

# Copy Number Variation of the Salivary Amylase Gene and Glucose Metabolism in Healthy Young Japanese Women

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## Abstract

**Background:** Many studies have shown that low copy number variation (CNV) of the salivary amylase gene (*AMY1*) and low serum amylase concentration are associated with impaired glucose metabolism and obesity. We aimed to clarify the conflicting results of previous studies by examining *AMY1* expression and metabolic indices in a homogenous group of healthy participants.

**Methods:** Sixty healthy non-obese young Japanese women aged 20 - 39 years were examined for *AMY1* CNV, salivary amylase, body mass index (BMI) and serum parameters including glycated hemoglobin (HbA1c), ketones, and total, salivary and pancreatic amylase. Respiratory quotient at rest and changes in blood glucose after starch loading were also examined.

**Results:** *AMY1* CNV (range, 4 - 14) and the level of serum salivary amylase were correlated inversely with HbA1c ( $r = -0.36$ ,  $P = 0.003$  and  $r = -0.30$ ,  $P = 0.02$ , respectively), whereas the percentage of serum salivary amylase in total serum amylase was positively correlated with blood glucose at 30 and 45 min after starch loading ( $r = 0.38$ ,  $P = 0.004$  and  $r = 0.27$ ,  $P = 0.04$ , respectively). The level of serum total amylase, but not *AMY1* CNV, was correlated inversely with BMI ( $r = -0.29$ ,  $P = 0.02$ ). Logistic regression analysis showed that low *AMY1* CNV (4 - 7) was significantly associated with an HbA1c of  $\geq 5.4\%$  (34 mmol/mol) even after adjustment for age, BMI and energy consumption, compared with high *AMY1* CNV (8 - 14).

**Conclusions:** Although a higher percentage of serum salivary amylase was associated with higher levels of blood glucose at the early stage after starch loading, low *AMY1* CNV was associated with

chronic unfavorable glucose metabolism in healthy non-obese young women in Japan.

**Keywords:** Amylase gene; Salivary amylase; BMI; HbA1c; Serum amylase; Starch loading

## Introduction

In recent decades, several studies have shown that low copy number variation (CNV) of the salivary amylase gene (*AMY1*) and low serum amylase concentration are associated with obesity and impaired glucose metabolism [1-10]. However, the results of these studies are conflicting, because some studies have shown no association of *AMY1* CNV with obesity and impaired glucose metabolism [11-16]. These conflicting results may be due to the heterogeneity in patient samples in terms of racial and genetic background, various complications and acquired physical conditions, and the use of different study methods particularly for the assessment of *AMY1* CNV [17].

Although most of the previous studies have focused on *AMY1* CNV and obesity/diabetes [1-10], few studies have considered *AMY1* expression in saliva, which is reflected in measurements of oral and serum salivary amylase, and is a main contributor to total serum amylase. Furthermore, serum amylase, which comprises the salivary and pancreatic isoforms of amylase, has rarely been considered in the interplay of *AMY1* CNV, obesity and glucose metabolism.

We aimed, therefore, to investigate the fundamental relationships of *AMY1* CNV, salivary amylase and serum salivary/pancreatic amylase with metabolic indices including body mass index (BMI) in a homogenous sample of healthy non-obese young women.

## Materials and Methods

### Design and participants

We recruited 60 apparently healthy female non-smokers of Japanese origin, aged 20 - 39 years, with normal BMI ( $< 25.0$  kg/m<sup>2</sup>) and no history of metabolic disorders. This study was approved by the Ethics Committee of Kanagawa University of Human Services (ID number 71-31).

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**Table 1.** Characteristics of the Study Participants

Characteristics	Means $\pm$ SD or median (IQ)
Age (years)	23.0 $\pm$ 4.2
BMI, kg/m <sup>2</sup> (range)	20.1 $\pm$ 1.6 (17.5 - 24.3)
TG, mg/dL (IQ)	52 (38 - 68)
HDL-C, mg/dL	73 $\pm$ 15.7
HbA1c, % (range)	5.25 $\pm$ 0.19 (4.9-5.6%)
mmol/mol (range)	33 (30 - 37)
Total ketone bodies, $\mu$ mol/L (IQ)	39.5 (29 - 66)
3-Hydroxybutyric acid, $\mu$ mol/L (IQ)	24 (15.5 - 42)
Acetoacetic acid, $\mu$ mol/L (IQ)	17 (11.5 - 24)
Respiratory quotient	0.77 $\pm$ 0.05
Energy consumption, kcal	1737 $\pm$ 350
Carbohydrate energy, % in total energy	52.8 $\pm$ 5.2
Alcohol consumption, kcal (IQ)	14 (0.0 - 29.0)
<i>AMY1</i> CNV (range)	8.3 $\pm$ 2.3 (4 - 14)
Salivary amylase, (U/L) IQ*	100 (62 - 148)
Total serum amylase (U/L)	91 $\pm$ 27.9
Serum salivary amylase(U/L)	61.3 $\pm$ 25.5
Serum pancreatic amylase (U/L)	29.9 $\pm$ 10.4
Percentage of serum salivary amylase (range)	66.0 $\pm$ 10.8 (40 - 86)

\*Divided by the protein concentration in each saliva sample. SD: standard deviation; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; HbA1c: glycated hemoglobin; *AMY1*: salivary amylase gene; CNV: copy number variation; IQ: interquartile range; TG: triglycerides; FPG: fasting plasma glucose.

## Measurements

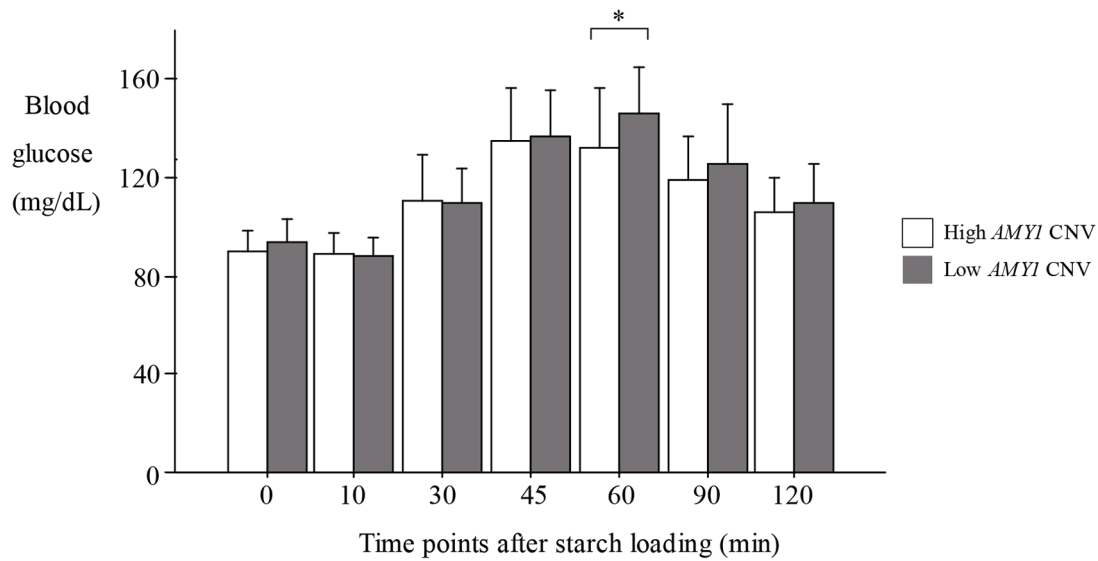
Laboratory measurements were performed in the morning following an overnight fast. Biochemical measurements including glycated hemoglobin (HbA1c), ketones and amylase (total serum, salivary and pancreatic) were performed using standard automated methods. Blood glucose concentration after starch loading with a 130-g rice ball was measured using a Glutest-Neo alpha glucose meter (Sanwa-Kagaku-Kenkyusho, Nagoya, Japan). We calculated areas under the blood glucose based on the trapezoid rule. *AMY1* expression was assessed by Droplet Digital PCR, which is preferred for its precise measurement [5, 17]. Briefly, TaqMan Copy Number Assays for *AMY1A* (*AMY1A*:Hs07226362\_cn) and restriction enzyme *DraI* (Anza 77 *DraI*; both from Thermo Fisher Scientific) were used. The concentrations of amylase and total protein in saliva samples were measured by a kinetic reaction assay (Salivary alpha-Amylase Assay Kit, Salimetrics, Carlsbad, CA, USA) and a bicinchoninic acid assay protein assay (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific), respectively. To estimate the respiratory quotient (RQ), respiratory monitoring for 5 min at rest was conducted after the adequate stabilization using an AR-1 portable gas monitor (ARCO SYSTEM Inc., Japan). To estimate an individual's consumption of energy, protein, lipids and carbohydrate, a food frequency questionnaire based on food groups [18] was used.

## Statistical analysis

Data are expressed as the mean  $\pm$  standard deviation (SD) or median (interquartile range). Difference in areas under the blood glucose between low (range, 4 - 7) and high *AMY1* CNV (range, 8 - 14) was tested by Mann-Whitney U test. Correlations between variables were tested by Spearman's correlation because of the high likelihood of non-parametric distributions in the parameters. Logistic regression analysis was used to test the association between low *AMY1* CNV (range, 4 - 7) and HbA1c  $\geq$  5.4% (34 mmol/mol), a criteria for gestational diabetes [19, 20], compared with high *AMY1* CNV (range, 8 - 14), considering potential confounders including age, BMI and energy consumption. Statistical analysis was performed using SAS Enterprise Guide (SAS-EG 7.1) in SAS version 9.4 (SAS Institute, Cary, NC, USA). A P value  $<$  0.05 was considered to indicate statistical significance.

## Results

The characteristics of the participants are shown in Table 1; all of the parameters were within normal ranges. The percentage of salivary amylase in total serum amylase was 66.0 $\pm$ 10.8%. Figure 1 shows the levels of blood glucose after starch loading according to high (8 - 14) and low *AMY1* CNV (4 - 7). All

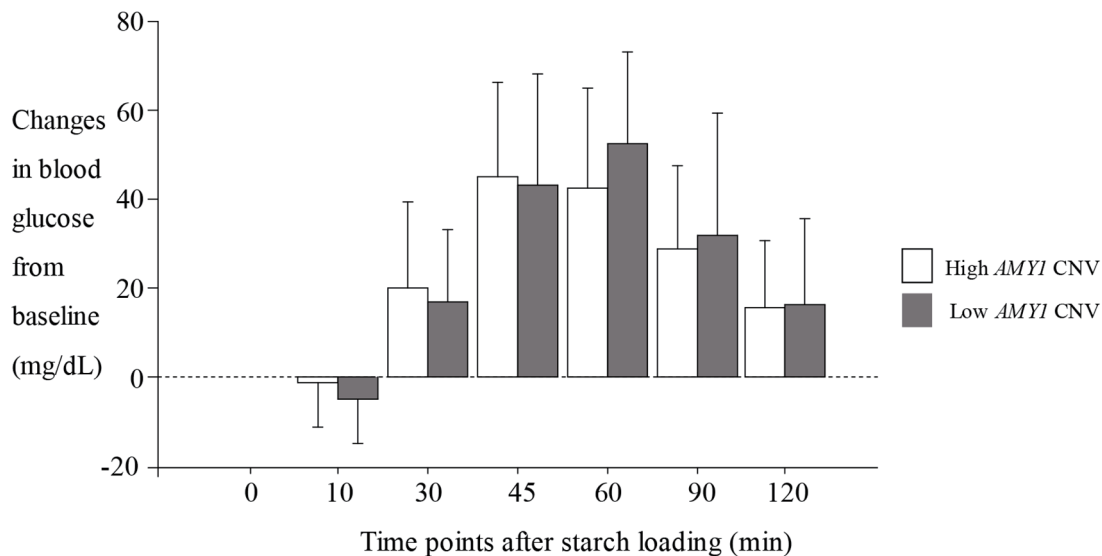


**Figure 1.** Levels of blood glucose after starch loading. Data are expressed as the mean ± SD. \*P < 0.05, t-test, high vs. low *AMY1* CNV. High *AMY1* CNV: 8 - 14; low *AMY1* CNV: 4 - 7. SD: standard deviation; CNV: copy number variation; *AMY1*: salivary amylase gene.

though significant difference was observed at 60 min between two groups, no difference was observed at other time points. Figure 2 shows the changes of blood glucose from baseline. No significant difference was observed at any time points between two groups. Simultaneously, we found no significant difference in the area under the blood glucose between two groups (medians (interquartile ranges), 3,102 (2,347 - 3,710) for high *AMY1* CNV and 3,305 (2,288 - 4,113) for low *AMY1*

CNV, P = 0.50, data are not shown).

Table 2 shows the results of correlation analysis among the parameters investigated. *AMY1* CNV (range, 4 - 14) and serum salivary amylase were inversely correlated with HbA1c (r = -0.36, P = 0.003 and r = -0.30, P = 0.02, respectively), whereas serum salivary amylase was positively correlated with blood glucose at 30 min after starch loading (r = 0.26, P = 0.04). The percentage of serum salivary amylase was also positively



**Figure 2.** Changes of blood glucose from baseline after starch loading. Data are expressed as the mean ± SD. High *AMY1* CNV: 8 - 14; low *AMY1* CNV: 4 - 7. SD: standard deviation; CNV: copy number variation; *AMY1*: salivary amylase gene.

**Table 2.** Correlation Coefficients Between the Study Parameters

	AMY1 CNV	Salivary amylase	Serum amylase				RQ
			Total	Salivary	Pancreatic	% Salivary	
BMI	-0.02	0.02	-0.29*	-0.22	-0.18	0.01	0.09
HbA1c	-0.36**	-0.13	-0.17	-0.30*	0.14	-0.37**	-0.12
Triglyceride	-0.15	0.23	-0.04	-0.05	-0.01	-0.04	0.10
HDL-C	-0.14	-0.17	-0.16	-0.20	0.06	-0.17	0.02
Uric acid	0.29*	0.09	-0.09	-0.06	-0.07	0.05	-0.15
Total ketone bodies	0.14	-0.04	-0.14	-0.10	-0.15	0.1	-0.44***
Acetoacetic acid	0.11	-0.01	-0.16	-0.13	-0.12	0.06	-0.36**
3-Hydroxybutyric acid	0.17	-0.06	-0.09	-0.05	-0.15	0.14	-0.45***
AMY1 CNV	-	-	-	-	-	-	-
Salivary amylase	0.39**	-	-	-	-	-	-
Serum amylase							
Total	0.29*	0.34**	-	-	-	-	-
Salivary	0.36**	0.36**	0.91***	-	-	-	-
Pancreatic	-0.08	0.13	0.40**	0.04	-	-	-
% Salivary	0.37**	0.24	0.36**	0.68***	-0.64***	-	-
RQ	-0.06	-0.02	0.21	0.12	0.29*	-0.13	-
Blood glucose time course							
Baseline	-0.14	-0.12	-0.11	-0.18	0.15	-0.22	0.08
10 min	-0.05	0.08	-0.03	0.02	-0.25	0.13	0.15
30 min	0.05	0.11	0.17	0.26*	-0.18	0.38**	0.11
45 min	-0.02	-0.06	0.04	0.14	-0.22	0.27*	-0.07
60 min	-0.14	0.04	-0.20	-0.10	-0.22	0.16	-0.18
120 min	-0.07	-0.02	-0.08	-0.07	0.003	-0.04	-0.09

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001. AMY1: salivary amylase gene; CNV: copy number variation; RQ: respiratory quotient; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; HbA1c: glycated hemoglobin.

correlated with blood glucose at 30 and 45 min ( $r = 0.38$ ,  $P = 0.004$  and  $r = 0.27$ ,  $P = 0.04$ , respectively). The level of total serum amylase, but not AMY1 CNV, was inversely correlated with BMI ( $r = -0.29$ ,  $P = 0.02$ ). Only serum pancreatic amylase was positively correlated with RQ, which was significantly correlated with three indices related to serum ketones.

Logistic regression analysis showed that low AMY1 CNV (4 - 7) was significantly associated with HbA1c of  $\geq 5.4\%$ , even after adjustment for age, BMI and energy consumption, compared with high AMY1 CNV (8 - 14) (Table 3). Furthermore, this association was strengthened by the further adjustment for salivary amylase adjusted for its concentration.

**Table 3.** Odds Ratios of Low AMY1 CNV for High HbA1c

	High AMY1 CNV (8 - 14) (N = 40)	Low AMY1 CNV (4 - 7) (N = 20)
High HbA1c $\geq 5.4\%$ (34 mmol/mol), n (%)	10 (25)	11 (55)
	<b>Odds ratios (95% CI)</b>	
Model 1	1 (reference)	3.67 (1.18 - 11.4)
Model 2	1 (reference)	4.32 (1.36 - 31.8)
Model 3	1 (reference)	6.31 (1.34 - 29.8)
Model 4	1 (reference)	8.31 (1.81 - 36.6)
	<b>P values</b>	
Model 1		0.02
Model 2		0.02
Model 3		0.02
Model 4		0.006

Model 1: unadjusted. Model 2: adjusted for age and BMI. Model 3: model 2 plus adjustments for energy consumption (kcal) and serum total amylase. Model 4: model 2 plus adjustments for energy consumption (kcal) and salivary amylase for its concentration. AMY1: salivary amylase gene; CNV: copy number variation; CI: confidence interval; HbA1c: glycated hemoglobin; BMI: body mass index.

## Discussion

Several studies published in the last decade have shown that low *AMY1* CNV is associated with obesity [1-10]. In this study, however, no significant direct association between *AMY1* CNV and BMI was observed. This result may have been influenced by our selection of non-obese female participants because the common association between BMI and HbA1c was also not observed ( $r = -0.09$ ,  $P = 0.48$ , data not shown). By contrast, only the total serum amylase concentration was inversely associated with BMI, which is consistent with our previous study [6, 21]. Therefore, roughly speaking, the relationship of *AMY1* CNV with metabolic indices may differ from that of total serum amylase and salivary amylase.

High *AMY1* CNV is generally considered to indicate a high level salivary amylase that takes advantage of a large amount of starch by digesting it into oligosaccharides. In consistent with this, high levels of serum salivary amylase and a high percentage of salivary amylase in total serum amylase were associated with high blood glucose levels at an early stage after starch loading in this study. This finding may be consistent with a study by Atkinson et al [13], but in contrast to a study by Mandel et al [1]. Nevertheless, *AMY1* CNV and serum salivary amylase were inversely correlated with HbA1c, an index for glucose metabolism over a period of months. More specifically, low *AMY1* CNV was associated with HbA1c  $\geq 5.4\%$ , independent of potential confounders. A plausible explanation for this is that healthy, non-obese individuals with high *AMY1* CNV and serum salivary amylase represent the default condition, i.e. a readiness to absorb as many carbohydrates as possible by means of a high amount of salivary amylase in the oral cavity, which might be accelerated in those without impaired glucose metabolism, i.e. lower HbA1c. Until the current century, most of the human beings who ate grains lived under such conditions, wherein the supply of grains was an inadequate source of energy.

By contrast, when individuals living in modern times chronically consume large amounts of carbohydrate, which leads to high HbA1c, a high level of salivary amylase may be unnecessary to digest a large intake of starch, suggesting that the fundamental relationship between *AMY1* CNV and glucose metabolism might have become modified.

Alternatively, healthy non-obese individuals with low *AMY1* CNV who lack the perception for sweet taste may overeat, and an absent cephalic response to the early secretion of insulin may lead to a higher peak level of blood glucose after starch loading [6, 7]. Both of these conditions predispose the individual to increasing risks for obesity and impaired glucose metabolism.

Furthermore, the inverse association between serum amylase and BMI may suggest a specific condition of “amylase resistance” in individuals with obesity and diabetes, who by definition also have insulin resistance. The action of insulin is crucial for the production of amylase in the pancreas, but probably also in the salivary glands [22, 23]. Therefore, it is possible that insulin resistance leads to decreased secretion of amylase from the saliva and pancreas [6, 7], and could be a physiological response in people with diabetes and obesity.

With regard to the correlations of serum pancreatic amylase with HbA1c, RQ and blood glucose after starch loading, it is noteworthy to consider that serum pancreatic amylase might have the opposite effect on glucose metabolism that serum salivary amylase has. This would strengthen the correlations between the percentage of serum salivary amylase and serum pancreatic amylase and blood glucose after starch loading. Therefore, the effect of salivary amylase on metabolic indices may be influenced by the level of pancreatic amylase.

Finally, in this study, all participants were non-obese young women without overt impaired glucose metabolism. Furthermore, the percentage of salivary amylase was higher (66%) than previously considered (around 50%) [6, 7]. Therefore, the current observations may not be applicable to other populations, such as men, elderly people and those with obesity, and as such caution should be exercised in the interpretation of the results.

In conclusion, although a higher percentage of serum salivary amylase was associated with higher levels of blood glucose early after starch loading, our finding that low *AMY1* CNV was associated with chronic unfavorable glucose metabolism in a population of healthy non-obese young Japanese women deserves further study.

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## Conflict of Interest

The authors declare that there is no conflict of interest associated with this manuscript.

## Informed Consent

Written informed consent was obtained from each participant.

## Author Contributions

KN contributed to the overall study design. KN and RH contributed to the interpretation of the initial analysis and the discussion of the literature. RH, TI and AI measured parameters and collected the serum data and others including RQ, salivary amylase and their proteins. KN prepared the first draft of the manuscript, and all authors read and approved the manuscript.



## Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author.

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