leisure time physical activities and previous medical history. Smoking status was classified as current smokers and currently non-smokers; the latter included never smokers and past smokers who had quit smoking at least two months prior to study participation. Regular alcohol consumption was defined as drinking > 8.5 g of alcohol per week. Non-regular alcohol consumption was defined as drinking ≤ 8.5 g of alcohol per week. Leisure time physical activities were categorized as low, moderate and high according to the International Physical Activity Questionnaire (IPAQ) long form, and the moderate and high categories were combined. The participants' characteristics are presented in Table 1. We additionally administered a dietary questionnaire among 206 participants and derived the daily calorific intake (kcal/day) and carbohydrate intake (g/day).

Copy number estimation

We performed quantitative real-time PCR using the general protocol with the Applied Biosystems 7900HT instrument (Applied Biosystems, Carlsbad, CA, USA) to measure the copy number of *AMY1* gene. We used a TaqMan Copy Number Assay and Copy Number Reference Assay duplex reaction system. In order to target the *AMY1* gene, we used

Table 1 Clinical characteristics of subjects

| | HOMA–IR | | | |
	≤ 1.72	> 1.72	Total	P
N	746	511	1257	
AMY1 copy number, mean (SD)	6.58 (2.39)	6.31 (2.02)	6.47 (2.25)	0.037
Age, mean (SD), year	49.0 (6.94)	48.8 (6.74)	48.9 (6.86)	0.49
BMI, mean (SD), kg/m^2	23.6 (2.37)	26.0 (2.60)	24.5 (2.73)	< 0.001
Waist circumference, mean (SD), cm	85.6 (6.83)	92.1 (6.79)	88.3 (7.51)	< 0.001
Systolic BP, mean (SD), mmHg	121 (13.9)	127 (14.1)	124 (14.2)	< 0.001
Diastolic BP, mean (SD), mmHg	77.1 (10.3)	81.4 (10.3)	78.9 (10.5)	< 0.001
Fasting plasma glucose, mean (SD), mmol/l	4.95 (0.63)	5.77 (1.22)	5.28 (1.00)	< 0.001
HbA$_{1c}$, mean (SD), mmol/mol	39.2 (4.33)	42.4 (8.57)	40.5 (6.59)	< 0.001
HbA$_{1c}$, mean (SD), %	5.73 (0.397)	6.03 (0.784)	5.85 (0.603)	< 0.001
Insulin, mean (SD), μIU/ml	4.62 (1.79)	11.2 (4.66)	7.27 (4.59)	< 0.001
HOMA–IR, mean (SD)	1.02 (0.414)	2.88 (1.56)	1.78 (1.38)	< 0.001
Total cholesterol, mean (SD), mmol/l	5.10 (0.88)	5.34 (0.96)	5.18 (0.92)	< 0.001
Triacylglycerol, mean (SD), mmol/l	1.20 (0.76)	1.88 (1.25)	1.47 (1.05)	< 0.001
HDL-C, mean (SD), mmol/l	1.34 (0.30)	1.25 (0.30)	1.31 (0.30)	< 0.001
LDL-C, mean (SD), mmol/l	3.37 (0.83)	3.50 (0.81)	3.42 (0.83)	0.0048
Current smoking, frequency (%)				
Non-current smokers*	478 (59.6)	324 (40.4)	802 (100)	0.81
Current smokers	268 (58.9)	187 (41.1)	455 (100)	
Alcohol consumption, frequency (%)				
Non-regular drinkers (≤ 8.5 g/week)	144 (56.3)	112 (43.8)	256 (100)	0.26
Regular drinkers (> 8.5 g/week)	602 (60.1)	399 (40.0)	1001 (100)	
Physical activity, frequency (%), category†				
Mild	291 (55.5)	233 (44.5)	524 (100)	0.022
Moderate to high	453 (62.0)	278 (38.0)	731 (100)	

Statistical analysis to test the difference between the group of HOMA–IR ≤ 1.72 and > 1.72 in continuous variables was carried out by *t*-test. For categorical variables such as physical activity, alcohol consumption, smoking, Student's chi-squared test was used.
*Non-current smokers included never smokers and past smokers who quit smoking at least two months before participating in the study.
†Degree of physical activity was categorized into low, moderate and high by International Physical Activity Questionnaire (IPAQ) long form. Moderate and high categories were combined.

the primer and probe of the TaqMan qPCR assay (Hs07226362_cn). For adjustment of DNA dilution quantity variation, we performed TaqMan Copy Number Reference Assay (*RNaseP*). Samples were run in triplicate. We estimated copy numbers with CopyCaller (v. 1.0 software, Applied Biosystems) by ΔΔCt method with the reference DNA sample NA18998, seven copies of which had been previously validated by fibre-fluorescence in situ hybridization (FISH) [5].

Statistical analysis

The participants were divided into two groups: HOMA-IR ≤ 1.72 and HOMA-IR > 1.72. The cut-off value was selected as described elsewhere [21] and was comparable with other studies [22,23]. A *t*-test was used to test differences between the two groups in terms of continuous variables such as BMI, waist circumference, BP, fasting plasma glucose, insulin and lipid panel. The chi-square test was used to analyse categorical variables such as smoking status, alcohol consumption and physical activities. Multivariate linear regression and logistic regression analyses were used to test the association between *AMY1* CNVs and HOMA-IR. For the logistic regression, HOMA-IR was classified around a cut-off value as either

≤ 1.72 or > 1.72. We used three models to verify the association; the first was unadjusted, the second was adjusted for age and BMI, and the third was adjusted for age, BMI, systolic BP, triacylglycerol, current smoking status, alcohol consumption status and leisure time physical activities. Covariate selection was conducted via backward stepwise regression with a *P*-value of 0.2. We used STATA v. 13.1 software (StataCorp LP, College Station, TX, USA) for the statistical analysis. A significance value of *P* < 0.05 was used.

Results

The participants' clinical characteristics are described in Table 1. The estimated *AMY1* CNVs varied from 2 to 19 copies (median: six copies) (Fig. S1). Differences were observed between participants with a HOMA-IR ≤ 1.72 and > 1.72 in terms of the *AMY1* copy numbers, BMI, waist circumference, systolic and diastolic BP, fasting plasma glucose, insulin, HOMA-IR, lipid panel (total cholesterol, triacylglycerol, HDL-C, LDL-C) and leisure time physical activity. The smoking and alcohol consumption status did not differ between the groups.

The association between estimated *AMY1* CNVs and HOMA-IR was tested via multivariate linear regression and

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logistic regression in the three models, as shown in Table 2. In the linear regression analysis, HOMA-IR correlated negatively with *AMY1* CNVs in model 1 (unadjusted; β = −0.035, P = 0.044), model 2 (adjusted for age and BMI; β = −0.041, P = 0.008) and model 3 (adjusted for age, BMI, systolic BP, triacylglycerol, current smoking status, regular alcohol consumption and leisure time physical activities; β = −0.036, P = 0.013). In the logistic regression analysis, the negative correlation remained consistent and statistically significant in all three models [model 1: odds ratio (OR) = 0.95, 95% confidence interval (95% CI): 0.90–1.0; model 2: OR = 0.92, 95% CI: 0.87–0.98; model 3: OR = 0.92, 95% CI: 0.86–0.98]. This indicates that a subject with a 1 SD increase in the *AMY1* copy number (equivalent to 2.25 copy number increases) is likely to belong to the HOMA-IR ≤ 1.72 group (Table 2).

The linear regression and logistic regression analyses were repeated in subgroups classified according to the current smoking and alcohol consumption statuses. In non-current smokers, the HOMA-IR was consistently and negatively correlated with the estimated *AMY1* CNVs (model 1: β = −0.047, P = 0.037; model 2: β = −0.048, P = 0.018; model 3: β = −0.036, P = 0.046). In the multivariate logistic regression, a 1 SD increase in the *AMY1* copy numbers correlated with a HOMA-IR ≤ 1.72 among non-current smokers (model 1: OR = 0.93, 95% CI: 0.87–0.99; model 2: OR = 0.90, 95% CI: 0.84–0.97; model 3: OR = 0.90, 95% CI: 0.84–0.98). Among current smokers, the associations between *AMY1* CNVs and HOMA-IR were not significant in either the linear or logistic regression analysis (Table 3).

Among non-regular alcohol drinkers (alcohol consumption ≤ 8.5 g/week), the correlation between HOMA-IR and *AMY1* CNVs was not statically significant. However, among regular drinkers (alcohol consumption > 8.5 g/week), HOMA-IR correlated negatively with *AMY1* CNVs in models 2 and 3 of the linear regression analysis (model 2: β = −0.031, P = 0.042; model 3: β = −0.028, P = 0.052) and in all three models of the logistic regression analysis (model 1: OR = 0.93; 95% CI: 0.88–0.99; model 2:

OR = 0.91, 95% CI: 0.85–0.97; model 3: OR = 0.90, 95% CI: 0.84–0.96; Table 3).

For those 206 participants who answered the dietary questionnaire, we conducted the same multivariate linear and logistic regression analyses according to models 1, 2 and 3, then additionally adjusted for daily caloric intake (model 4) and daily carbohydrate intake (model 5) (Tables S1 and S2). In logistic regression for models 4 and 5, HOMA-IR exhibited a negative correlation with *AMY1* CNVs in the 206 participants, and this correlation was consistent among non-smokers. In the subgroups stratified by alcohol consumption, however, these correlations were not observed.

Discussion

In this study, individuals with low *AMY1* copy numbers were likely to have a high HOMA-IR independent of age, BMI, systolic BP, lipid panel and lifestyle factors such as smoking, alcohol consumption and physical activities. Furthermore, the negative correlation between *AMY1* CNVs and HOMA-IR was consistent and significant in the subgroup of non-smokers and regular drinkers, but was not statistically significant among current smokers and non-regular drinkers.

α-Amylase is an enzyme that participates in the first stage of starch metabolism. Starch is initially hydrolysed by α-amylase in the oral cavity to produce oligosaccharides such as maltose, maltotriose and dextrin [2]. These oligosaccharides protect the actions of salivary amylase in the acidic gastric environment. Following its generation by α-amylase in the oral cavity, maltose binds to sweetness receptors in the upper gastrointestinal tracts to signal the secretion of GLP-1, PYY and pancreatic amylase [2,24].

The amylase gene comprises *AMY1A*, *1B*, *1C*, *2A*, *2B* and *P*, all of which are located on chromosome 1p21. *AMY1* encodes salivary amylase and is known to exhibit CNVs from 2 to > 16 copies, whereas *AMY2*, which encodes pancreatic amylase, showed CNVs from 1 to 3 only [25,28]. *AMY1* CNVs are known to be greater in populations that consume high-starch diets (agricultural societies) than in populations

Table 2 Relationship between HOMA–IR with estimated *AMY1* CNVs

	Model 1			Model 2			Model 3		
	Coefficient (SE) OR (95% CI)		P	Coefficient (SE) OR (95% CI)		P	Coefficient (SE) OR (95% CI)		P
Total									
HOMA–IR*	−0.035 (0.017)		0.044	−0.041 (0.016)		0.008	−0.036 (0.015)		0.013
HOMA–IR > 1.72[†]	0.95 (0.90–1.0)		0.037	0.92 (0.87–0.98)		0.006	0.92 (0.86–0.98)		0.006

Model 1, unadjusted; model 2, adjusted for age and BMI (kg/cm^2); model 3, adjusted for age, BMI (kg/cm^2), systolic BP (mmHg), triacylglycerol (mmol/l), alcohol consumption (≤ 8.5 g/week vs. > 8.5 g/week), smoking (non-smoking vs. current smoking) and physical activity (low vs. moderate to high according to International Physical Activity Questionnaire long form).
*Multivariate linear regression was performed to yield coefficients.
[†]Logistic regression was performed to produce odds ratios of HOMA–IR > 1.72.

Table 3 Relationship between HOMA–IR with estimated *AMY1* CNVs in subgroups stratified by current smoking and regular alcohol consumption

	Model 1			Model 2			Model 3		
	Coefficient (SE) OR (95% CI)		*P*	Coefficient (SE) OR (95% CI)		*P*	Coefficient (SE) OR (95% CI)		*P*
Non-smokers									
HOMA–IR	−0.047 (0.022)		0.037	−0.048 (0.020)		0.018	−0.036 (0.018)		0.046
HOMA–IR > 1.72	0.93 (0.87–0.99)		0.022	0.90 (0.84–0.97)		0.007	0.90 (0.84–0.98)		0.010
Smokers									
HOMA–IR	−0.011 (0.027)		0.68	−0.028 (0.024)		0.24	−0.025 (0.023)		0.28
HOMA–IR > 1.72	0.98 (0.90–1.1)		0.71	0.95 (0.87–1.0)		0.32	0.94 (0.85–1.0)		0.22
Non-drinkers									
HOMA–IR	−0.072 (0.054)		0.19	−0.080 (0.050)		0.11	−0.063 (0.042)		0.13
HOMA–IR > 1.72	1.01 (0.90–1.1)		0.84	1.0 (0.87–1.1)		0.97	0.99 (0.86–1.1)		0.91
Regular drinkers									
HOMA–IR	−0.025 (0.017)		0.14	−0.031 (0.015)		0.042	−0.028 (0.014)		0.052
HOMA–IR > 1.72	0.93 (0.88–0.99)		0.018	0.91 (0.85–0.97)		0.003	0.90 (0.84–0.96)		0.002

Model 1, unadjusted; model 2, adjusted for age and BMI (kg/cm^2); model 3, adjusted for age, BMI (kg/cm^2), systolic BP (mmHg), triacylglyerol (mmol/l), alcohol consumption (≤ 8.5 g/week vs. > 8.5 g/week), smoking (non-smoking vs. current smoking) and physical activity (low vs. moderate to high according to International Physical Activity Questionnaire long form). Multivariate linear regression was performed to yield coefficientsand logistic regression was performed to produce odds ratios of HOMA–IR > 1.72. Adjustment of smoking was omitted in the subgroup by smoking. Adjustment of alcohol consumption was omitted in the subgroup by alcohol consumption.

that consume low-starch diets (hunter–gatherer societies) [5]. The fact that salivary amylase, but not pancreatic amylase, copy numbers are naturally selected according to diet suggests the importance of the interaction between salivary amylase and carbohydrate metabolism [4].

Mandel and colleagues found that individuals with higher amylase activity levels exhibited faster starch digestion in the oral cavity [3]; thus, they expected that a decrease in salivary amylase pre-digestion would also decrease starch digestion, resulting in a lower postprandial glucose level. However, individuals with higher salivary amylase levels, exhibited lower postprandial glucose levels and an early surge of insulin [4]. Also, salivary amylase activity was positively correlated with *AMY1* copy numbers. This finding suggests that early in digestion, mostly during the oral phase before glucose absorption, insulin secretion from pancreatic β-cells is promoted in individuals with high *AMY1* copy numbers and high salivary amylase activities. Evidence suggests that amylolytic activity in the oral cavity induces signalling to promote insulin secretion in acinar cells through oligosaccharide binding to lingual taste receptors [26]. Mandel and Breslin suggested that '*AMY1* gene copy numbers may play a role in the development of insulin resistance' [4], which we have verified partly in this study.

As mentioned earlier, negative correlations between *AMY1* CNVs and HOMA-IR presented differently in subgroups stratified by current smoking and alcohol consumption statuses. Because smoking can affect the independent variable salivary amylase activity, as well as the dependent variable insulin resistance, the specific mechanism by which smoking affects the association between *AMY1* CNVs and insulin resistance requires further investigation. Cigarette smoking inhibits or significantly reduces salivary amylase activity via toxic effects of cigarette components [27]. For example, salivary amylase was inactivated immediately after smoking, by chemical damage of salivary proteins when saliva was exposed to unsaturated aldehydes produced during smoking. Cigarette smoking also increases insulin resistance [8–11] and the risk of Type 2 diabetes [12–14]. Smoking causes acute hyperglycaemic and hyperinsulinaemic responses and also promotes adrenergic activity that inhibits the glucose-stimulated release of insulin from the pancreas [8,11]. Therefore, in our study, smoking might have attenuated the advantageous effects of high *AMY1* copy numbers with respect to reducing insulin resistance (Fig. 1).

There is evidence that alcohol consumption is known to have a protective effect on insulin sensitivity. In Japanese studies, alcohol consumption correlated inversely with insulin resistance [17,18] and in other studies, moderate alcohol consumption was associated with improved insulin sensitivity [15,16]. Mechanisms by which moderate alcohol consumption improves insulin sensitivity have been addressed in relation with adiponectin, the hormone secreted by adipose tissue that is known to enhance insulin sensitivity [18,19]. Moderate alcohol consumption increased plasma adiponectin, and insulin sensitivity improved in a insulin-resistant subgroup [19]. Therefore, it is possible that the negative correlation between *AMY1* CNVs and HOMA-IR was strengthened in the subgroup of regular alcohol consumers (Fig. 2).

Although there is a correlation between *AMY1* copy numbers and insulin resistance, the absolute value of the coefficient in regression analysis is very small and OR is close to 1, suggesting that the contribution of *AMY1* copy numbers to development of insulin resistance may be quite

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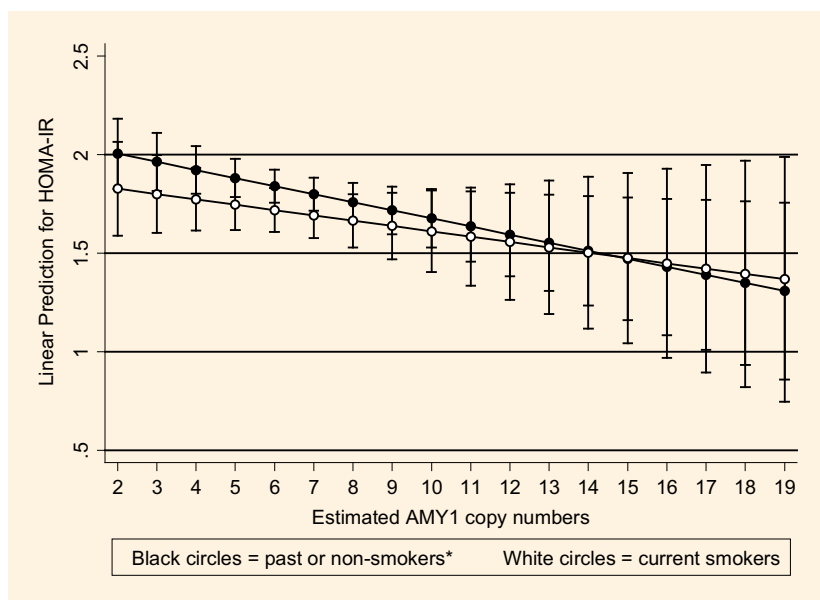
FIGURE 1 Estimated linear prediction for HOMA–IR according to *AMY1* copy numbers in subgroups by current smoking. Linear prediction was derived from multivariate linear regression analysis based on model 3 in Table 3, adjusted for age, BMI (kg/cm^2), systolic BP (mmHg), triacylglyceride (mmol/L), smoking (non-smoking vs. current smoking) and physical activity (low vs. moderate to high according to International Physical Activity Questionnaire long form). *Significant *P*-value < 0.05.
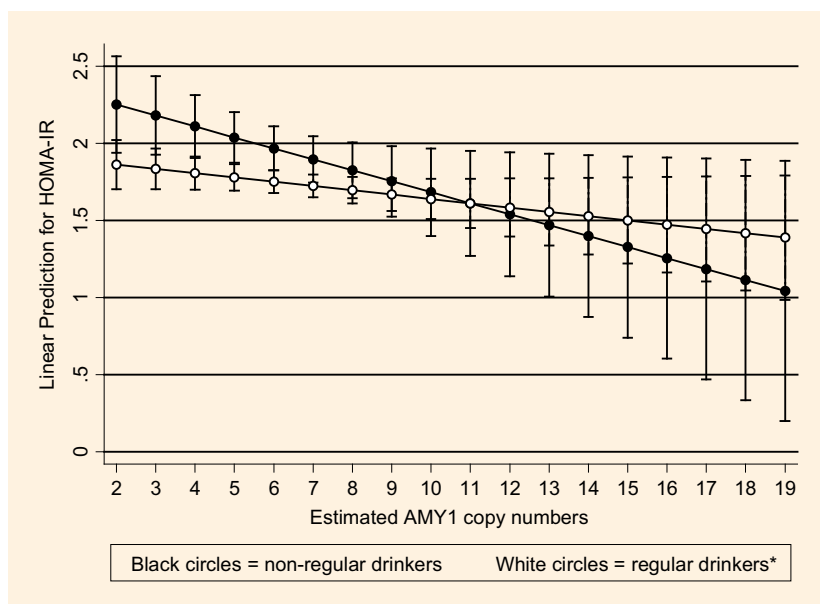
FIGURE 2 Estimated linear prediction for HOMA-IR according to *AMY1* copy numbers in subgroups by regular alcohol consumption. Linear prediction was derived from multivariate linear regression analysis based on model 3 in Table 3, adjusted for age, BMI (kg/cm^2), systolic BP (mmHg), triacylglyceride (mmol/L), smoking (non-smoking vs. current smoking) and physical activity (low vs. moderate to high according to International Physical Activity Questionnaire long form). *Significant *P*-value < 0.05.

limited. However, *AMY1* CNVs may contribute to the elucidation of one of the pathways to developing insulin resistance. Recent studies have reported that low *AMY1* copy numbers increased the risk of obesity [28,29]; however, we found no significant associations between *AMY1* copy numbers and obesity markers such as BMI and waist circumference in our study (data not shown). Because

AMY1 copy numbers and insulin resistance were inversely related after adjusting for BMI, we postulate the existence of an independent pathway via which salivary amylase influences insulin resistance while bypassing the mechanisms that involve obesity. The salivary amylase level is not solely determined by *AMY1* CNVs; single nucleotide polymorphisms and epigenetic factors such as methylation or the

enhancer status can also influence salivary amylase activity. Furthermore, transcription or translation efficiency may differ among different haplotypes. Salivary amylase activity is also influenced by emotional stress and starch consumption habits [25]. Nevertheless, the *AMY1* CNVs appear to be a meaningful factor that determines salivary amylase activity and influences insulin resistance.

Perry *et al.* reported higher *AMY1* gene copy numbers in populations with a high starch diet [5]. South Korea can be viewed as a high starch diet population because starch consumption comprised 44% of total energy intake in 2011, which is comparable with Japan (41%) and the USA (25%) [30], and because Japanese and European Americans were categorized as populations with a high starch diet in Perry's study, South Koreans can be also. We analysed the correlation between daily carbohydrate intake and *AMY1* CNVs; however, no such correlation was observed (data not shown). The effect of diet on the relationship between *AMY1* CNVs and insulin resistance is indeterminate, as demonstrated earlier (Tables S1 and 2), and thus requires further investigation.

It is noteworthy that an association between HOMA-IR and *AMY1* CNVs was observed even in asymptomatic adults. Because HOMA-IR is a factor predictive of the risk of Type 2 diabetes, *AMY1* CNVs might have a predictive value in asymptomatic populations. Because the most frequently observed *AMY1* copy numbers range from 4 to 10 (Fig. S1), an interpretation of linear HOMA-IR predictions in subgroups stratified by smoking and alcohol consumption might focus specifically on this range (Figs 1 and 2). For example, because the linear HOMA-IR prediction slope was steeper among non-smokers ($\beta = -0.036$, $P = 0.046$) than among smokers ($\beta = -0.025$, $P = 0.28$), individuals with higher *AMY1* copy numbers would derive a greater advantage from not smoking in terms of insulin sensitivity. However, this interpretation requires caution in very high CNVs because standard deviation becomes very large as CNVs increase (Fig. 1). This might be attributed to the right-tailed distribution of CNVs, in which only a small number of participants with very high copy numbers exists (Fig. S1). We repeated the analysis with CNVs outliers ≥ 15 and ≥ 13 consecutively omitted (Table S3). As a result, the coefficients were similar regardless of omitted outliers, but the significance decreased, which seems to be due to a smaller sample size.

The limitation of this study is that the participants were confined to two healthcare centres in Korea; therefore, ethnic and cultural differences could not be studied in depth. Given that *AMY1* CNVs vary among different ethnic groups, similar studies will need to be conducted in other ethnic groups to confirm this inverse relationship between *AMY1* copy numbers and insulin resistance.

Funding sources

Competing interests

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Distribution of estimated *AMY1* copy numbers.
Table S1. Relationship between HOMA-IR and estimated *AMY1* CNVs with additional adjustment for dietary habit.
Table S2. Relationship between HOMA-IR and estimated *AMY1* CNVs with additional adjustment for dietary habit in subgroups by smoking and alcohol consumption.
Table S3. Relationship between HOMA-IR and estimated *AMY1* CNVs in subgroups stratified by current smoking and regular alcohol consumption with CNVs outliers omitted.