Salivary Amylase Gene (AMY1) Copy Number Variation Has Only a Minor Correlation with Body Composition in Chinese Adults

Zhang Xinming[1*†], Colin Moran[3†], Wang Ruiyuan[2†], Zhou Yue[2] and Naomi Brooks[3]

1 School of Sport Science, Nantong University, Jiangsu Province, China.

2 School of Sport Science, Beijing Sport University, Beijing City, China.

3 School of Health Sciences and Sport, University of Stirling, Scotland, The United Kingdom

*Corresponding authors. E-mail(s): smile-carrie@hotmail.com;

Contributing authors: E-mail(s): colin.moran@stir.ac.uk;

†These authors contributed equally to this work.

Abstract

Background: According to the WHO, about 39% of the global adult population were overweight or obese in 2016. Obesity has high heritability, with more than 1000 variants so far identified. There have been reports indicating that salivary amylase gene (AMY1) copy number was one of these variants, yet its association with obesity remains controversial.

Objective: Our research aimed to provide more evidence on the relationship of AMY1 copy number variation (CNV) with body mass index (BMI) and body composition.

Methods: We recruited 133 Chinese adults (65 males, 68 females, 18 - 25 years old) with normal fasting blood glucose and blood pressure levels. 19 males were selected for a 10-week intervention to change body composition. After anthropometric measurements, BMI was calculated, and body composition was measured using dual energy X-ray absorptiometry (DEXA). For the 19 selected participants, we collected their height, weight, and body composition data one more time after intervention. All participants were required to leave their saliva samples and their AMY1 copy number was determined by real-time fluorescence quantitative PCR.

Results: We failed to find any significant difference in BMI and body composition between different copy number groups. Only a weak correlation was found between body muscle mass and body fat mass. After adjusted for height and weight, AMY1 CNV explained 4.83% of the variance and one single increase in AMY1 CNV can increase 0.214kg of the body muscle mass, while one single increase in AMY1 CNV can decrease 0.217kg of the body fat mass and explained 4.69% of the variance.

Conclusions: As a genetic factor, the AMY1 gene copy number variation has only a minor correlation with BMI and body composition, and its effect can easily be hidden by other factors such as individual diet and exercise habit.

Key words: Salivary Amylase Gene, Copy Number Variation, BMI, Body Composition

1. Introduction

Obesity has been regarded as a worldwide health problem for decades. According to the World Health Organization (WHO), worldwide obesity has nearly tripled since 1975. In 2015, the percentage of the obesity population among adults has reached 35% in North America (Smith et al., 2016). Many studies showed that the prevalence of obesity was also notable in the Chinese population. For example, the proportion of the overweight population in Jilin Province was 32.3%, including the obese population 14.6% (Wang et al., 2016). Another study showed that females with obesity in Chinese rural area had reached 16.5% between 2010 and 2014 (He et al., 2016). Obesity kills more people than underweight in most countries. It is the cause of numerous kinds of chronic diseases, such as diabetes, hypertension, and other cardiovascular diseases (Leong et al., 1999; Després et al., 2006; Sowers, 2003), which can all seriously affect normal life, making obesity one of the most severe health threats all over the world.

Obesity has already been proved to be highly heritable. Earlier in 2013, Moustafa (Mostafa et al., 2013) found that the possibility of BMI been affected by genetic factors could reach 40-70%. Among all genetic factors, DNA copy number variation (CNV) has been frequently reported in recent studies, involving genes such as *11q11, 1p21.1, 10q11.22, 10q26.3, 16q12.2, 16p12.3,* and *4q25* (Phillips et al., 2020). The generally accepted nongenetic factors include a high glucose diet, overeating, low physical activity, and high sedentary behavior. Exceeding intake of complex carbohydrates is one of the common nongenetic of obesity.

The salivary amylase gene copy number variation (AMY1 CNV), located on *1p21.1*, has been confirmed to be associated with salivary enzyme activity and the amount of protein in saliva (Mandel et al., 2010). Since complex carbohydrate is digested first by amylase in saliva, many researchers believed that low AMY1 CNV should be responsible for obesity. In 2014, research published by Falchi (Falchi et al., 2014) in Nature Genetics showed that the risk of obesity for participants with lower AMY1 copy number is 7 times higher than participants with higher AMY1 copy number. However, the results of several other studies followed failed to show a relationship between AMY1 CNV and BMI (Usher et al., 2015; Yong et al., 2016).

The inconsistency among these results has drawn our attention. To provide more evidence on how AMY1 CNV is associated with obesity, we designed this study to find more information about its relationship with BMI and body composition. Except for a simple comparison, we added a diet and training intervention to explore whether there would be a dynamic difference in BMI and body composition between different CNV groups.

2. Methods

2.1 Selection of participants

133 adults were randomly selected by posters on campus or online advertisements. Participants were Chinese, Han nationality, aged 18 to 25 years, with fasting blood glucose lower than 6.1 mmol/L and normal blood pressure level (resting systolic pressure between 100 and 130 mmHg as well as resting

diastole between 60 and 80 mmHg).

Afterward, 19 males were chosen for an intervention with high protein diet and strength training that lasted 10 weeks to build muscle.

All data were collected from 8: 00 am to 10 am to reduce errors. Before the experiment, participants would receive a printed informed consent that listed the whole experimental process and risks. They all agreed and signed it before they officially involved in the study. Our protocol has been approved by the School of Sport Research Ethics Committee at University of Stirling (code SSREC number #880).

2.2 Anthropometric measurement and body composition

2.2.1 Anthropometric measurement

In this part of the study, participants were requested to wear thin and tight clothing and keep shoes off during all tests. When measuring height and weight, participants were instructed to stand on the height and weight measurement instrument (DS-103M, DONG SAHN JENIX, South Korea), keep their bodies straight and their eyes looking forward with their chins parallel to the floor. The results were recorded immediately after the number on the screen was stable. BMI was later calculated using standard formula [BMI (kg/m^2) = Weight (kg) / Height $(m)^2$]. For waist and hip circumference, a tape measure with a maximum range of 150 cm was used for measurement following the IASK guidelines, and the waist-hip ratio was calculated as waist circumference dividing hip circumference.

2.2.2 Body composition

Body composition was evaluated using a dual energy X-ray absorptiometry (DEXA) scanner (Lunar iDXA scanner; General Electric Healthcare, USA). Participants were asked not to wear clothing with metal decorations when attending DXA scanning. All jewelry and other metal items were also required to be removed before testing.

2.3 Diet and training intervention

The 19 selected males received a controlled amount of diet with 2 gram of protein per kilogram of weight and 5 gram of carbohydrate per kilogram of weight each day during 10 weeks of intervention (Norton, 2009). All diets were under strict supervision for muscle building. The parts of muscles involved in the strength training included the chest, back, shoulder, arms, and thighs. Each part was trained specifically on one day with an intensity of 12 repetition maximum (RM). The training time for each day was 70 to 80 minutes. Participants received strength training 5 days a week to make sure that all the parts mentioned above were involved and took rest for the rest 2 days. After the intervention, height, weight, and body composition were measured again for later comparison.

2.4 AMY1 copy number variation measurement

2.4.1 DNA collection and extraction

Participant saliva samples were collected in sample collection tubes (Zhishan Biology Company, Xiamen, China). DNA was extracted using prepIT•L2P (DNA Genotek, Canada), and its quantity and quality of DNA were determined by the DS-11FX Spectrophotometer/Fluorometer (Denovix, USA).

2.4.2 AMY1 copy number determination

Real time fluorescence quantitative Polymerase Chain Reaction (RTFQ PCR) was performed to

estimate AMY1 CNV for each participant. We used Taqman copy number assay (*Hs07226361_cn*, Thermo Fisher, USA) as the target detector, and Taqman copy number reference assay (RNaseP) (*Hs07226361_cn*, Thermo Fisher, USA) was applied to detect the sequence on the other chain in Applied Biosystems™ ViiA 7 Real Time PCR System (Thermo Fisher, USA). The ΔΔCT results were then calculated in Applied Biosystems™ ViiA 7 software v2.0 (Thermo Fisher, USA).

2.5 Statistical analyses

All statistical analyses were performed with SPSS software (version 24.0, IBM Corporation, USA), and the related figures were drawn with Graphpad Prism 8 (GraphPad Software Inc., USA).

The difference in anthropometric measurement and body composition data between each copy number group was compared using two methods. First, we used two-way analysis of variance (two-way ANOVA), with CNV and gender as fixed factors, and the Tukey test was then conducted as a post hoc test to see the difference between different groups. Second, an independent t-test was performed for group comparison in each gender. For those who participated in the intervention part of the study, we used an independent t-test for group comparison and a paired sampled t-test to determine individual changes.

The linear regression model was used to find the correlation between AMY1 CNV and all the measured indexes. We built two models to verify this association. The first was adjusted for gender, with CNV only as a dependent variable. We used this model to find a general correlation between AMY1 CNV and all the measured indices. Since the absolute value of muscle and fat mass is strongly associated with height and weight, the second model was created, which was also adjusted for gender while height and weight joined CNV as the other two dependent variables.

For each index with significant difference, we calculated their statistical power using the G-power calculator (α=0.05).

3. Results

3.1 Distribution of AMI CNV in participants and determination of group

3.1.1 Distribution of AMI CNV in participants

The distribution of AMY1 CNV in the participants (ranged from 1 to 16) is shown in Figure 1. Gender will not be a factor affecting the AMY1 CNV results proved by the chi-square test(P=0.895). As we calculated, the average of CNV was 4.71, the median was 4, and the mode was 3.

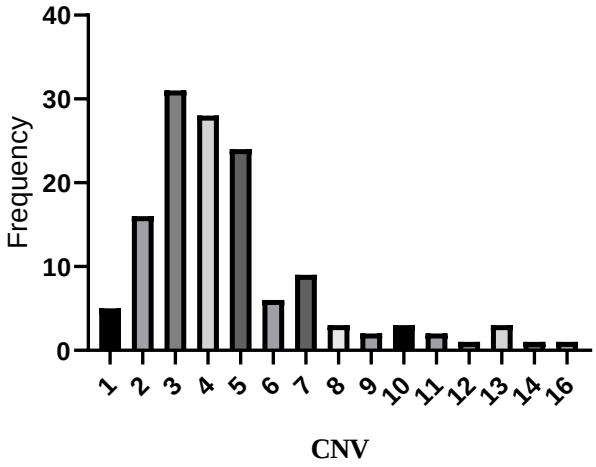

Fig.1 Distribution histogram of the estimated AMY1 copy number in 133 unrelated Chinese samples

3.1.2 Group determination

From the distribution situation, it was obvious that CNV 3, 4 and 5 have most samples. Since 3 is the mode number, we decided to set CNV=1 and 2 as the low copy number group. For the high copy number group, we first considered to balance the number of samples as the low copy number group to decide the cutting point. However, the sharp drop in participant number between CNV=5 and CNV=6 was hard to ignore. For these reasons, we finally use CNV=6 as the cutting point of high copy number.

Since the number of participants who took part in the intervention was limited and it was hard to divided them into 3 groups, we decided to use the median number as the cutting point, making only two groups in this case.

Finally, we got two grouping situations. For the total of 133 participants, we divided them into 3 groups, low copy number variation (L-CNV, CNV≤2), middle copy number variation (M-CNV，3≤CNV≤5) and high copy number variation (H-CNV，CNV≥6) (Table 1).

Table 1 The number of all the participants in each group (n=133)

Gender	Group		
	L-CNV	M-CNV	H-CNV
Female	10	41	17
Male	10	41	14
Total	20	82	31

For those who participated in the intervention, due to the limited number of participants, we divided them into two groups according to the median number of their AMY1 gene copy numbers, that is, low-middle copy number variation (LM-CNV, CNV≤3) and high-middle copy number variation (HM-CNV，

CNV≥4) (Table 2). The reason we chose males only in this part is that males gain muscle faster than females due to their higher testosterone level.

Table 2 The number of participants with intervention in each group (n=19)

Gender	CNV group	
	HM-CNV	LM-CNV
Male	10	9

3.2 Anthropometric measurement and body composition

3.2.1 Anthropometric measurement

When comparing each group, no significant differences were observed in all the results involving (P > 0.05). However, after Tukey test, we observed that the height of the L-CNV group was higher than that of the M-CNV group (P=0.039). For a single gender comparison, the height of male participants in the M-CNV group was significantly lower than that of the L-CNV group (P=0.005) and of the H-CNV group (P=0.020). As for female participants, we found no significant differences in all measured indices (Table 3).

Table 3 The results of anthropometric measurement of all the participants in each group (n=133)

	Gender	Group		
		L-CNV	M-CNV	H-CNV
Age（yr.）		22.64±2.79	23.04±3.23	22.60±3.53
Height(cm)	M	178.68±4.08[1]	174.00±4.61	177.64±5.63[2]
	F	164.84±5.11	163.54±4.55	162.76±5.50
Weight(kg)	M	74.75±11.79	70.25±9.65	74.54±8.70
	F	54.51±6.81	56.40±6.41	55.72±5.98
BMI	M	23.35±3.04	23.17±2.78	23.59±2.159
	F	20.07±2.49	21.08±2.20	21.01±1.72
Waist Circumference (cm)	M	81.80±7.42	79.48±7.91	79.79±6.419
	F	67.05±4.39	69.19±4.71	69.54±4.72
Hip Circumference（cm）	M	96.85±7.84	96.12±6.05	97.93±9.51
	F	91.75±5.83	93.01±5.15	92.59±3.87

M: male, F: Female

BMI: Body Mass Index

[1] L-CNV compared to M-CNV, *P*=0.005, Power=0.96

[2] H-CNV compared to M-CNV, *P*=0.020, Power=0.83

For those who participated in the intervention, we were only able to see an obvious increase in weight and BMI in the HM-CNV group (P<0.05), which did not appear in the LM-CNV group. However, due to the low statistic power, we were unable to agree that these differences were significant (Table 4).

Table 4 Comparison of anthropometric measurement of participants with intervention in each group (n=19)

		Group	
		HM-CNV	LM-CNV
Age（yr.）		21.89±3.18	21.50±1.51
Height（cm）		176.20±6.70	176.50±2.73
Weight（kg）	B	71.36±10.49	72.16±7.86
	A	75.38±10.45[1]	76.05±4.00
BMI（kg/m^2）	B	22.91±2.39	23.37±2.52
	A	24.01±2.23[2]	24.56±1.88

B: Before intervention, A: After intervention

[1] Compared to before, *P*=0.021, Power=0.23

[2] Compared to before, *P*=0.021, Power=0.33

In the first linear regression model, we were unable to find any correlation between AMY1 CNV and height, weight, or BMI(P > 0.05).

3.2.2 Body Composition

For the body composition of the 133 participants, in both the two-way ANOVA and the independent t-test, we found no significant differences either in the whole body or in each part of the body between each group (P > 0.05) (Table 5).

Table 5 The results of the body composition of all the participants in each group (n=133)

	Gender	Group		
		L-CNV	M-CNV	H-CNV
Whole Muscle Mass (kg)	M	55.22±5.53	54.12±6.37	58.95±7.01
	F	36.09±4.24	36.91±4.17	36.88±4.29
Whole Fat Mass (kg)	M	15.57±6.70	12.67±5.78	14.50±3.93
	F	16.15±3.72	16.91±4.16	15.94±3.39
Whole Bone Mass (kg)	M	3.07±0.39	2.95±0.33	3.16±0.48
	F	2.27±0.19	2.26±0.24	2.26±0.29
Ratio of Muscle Weight to Body weight	M	0.75±0.05	0.78±0.06	0.77±0.05
	F	0.66±0.04	0.66±0.05	0.67±0.05
Ratio of Fat Weight to Body Weight	M	0.20±0.06	0.18±0.068	0.19±0.05
	F	0.29±0.05	0.30±0.05	0.29±0.05
Ratio of Bone Weight to Body Weight	M	0.04±0.01	0.04±0.00	0.04±0.00
	F	0.04±0.00	0.04±0.00	0.04±0.00

M: male, F: Female

BMI: Body mass index

Although no changes were discovered between the different groups, we still found some changes after the intervention within each group. Both groups had a significant increase in muscle mass, which was congruous with the result of body weight. The most obvious change was found in the percentage of bone weight, which decreased after intervention in the HM-CNV group, while no change was shown in the LM-CNV group. Since the statistic powers of the absolute bone mass value in both groups were quite low, their significant differences were not credible (Table 6).

Table 6 Comparison of body composition of participants with intervention in each group (n=19)

	Gender	Group	
		HM-CNV	LM-CNV
Whole Muscle Mass (kg)	B	54.02±8.42	53.21±3.12
	A	59.09±9.43[1]	58.06±2.13[2]
Whole Fat Mass (kg)	B	12.60±4.77	14.92±6.04
	A	13.33±3.58	15.01±3.99
Whole Bone Mass (kg)	B	2.93±0.53	2.90±0.32
	A	2.96±0.55[3]	2.98±0.27[4]
Ratio of Muscle Weight to Body weight	B	0.78±0.05	0.76±0.06
	A	0.78±0.05	0.77±0.04
Ratio of Fat Weight to Body Weight	B	0.18±0.06	0.20±0.06
	A	0.18±0.05	0.20±0.05
Ratio of Bone Weight to Body Weight	B	0.04±0.00	0.04±0.00
	A	0.04±0.00[5]	0.04±0.00

[1] Compared to before，*P*=0.002, Power=0.73

[2] Compared to before，*P*=0.003, Power=0.99

[3] Compared to before，*P*=0.001, Power=0.05

[4] Compared to before，*P*=0.032, Power=0.14

[5] Compared to before，*P*=0.001, Power=0.96

Although there were no significant differences between each group of the 133 participants in body composition, we can still find a weak correlation between AMY1 CNV and muscle or fat mass in different body parts.

In the first linear regression model, we found that CNV was positively related to the whole muscle mass and trunk muscle mass after adjusted by gender. In the parameter of whole muscle mass, AMY1 CNV alone explained 3.96% of the variance (see squared partial correlation) between the participants. Moreover, one single increase in AMY1 CNV can lead to an increase of 0.386kg (see parameter estimation) of the whole muscle mass. The explanation was the same for the correlation between AMY1 CNV and trunk muscle mass (Table 7 and Fig. 3).

Table 7 Linear regression between CNV and body composition with AMY1 CNV as a dependent variable (n=133)

CNV	Correlation coefficient(r)	Parameter Estimation[2]	Squared Partial Correlation[1] (%)	Pr > |t|
Whole Muscle Mass	0.199	0.386	3.96	0.044
Trunk Muscle Mass	0.207	0.191	4.30	0.036

[1] Squared partial correlation explains how much AMY1 CNV can explain the whole variance.

[2] Parameter estimation shows how much one single increase in AMY1 CNV can lead to changes in body composition.

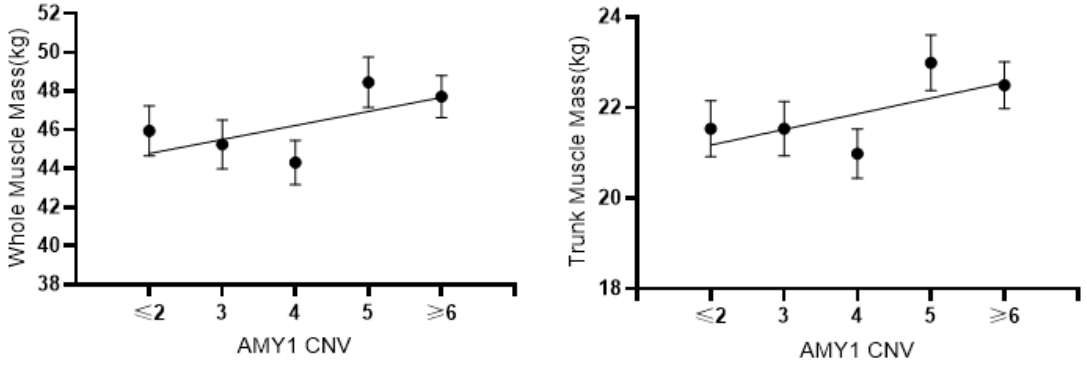

Fig 3. The linear regression between AMY1 CNV and some parameters of body composition in the first model. This figure only showed the average and standard deviation in each group.

All data related with muscle was positively correlated to AMY1 CNV.

For the second linear regression model, more correlations were discovered in different parts of the body. Except for the whole muscle mass and trunk muscle mass, some fat parameters (thigh fat mass, gynoid fat mass, the whole fat mass) were also observed to be associated with AMY1 CNV, except that their correlations were negative (Table 8 and Fig.4).

Table 8 Linear regression between CNV and body composition with AMY1 CNV, height, and weight as dependent variables (n=133)

CNV	Correlation coefficient(r)	Parameter Estimation[2]	Squared Partial Correlation[1] (%)	Pr > |t|
Whole Muscle Mass	0.220	0.214	4.83	0.028
Trunk Muscle Mass	0.223	0.123	4.96	0.025
Thigh Fat Mass	0.221	-0.076	4.86	0.027
Gynoid Fat Mass	0.235	-0.040	5.54	0.018
Whole Fat Mass	0.216	-0.217	4.69	0.030

[1]Squared partial correlation explains how much AMY1 CNV can explain the whole variance.

[2]Parameter estimation shows how much one single increase of AMY1 CNV can lead to changes in body composition.

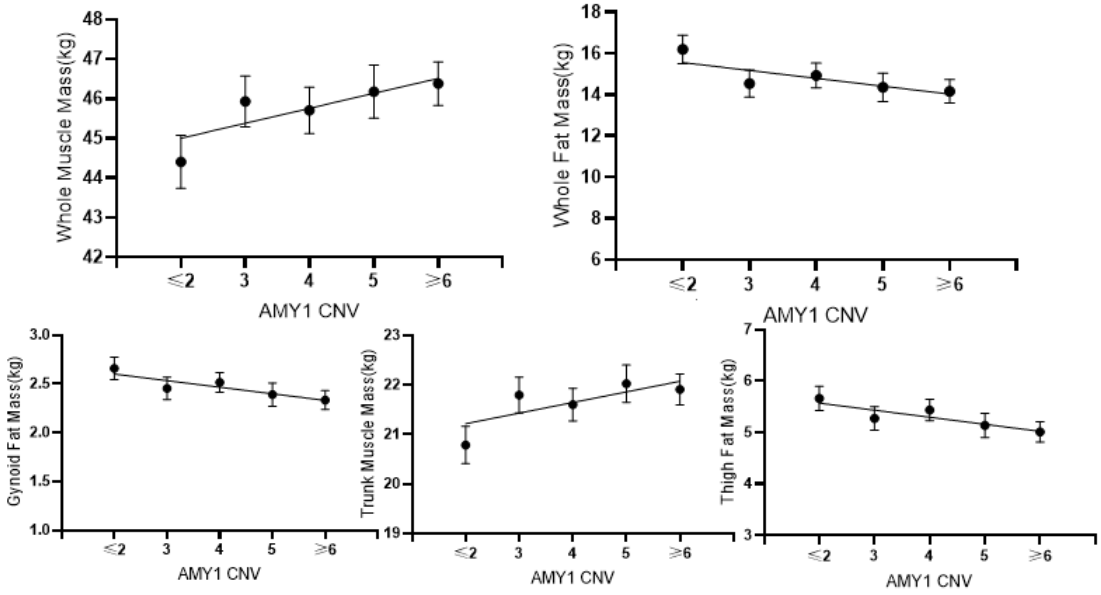

Fig 4. The linear regression between AMY1 CNV and some parameters of body composition in the second model. This figure only showed the average and standard division in each group.

All data related to muscle were positively correlated with AMY1 CNV, and those related to fat were negatively correlated.

4. Discussion

4.1 Validity of participant amount

In previous studies related to AMY1 CNV, Perry (Perry et al., 2007) chose 17 to 50 samples in each

race involved, Santos (Santos et al., 2012) had 89 participants for his nutrition-related study, and Viljakainen (Viljakainen et al., 2015) had 132 samples when dealing with the relationship between AMY1 CNY and early-onset female obesity. The number of participants we had in our study was similar to those study mentioned above, making it efficient to achieve our research objective.

4.2 Distribution of AMI CNV in participants

In our result of the AMY1 CNV distribution, we failed to find results reported by Yong (Yong et al., 2016), Rukh (Rukh et al., 2017) and Carpenter (Carpenter et al., 2017) that the larger number of participants carried even copy numbers. When comparing our results with previous studies involving only East Asians, although the range appeared to be similar to the others（1-16）, the average AMY1 CNV was lower in our results(4.71) (Yong et al., 2016; Perry et al., 2007; Yang et al., 2015; Choi et al., 2015; Inchley et al., 2016). Most numbers were located between copy number 3 to 5 (82 out of 133).

4.3 Anthropometric measurement and body composition

4.3.1 Anthropometric measurement

Unlike most previous studies, we were unable to see the most significant difference in our results among different CNV groups except for height. Surprisingly, instead of expecting difference between high CNV and low CNV group, the difference could only be observed in male participants, and both low CNV and high CNV group had higher height than M-CNV group.

4.3.2 Body composition

Although we did not find significant differences in body composition data between each group using two-way ANOVA, the linear regression results showed that there was still a weak association between AMY1 CNV and body mass. More correlations were discovered when we added height and weight as dependent variables. What we found in the results was that higher AMY1 CNV could contribute to muscle gain and fat loss. However, the percentage that AMY1 CNV could explain the whole variance was no more than 6%, and one single increase of AMY1 CNV could only lead to no more than 0.4 kg of changes in body composition.

4.3.3 Changes after intervention

In our study, we also tried to change body composition using a high-protein diet and weight training intervention, which had been proved to be valid by other studies (Antonio et al., 2015). From our result, both groups have an obvious increase in their muscle mass. It seemed that higher AMY1 CNV could contribute more to weight gain, but low statistic power indicates that more samples are required to verify this result. Instead of fat or muscle mass, only the percentage of bone mass showed difference between two groups. The increase in body weight in higher AMY1 CNV group might explain the decreasing bone mass ratio, but due to the low statistic power, this hypothesis should also be proved by expanding the number of participants.

4.4 Hypotheses and inference

In our study, since no body composition differences were found between each AMY1 CNV group, combined with the linear regression results, we can try to establish the hypothesis that the contribution of AMY1 CNV could probably be hidden by other factors such as diet or exercise. This hypothesis is

accordant with many previous studies. Most studies with positive results used children as participants (Marcovecchio et al., 2016; Mejia-Benítze et al., 2015)], or at least involving childhood-onset obese (Viljakainen et al., 2015). In contrast, most studies whose conclusions were against Falchi's research selected adults as participants. The possible reason could be explained as, for adults, their diet and exercise habits have lasted for a relatively long time, enough to surpass the effect caused by AMY1 CNV. For children, the AMY1 CNV might play a more important role since their diet and exercise habit have not yet been shaped. This may also explain why the heritability of BMI was higher in childhood rather than in adulthood (Koziel et al., 2013).

4.5 Limitations of the study

Due to our number of participants in the intervention part, we divided them only into two groups. It would be more supportive to our hypothesis if we expanded the saliva sample size, selected participants equally from each AMY1 CNV group, and added gender as one of the dependent factors.

5. Conclusions

Considering all mentioned above, we can try to explain that AMY1 copy number variation only has a minor correlation with obesity and obese as a genetic factor, and its function could easily be concealed by factors such as diet or exercise.

Compliance with Ethical Standards

Conflict of interest

Zhang Xinming, Colin Moran, Wang Ruiyuan, Zhou Yue and Naomi Brooks declare that they have no conflict of interest.

Ethical approval

This study had been approved by the School of Sport Research Ethics Committee at University of Stirling (code SSREC number #880). Informed consent was obtained from all individual participants included in the study.

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