Original Article

Association of dopamine receptor D2 gene (*DRD2*) Taq1 polymorphisms with eating behaviors and obesity among Chinese and Indian Malaysian university students

Fang-Ying Lek BSc, Hing-Huat Ong MSc, Yee-How Say PhD

Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR), Kampar Campus, Kampar, Perak, Malaysia

Background and Objectives: This study investigated the association of *DRD2* Taq1A, Taq1B and Taq1D gene polymorphisms with eating behavior, the preference/intake frequency/craving of high-fat foods and obesity in 394 Malaysian adults (161 males, 233 females; 308 Chinese, 86 Indians; 67 obese, 327 non-obese). **Methods and Study Design:** Eating behaviors namely Cognitive Restraint, Uncontrolled Eating and Emotional Eating scores were assessed by the Three Factor Eating Questionnaire-R18. The preference/intake frequency/craving of 26 common high-fat Malaysian foods was assessed using a 7-point hedonic scale. Anthropometric measurements were taken and Taq1 gene polymorphisms were genotyped by PCR-Restriction Fragment Length Polymorphism using DNA extracted from mouthwash samples. **Results:** The overall minor allele frequencies of Taq1A, Taq1B and Taq1D according to ethnicities (Chinese/Indian) were 0.37/0.29, 0.39/0.28, 0.06/0.30, respectively; genotype and allele distributions of Taq1B and Taq1D were significantly different between ethnicities. Eating behaviorscores were not significantly different between gender and ethnicities. Those with A1 or B1 allele had lower Cognitive Restraint score and higher Uncontrolled Eating score, while those with A1/A1 or B1/B1 genotype had higher fast food preference. D1 allele was associated with increased starchy food craving and *mamak* (Malaysian Indian-Muslim) food preference, but not eating behavior scores. All three gene variants were not associated with obesity and adiposity. **Conclusion:** Taken together, we posit that three *DRD2* Taq1 gene polymorphisms influence the eating behavior and preference/intake frequency/craving of certain high-fat foods in Malaysian adults, but their role in obesity and adiposity is still inconclusive and needs further investigation.

Key Words: *DRD2* gene Taq1 polymorphisms, eating behavior, obesity, Malaysia, university students

INTRODUCTION

Obesity is defined as abnormal or excessive body fat accumulation that presents a risk to health.[1] The rising prevalence of obesity worldwide has been described as a global pandemic. The 2013 global systemic analysis reported that the prevalence of overweight and obesity was 43.8% and 48.6% among adults >20 years in Malaysia, respectively.[2] Meanwhile, the Malaysian National Health and Morbidity Survey 2015 reported that the prevalence of overweight and obesity was 33.4% and 30.6% among adults, respectively (Malaysian Clinical Practice Guidelines of Obesity 2004).[3] Obesity can be considered as a disease of food addiction, as the symptoms of uncontrolled eating and emotional eating correspond to the symptoms in drug abuse and drug dependence.[4] Both food consumption and drug use activate the same mesolimbic dopamine reward system - a neurotransmitter system that plays a major role in the control of energy intake and body weight.[5]

The D2 dopamine receptor (D2R) is one of the five subtypes of dopamine receptors (D1R-D5R). The gene encoding for it, *DRD2*, has eight exons and several of the single nucleotide polymorphisms (SNPs) have been screened for association with schizophrenia,[6] alcohol and drug addiction,[7] obesity,[8] eating behaviors,[9-11] albeit with conflicting findings in different world populations. Three common *DRD2* SNPs include missense SNP Taq1A (NM_178510.1:c.2137G>A; also known as rs1800497, C/T, and Glu713Lys), intronic SNP Taq1B (NM_016574.3:c.-31-882G>A; also known as rs1079597 and C/T), and intronic SNP Taq1D (NM_016574.3:c.286-2730C>T; also known as rs1800498 and G/A). *DRD2* Taq1A and Taq1B SNPs support hypodopaminergic activity as *in vitro* and *in vivo* studies suggest that individuals with the variant alleles have reduced D2R density in the striatum.[12,13]

Dutch adolescents carrying at least one *DRD2* Taq1A A1 allele have been shown to have increased emotional eating in relation to high parental psychological control.[9]

Corresponding Author: Dr Yee-How Say, Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR) Kampar Campus, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia.
Tel: +605-4688888 ext. 4505; Fax: +605-4661676
Email: sayyh@utar.edu.my
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However, Caucasian adults carrying Taq1A A2/A2 genotype had significantly higher scores on binge eating, hedonic eating, emotional eating, and food cravings compared to those carrying the A1 allele.[10] To date, there are scarce studies investigating the association of *DRD2* SNPs with eating behaviors and obesity in Asian populations, as the majority of studies were mostly conducted in Caucasians. Only a single study recently showed that Asian Americans carrying the A1 allele had greater carbohydrate and fast food craving.[11] Therefore, the objective of this study was to investigate the prevalence of *DRD2* Taq1A, Taq1B and Taq1D SNPs in Malaysian students, and to associate the SNPs with eating behavior, the preference/intake frequency/craving of high-fat foods, and obesity.

PARTICIPANTS AND METHODS

Subjects, questionnaire and anthropometric measurements

Convenience sampling was performed in this study to recruit students of Universiti Tunku Abdul Rahman, Kampar Campus, Malaysia from Jan–June 2014. The exclusion criteria included those with psychiatric history or who took medications affecting appetite like steroids and antipsychotics. The demographic data included in this questionnaire were age, gender and self-identified ethnicity. Due to the student composition of the institution being historically predominantly non-Malay, we did not successfully recruit any Malay subjects. Anthropometric measurements - waist and hip circumferences (WC and HC), height, weight, Body Mass Index (BMI) and Total Body Fat (TBF) were taken. WC was measured using a stretch-resistant tape measure to the nearest 0.1 cm between the lowest rib and the iliac crest with the subjects in standing position, while HC was measured to the nearest 0.1 cm around the widest portion of the buttocks. A bio-impedance body fat weighing scale (HBF-362 Omron Karada, Japan) was used to determine the weight (to nearest 0.25 kg), BMI (to nearest 0.1 kg/m^2) and TBF (to nearest 0.1%). The cut-off points for obesity (based on BMI), overall adiposity (TBF) and central adiposity (Waist-to-Hip Ratio; WHR) were ≥25 kg/m^2,[1] 20% (males) or 30% (females)[14] and 0.90 (males) or 0.85 (females),[15] respectively. The UTAR Scientific and Ethnical Review Committee approved this study (UTAR/SERC/23Dec2013), all individuals participating in this study signed informed consent forms and all samples were taken in accordance with the Declaration of Helsinki (as revised in Seoul 2008).

Three Factor Eating Questionnaire-R18 (TFEQ-R18)

TFEQ-18[16] consists of eighteen items related to eating behaviors in a person. The responses were "definitely true", "mostly true", "mostly false" or "definitely false". The three subscales of TFEQ-18 were cognitive restraint (CR), uncontrolled eating (UE) and emotional eating (EE).[16] The raw scale scores were transformed to a 0-100 scale [(raw score – lowest possible raw score)/possible raw score range) × 100]. The reliability of these subscales was proven by high Cronbach's α-values.[17] These subscales were further categorized into tertiles of Low (scores 0-33), Medium (scores 34-67) and High (scores 68-100). Higher scores in the respective scales indicate greater CR, UE or EE.[16]

Hedonic assessment of high-fat food preference, intake frequency and craving

A self-administered questionnaire on the preference, intake frequency and craving of a list of 26 foods/dishes which contain a high proportion of fat was presented. The list was determined by surveying around the university campus food/dishes that are prepared using high amounts of oil (deep-frying, stir-frying with lots of oil) and/or have a high proportion of fats and/or oils. The preparation methods, serving sizes/amount and total fat content are presented in Supplementary Table 1. These include burger, cheese cake, chocolate bar, creamed soup, curry noodles, French fries, fried chicken chop (Taiwanese snack),

Table 1. Demographic, anthropometric characteristics and TFEQ-R18 scores of the subjects according to gender

Variables	Male (*n*=161)	Female (*n*=233)	χ^2	*p*
Mean age	20.9±0.13	20.9±0.11		
Ethnicity			0.28	0.60
Chinese	128 (79.5)	180 (77.3)		
Indian	33 (20.5)	53 (22.7)		
BMI class			15.9	<0.001*
Non-obese	119 (73.9)	208 (89.3)		
Obese	42 (26.1)	25 (10.7)		
WHR class			14.1	<0.001*
Normal	37 (23.0)	96 (41.2)		
High	124 (77.0)	137 (58.8)		
TBF class			1.74	0.19
Normal	109 (67.7)	172 (73.8)		
High	52 (32.3)	61 (26.2)		
TFEQ-R18 scores				
CR100	53.9±1.75	50.0±1.18		0.07
UE100	55.1±1.92	58.2±1.30		0.11
EE100	66.0±2.11	67.6±1.45		0.75

TFEQ-R18: Three Factor Eating Questionnaire-R18; BMI: Body Mass Index; WHR: Waist-Hip Ratio; TBF: Total Body Fat percentage; CR100: Cognitive Restraint Score 100; UE: Uncontrolled Eating Score 100; EE: Emotional Eating Score 100.
Parentheses indicate percentage within the same gender.
p-values by Pearson's Chi-Square test or Mann-Whitney *U* test ; *p-value significant at <0.05.

fried chicken meal (fast food), fried fish, fried noodles, fried nuggets/meatballs/fishballs, fried rice, fried squid, fried sweet potato/banana, hot dog, ice-cream cone/sundae, fried noodles *mamak* (Malaysian Indian-Muslim) style, *mee rebus mamak* (yellow noodles served with a thick spicy potato-based gravy and garnished with a hard-boiled egg, spring onions, bean sprouts, fried shallots, dried/ fried beancurd and lime juice), soup noodles, briyani rice set (with a piece of curry chicken and pickled vegetables), *nasi lemak* (Malaysian coconut milk rice, served with *sambal*, fried crispy anchovies, toasted peanuts, and cucumber), pizza, potato chips, *rojak mamak* (contains fried dough fritters, bean curds, boiled potatoes, prawn fritters, hard boiled eggs, bean sprouts, cuttlefish and cucumber mixed with a sweet thick, spicy peanut sauce), *roti telur* (Indian flatbread with egg; normally served with dhal or lentil curry) and spaghetti with sauce. In order to aid a better recall of the preference, intake frequency and craving of foods, subjects were also presented with images of the foods, with appropriate serving sizes to reflect high fat content. For example, fried chicken meal (fast food) was presented as two pieces of standard size fried chicken, a 3" × 3" bun, a cup of mashed potato and a cup of coleslaw. Subjects were required to rate the high-fat foods based on how much do they like the item (preference), how frequent do they consume the item (intake frequency), and how often do they experience a craving for the item over the past month (craving) using a 7-point hedonic scale.[18] The response alternatives for preference were 1 = very unpleasant, 2 = fairly unpleasant, 3 = slightly unpleasant, 4 = neither pleasant nor unpleasant, 5 = slightly pleasant, 6 = fairly pleasant and 7 = very pleasant; intake frequency were 1 = never, 2 = once a month or less often, 3 = 1-2 times a month, 4 = once a week, 5 = a couple of times a week, 6 = almost everyday and 7 = at least once a day and for craving, 1 = never, 2 = very rarely, 3 = rarely, 4 = sometimes, 5 = often, 6 = very often and 7 = always/almost everyday.

Genotyping

Participants were asked to rinse their mouth vigorously with 5 mL of 3% sucrose solution for 1 min and the mouthwash samples were preserved in 3 mL TNE buffer [17 mMTris/HCl (ph 8.0), 50 mMNaCl, 7 mM EDTA]. DNA extraction protocol was then conducted using the Rapid Blood Genomic DNA Extraction Kit (Biobasic, Canada) according to manufacturer's protocols. The *DRD2* Taq1A, Taq1B and Taq1D SNPs were genotyped by PCR – Restriction Fragment Length Polymorphism (PCR - RFLP) using the primers, reagents, and conditions adopted from Vijayan et al (2007),[6] with minor modifications. PCR was performed in a 20 µL total reaction mixture containing 1× Taq buffer without $MgCl_2$ (PhileKorea, Korea), 1.0 mM $MgCl_2$, 10 µM dNTPs (PhileKorea, Korea), 10 pmol of forward primer, 10 pmol of reverse primer, 100 ng of DNA template, 1 U of Taq polymerase (PhileKorea, Korea), and topped up with an appropriate volume of sterile deionized water. The PCR amplification protocol was carried out using the Mastercycler Gradient PCR machine (Eppendorf, Germany) which began with initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 68°C (for all SNPs) for 45 sec, extension at 72°C for 1 min, and final extension at 72°C for 5 min. The genotyping of all three SNPs was performed by digesting PCR products using 1U of *Taq*I (Thermo Scientific, MA, USA) at 65°C for 16 h. RFLP products were electrophoresed on 2% agarose gel, then stained with ethidium bromide and visualized under UV light after electrophoresis. The sizes of the bands for Taq1A genotypes were A1/A1 310 bp (uncleaved); A1/A2 310, 180, 130 bp; A2/A2 180, 130 bp, for Taq1B genotypes were B1/B1 459 bp (uncleaved); B1/B2 459, 267, 192 bp; B2/B2 267, 192 bp and for Taq1D were D1/D1 419 bp (uncleaved); D1/D2 419, 272, 147 bp; D2/D2 272, 147 bp. Three genotypes from each SNP were verified by DNA sequencing of PCR products (First BASE Laboratories Sdn. Bhd., Malaysia).

Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics software version 16.0 (IBM, NY, USA). Data for continuous variables were presented as means ± standard error of the means (SEM), and as frequency for categorical variables. The normality of the sample distribution was examined using the Kolmogorov-Smirnov Test. Allelic frequencies were determined by direct gene counting, and the distribution of genotypes was tested for the Hardy-Weinberg equilibrium using the Chi-square ($\chi 2$) test. Linkage disequilibrium (LD) analysis of the three SNPs was performed using Haploview (Broad Institute, Cambridge, MA). Logistic regression analysis (enter method) was performed with adjustment for covariate gender (for BMI and WHR) and ethnicity (for TBF) to investigate the association of *DRD2* Taq1 genotype and allele distribution with anthropometric classes. BMI, WHR and TBF were log-transformed and analysed by using univariate analysis of variance (General Linear Model) with adjustment for covariate gender (for BMI and WHR) and ethnicity (for TBF).

Principal Component Analysis (PCA) with Varimax rotation was used to portray the basic structure of continuous variables, i.e. Fast food preference, Starchy foods craving, *Mamak* food craving & intake frequency, *Mamak* food preference, Fast food intake frequency, Soup intake frequency, craving and preference, Chocolate intake frequency, craving and preference, Ice-cream intake frequency, craving and preference Soup noodle intake frequency, craving and preference, Curry noodles intake frequency, craving and preference, Fried noodles intake frequency, craving and preference, Spaghetti intake frequency, craving and preference, Fried rice intake frequency, craving and preference, Fried squid intake frequency, craving and preference and Fried fish intake frequency, craving and preference. Therefore, there were a total of 78 variables in the initial PCA based on the 26 food list × 3 (preference, intake frequency and craving). Correlation matrix (normed) was used to compute the PCA results. The conditions for the validity of PCA were adopted as described in our previous study.[19] Results from PCA showed that the Kaiser-Meyer-Olkin index was 0.82 and the Bartlett's test was highly significant (p<0.0001). In this study, 53 out of 78 variables were used in PCA, as 25 variables with the MSA values of less than 0.50 were removed. PCA with Varimax rotation resulted in 16 com-

ponents (factors) that had eigenvalues which contributed to an accumulative 74.3% of variance explained (Supplementary Table 2). All principal components by PCA are uncorrelated (orthogonal) to each other. After identifying the hidden variables as components, the factored mean for each principal component was calculated as described in our previous study.[19] The significant differences between demographic and anthropometric classes and *DRD2* genotypes and alleles for different components were determined by Mann-Whitney *U* or Kruskal-Wallis test.The *p*-value of <0.05 was considered as statistically significant.

RESULTS

The demographic, anthropometric characteristics and TFEQ-18 scores for CR, UE and EE of the 394 healthy and unrelated subjects are as shown in Table 1. Only BMI and WHR were significantly different between gender.

The genotype and allele distribution of *DRD2* Taq1A, Taq1B and Taq1D SNPs of subjects did not deviate from the Hardy-Weinberg equilibrium. LD analysis also showed that the three SNPs were in strong LD with each other (all r^2>0.87; data not shown). The minor allele frequencies (MAFs) of Taq1A, Taq1B and Taq1D according to ethnicities (Chinese/Indian) were 0.37/0.29, 0.39/0.28, 0.06/0.30, respectively. Taq1B and Taq1D genotype and allele distributions were associated with ethnicity – B1/B1, B1, D2/D2 and D2 were distributed higher among Chinese (data not shown). Table 2 shows that *DRD2*Taq1A, Taq1B and Taq1D genotype and allele distributions were overall not associated with BMI, TBF and WHR classes. Nevertheless, the Taq1D D2 allele was associated with having 0.55 times lower risk of having high central adiposity (WHR) compared with the D1 allele (Table 2). The adjusted means of BMI, WHR and TBF were also not significantly different between Taq1A, Taq1B and Taq1D genotypes and alleles (data not shown).

In this study, the overall TFEQ-18 scores for CR, UE and EE were 51.5±0.99, 57.0±1.09 and 67.0±1.20, respectively. CR was correlated with both UE (r=0.12, p=0.03) and EE (r=0.25, p<0.001), while UE was correlated with EE (r=0.39, p<0.001). CR, UE and EE scores were all not significantly different between gender (Table 1) and ethnicities. However, those who were non-obese and with normal TBF had significantly higher CR scores, while those with normal TBF also had significantly higher EE scores (Table 3). We also found that those with the A1 or B1 allele had lower CR scores but higher UE scores (Table 3), while EE scores were not significantly different between all alleles. Eating behavior scores were not significantly different between Taq1D alleles (Table 3).

Table 4 shows that 12 PCA components of high-fat food preference/intake frequency/craving were significantly different between UE tertile groups, while only one and two PCA components were significantly different between CR and EE tertile groups, respectively. Those with higher CR had lower preference/intake frequency/craving of soup noodles (Table 4). Interestingly, those that belong to the "high" tertile groups of UE and EE tend to have the lowest factored means of preference/intake frequency/craving of high-fat foods (except for cheese cake; Table 4).

Table 5 shows that the fast food preference, starchy food cravings and *mamak* food preference were significantly different between Taq1 genotypes and alleles. Those with A1/A1 or B1/B1 genotypes had the highest factored mean of fast food preference, while those with D1 allele rated significantly higher craving of starchy foods and preference of *mamak* foods (Table 5). All other PCA components of preference/intake frequency/craving of high-fat foods were not significantly different between

Table 2. Logistic regression analysis for the association of *DRD2* Taq1 genotype and allele distribution with anthropometric classes

DRD2 variant (*n*)	BMI Class†		TBF Class‡		WHR Class†	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Taq1A						
A1/A1 (48)	1.00	-	1.00	-	1.00	-
A1/A2 (183)	0.74 (0.33, 1.63)	0.45	0.75 (0.38, 1.51)	0.42	1.19 (0.57, 2.47)	0.64
A2/A2 (163)	0.65 (0.29, 1.47)	0.30	0.60 (0.29, 1.23)	0.16	0.79 (0.50, 1.24)	0.30
A1 (279)	1.00	-	1.00	-	1.00	-
A2 (509)	0.83 (0.56, 1.22)	0.33	0.79 (0.57, 1.09)	0.15	1.02 (0.74, 1.40)	0.91
Taq1B						
B1/B1 (55)	1.00	-	1.00	-	1.00	-
B1/B2 (181)	0.71 (0.33, 1.50)	0.37	0.82 (0.42, 1.60)	0.56	0.88 (0.46, 1.71)	0.71
B2/B2 (158)	0.59 (0.27, 1.28)	0.18	0.64 (0.32, 1.28)	0.21	0.94 (0.48, 1.85)	0.87
B1 (291)	1.00	-	1.00	-	1.00	-
B2 (497)	0.77 (0.53, 1.14)	0.19	0.80 (0.57, 1.11)	0.17	1.00 (0.73, 1.36)	0.99
Taq1D						
D1/D1 (10)	1.00	-	1.00	-	1.00	-
D1/D2 (69)	0.76 (0.14, 4.22)	0.75	0.57 (0.14, 2.26)	0.42	0.29 (0.03, 2.46)	0.25
D2/D2 (315)	0.81 (0.16, 4.06)	0.80	0.66 (0.17, 2.50)	0.54	0.19 (0.02, 1.53)	0.12
D1 (89)	1.00	-	1.00	-	1.00	-
D2 (699)	0.99 (0.54, 1.81)	0.98	0.98 (0.60, 1.62)	0.95	0.55 (0.33, 0.93)	0.03*

OR: odds ratio; CI: confidence interval
†Adjusted for co-variate: gender.
‡Adjusted for co-variate: ethnicity.
Values are by logistic regression enter method. *p-value significant at <0.05.

Table 3. TFEQ-R18 scores between BMI, TBF classes and *DRD2* Taq1 alleles

Categories (*n*)	CR	UE	EE
BMI Class			
Non-obese (327)	52.8±1.05	57.4±1.12	67.6±1.27
Obese (67)	41.9±2.44	54.5±3.81	62.6±3.73
p	<0.001*	0.47	0.20
TBF Class			
Normal (281)	53.4±1.13	57.7±1.21	68.5±1.33
High (113)	45.0±1.88	54.7±2.44	61.8±2.68
p	<0.001*	0.29	0.02*
Taq1A Allele			
A1 (279)	49.7±1.11	59.3±1.22	66.2±1.34
A2 (509)	52.6±0.89	55.7±0.98	67.5±1.10
p	0.03*	0.03*	0.23
Taq1B Allele			
B1 (291)	49.6±1.08	59.2±1.19	66.3±1.30
B2 (497)	52.7±0.91	55.6±1.00	67.5±1.12
p	0.02*	0.03*	0.27
Taq1D Allele			
D1 (89)	53.8±2.86	53.4±2.45	68.1±2.70
D2 (699)	51.3±0.72	57.3±0.81	66.9±0.89
p	0.30	0.16	0.94

CR: cognitive restraint, UE: uncontrolled eating, EE: emotional eating.
Data presented are Mean±SEM.
Significance was compared by Mann-Whitney *U* or Kruskal-Wallis test. * indicate significance at $p<0.05$; ** $p<0.01$.

Table 4. Factored means of PCA components of fat food preference/intake frequency/craving between TFEQ-R18 tertile groups

PCA components	Low (0-33 score out of 100)	Medium (34-67 score out of 100)	High (68-100 score out of 100)
CR			
FreCravLike Soup Noodles	7.54±0.34	7.17±0.19	6.08±0.36
UE			
Fast food preference	21.7±0.98	19.8±0.34	18.9±0.54
Starchy foods craving	7.13±0.50	6.42±0.18	5.83±0.24
Mamak food preference	11.0±0.62	11.3±0.21	10.2±0.31
Fast food frequency	6.99±0.36	6.52±0.17	5.96±0.18
Chocolate FreCravLike	7.69±0.48	8.50±0.18	7.63±0.23
Ice-cream FreCravLike	9.39±0.66	9.68±0.19	8.93±0.24
Fried noodles FreCravLike	8.21±0.68	7.20±0.19	6.38±0.24
Spaghetti FreCravLike	9.55±0.48	8.72±0.19	8.32±0.22
Fried rice FreCravLike	8.09±0.39	7.90±0.16	7.11±0.23
Cheese cake FreCravLike	6.91±0.60	7.95±0.20	6.98±0.25
Fried squid FreCravLike	7.70±0.56	7.10±0.19	6.19±0.26
Fried fish FreCravLike	7.41±0.39	6.98±0.16	6.39±0.20
EE			
Cheese cake FreCravLike	8.17±0.53	7.85±0.20	7.05±0.26
Fast food frequency	7.40±0.44	6.33±0.17	6.24±0.19

CR: cognitive restraint, UE: uncontrolled eating, EE: emotional eating, Fre: intake frequency, Crav: craving, Like: preference.
Data presented are factored Mean±SEM of selected PCA which are significantly different ($p<0.05$) from TFEQ-18 tertile groups. Significance was compared by Mann-Whitney *U* or Kruskal-Wallis test.

Taq1 genotypes and alleles (data not shown).

DISCUSSION

Among Malaysian Chinese subjects, the MAFs for Taq1A, Taq1B and Taq1D (0.37, 0.39, 0.06, respectively) are similar with those from the HapMap Chinese Han Beijing (CHB),[20,21] and 1000Genomes East Asians (EAS)[22] studies (0.35, 0.38 and 0.06, respectively). The non-significant association between *DRD2* SNPs with anthropometric measurements is consistent with a recent study among Asian Americans - where there was no significant difference in BMI and TBF between Taq1A genotypes.[11] According to the findings from mainly Caucasian populations, the association between *DRD2* genotypes and obesity are equivocal,[23] possibly due to sample size differences. For example, the study of Spitz et al (2000)[24] consisting of 37 obese and 139 non-obese reported that *DRD2* Al and B1 alleles were associated with adiposity. In contrast, another study among 1187 Pima Indians reported there was no significant association between *DRD2* genotypes and obesity.[8]

CR is defined as consciously restricting food intake in order to maintain body weight, UE is tendency to increase intake frequency of foods due to loss of control over consumption of food, while EE is characterized as the presence of overeating when in certain mood states such as

Table 5. Factored means of selected PCA components of fat food preference/craving between *DRD2* Taq1 genotypes and alleles

	Fast food preference	Starchy foods craving	*Mamak* food preference
Taq1A genotype			
A1/A1	21.2±0.60	6.58±0.40	10.7±0.46
A1/A2	19.0±0.42	6.00±0.20	10.9±0.25
A2/A2	20.1±0.44	6.59±0.22	11.2±0.27
p	0.03*	0.13	0.53
Taq1B genotype			
B1/B1	20.9±0.62	6.60±0.39	10.7±0.45
B1/B2	18.9±0.41	6.06±0.20	11.0±0.24
B2/B2	20.2±0.45	6.51±0.22	11.1±0.28
p	0.03*	0.26	0.68
Taq1D allele			
D1	20.9±0.80	7.12±0.32	12.2±0.48
D2	19.6±0.20	6.24±0.10	10.9±0.12
p	0.09	0.01*	0.02*

Data presented are Factored Mean±SEM.
Significance was compared by Mann-Whitney *U* or Kruskal-Wallis test. *Indicates significance at $p<0.05$.

anxiety or loneliness.[25] CR, UE and EE scores were all not significantly different between gender and ethnicities, in contrast with earlier findings stating that females had higher CR and EE scores.[16,17] Nevertheless, in this study, we found that obesity and adiposity are correlated with eating behaviors, consistent with an earlier finding stating that BMI are significantly correlated with CR, UE and EE scores.[17]

The association of Taq1A SNP with eating behaviors has been shown in previous studies - individuals with A1 allele had increased binge eating[8] and emotional eating.[9] Similarly in this study, we found that those with A1 or B1 allele had lower CR scores but higher UE scores, indicating poorer food restraint and more likely to binge-eat. Taq1D did not seem to influence eating behaviors in this study.

In this study, interestingly, we found that UE and EE tend to have the lowest factored means of high-fat food preference/intake frequency/craving. In contrast, UE was significantly positively correlated and EE was significantly inversely correlated with the liking and use-frequency of salty-and-fatty foods,[17] indicating that those with higher UE and lower EE will prefer and eat more salty-and-fatty foods. Discrepancy in these findings could be due to ethnic or socio-cultural differences between populations.

In this study, we found that fast food preference, starchy food cravings and *mamak* food preference were significantly associated with Taq1 genotypes and alleles. Similarly, in an Asian population in America, those with A1 allele had greater carbohydrate and fast food craving compared with A2 allele.[11] All other PCA components of high-fat food preference/intake frequency/craving were not associated with Taq1 genotypes and alleles. Therefore, Taq1 SNPs seem to have minimal influence in determining high-fat food preference/intake frequency/craving of among Malaysian subjects.

The mechanisms that mediate the effects of the three *DRD2* Taq1 SNPs on eating behavior, high-fat food preference/intake frequency/craving, body weight and fat mass are still elusive. Given three SNPs are in strong LD, the association patterns of these SNPs with eating behavior and body composition should be consistent or similar. But they are not. This is due to the SNP locations in the *DRD2* gene, which might have different functional effects independently. Taq1A missense and Taq1B intronic SNPs seem to be functional SNPs as their variant alleles are associated with reduced dopaminergic activity and reduced D2 density in the striatum.[12,13] Therefore, the functional significance of these SNPs particularly on other affected tissues apart from the nervous system (like the adipose tissue) warrants further investigation.

The present study had several limitations. First, the relatively small sample size could have limited the statistical power needed to achieve significant association, for example, between genotype and obesity traits. Also, the study was conducted in Chinese and Indian Malaysians restricted to a certain area only. It is unclear if the results could be generalized to the entire Malaysian population, especially Malays, the major ethnicity. *DRD2* is highly polymorphic, containing 3,477 SNPs, insertions and deletions, and duplications.[26] Therefore, other *DRD2* SNPs that have high LD with Taq1A, Taq1B and Taq1D could also be associated with eating behavior, obesity and adiposity.

In conclusion, the most-studied *DRD2* functional SNP Taq1A is associated with CR, UE and fast food preference, but not with obesity and adiposity in Malaysian subjects. Those with "dopamine-resistant" A1 allele had poorer conscious food restraint in order to maintain their body weight (CR), higher uncontrolled eating, while those with A1/A1 genotype had higher fast food preference. Another *DRD2* Taq1 SNP Taq1B, particularly B1 allele, is also associated with lower CR, higher UE and preferred fast food more. The third SNP Taq1D, particularly D1 allele, is associated with increased starchy food craving and *mamak* food preference. Taken together, while the three *DRD2* Taq1 SNPs seem to indeed influence eating behavior and the preference/intake frequency/craving of certain high-fat foods, their role in obesity and adiposity is still inconclusive and needs further investigation.

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AUTHOR DISCLOSURES

The authors have no potential conflicts of interest.

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Supplementary table 1. Preparation methods, serving sizes and amount and total fat content of foods/dishes used in the survey.

Food list	Preparation method and portion presented in photo	Serving amount (g or ml)	Total fat content (g)
Burger	Bun with beef patty, cheese, lettuce and pickled cucumber, 1 whole	124	14.3
Cheese cake	1 piece	125	28.0
Chocolate bar	1 medium bar (L x W x T, 11 x 3 x 1 cm)	50.0	16.4
Creamed soup	Cream of mushroom soup, 1 cup	248	6.00
Curry noodles	Wheat noodles with curry broth and condiments, 1 bowl	410	36.9
French fries	1 cup, small	90.0	16.1
Fried chicken chop (Taiwanese snack)	5 pieces	87.3	14.9
Fried chicken meal (fast food)	Two pieces of standard size fried chicken, a 3' × 3" bun, a cup of mashed potato and a cup of coleslaw	500	51.0
Fried fish	Indian mackerel, fried, 1 piece	50.0	5.79
Fried noodles	Stir-fried with soy sauce and condiments, 1 plate	170	9.01
Fried nuggets/meatballs/fishballs	4 pieces	64.0	12.7
Fried rice	Chinese style, 1 plate	392	51.7
Fried squid	Deep-fried calamari rings, 1 cup	350	29.0
Fried sweet potato/banana	Deep-fried with batter, 2 pieces	130	5.72
Hot dog	1 whole	82.0	11.5
Ice-cream cone/sundae	1 serving	150	21.0
Fried noodles *mamak*	Malaysian Indian-Muslim style, 1 plate	325	29.3
Mee rebus mamak	Yellow noodles served with a thick spicy potato-based gravy and garnished with a hard-boiled egg, spring onions, bean sprouts, fried shallots, dried/ fried beancurd and lime juice, 1 plate	325	19.0
Soup noodles	1 bowl	563	14.6
Briyani rice set	With a medium piece of curry chicken and pickled vegetables	370	26.7
Nasilemak	Malaysian coconut milk rice, served with sambal, fried crispy anchovies, toasted peanuts,and cucumber, 1 medium plate	306	11.0
Pizza	With chicken and pineapple, 1 piece (R x W x T, 11 x 10.5 x 2.8)	94.4	14.2
Potato chips	1 medium packet	50.0	16.1
Rojakmamak	Contains fried dough fritters, bean curds, boiled potatoes, prawn fritters, hard boiled eggs, bean sprouts, cuttlefish and cucumber mixed with a sweet thick, spicy peanut sauce	330	51.2
Roti telur	Indian flatbread with egg; normally served with dhal or lentil curry	135	13.5
Spaghetti with sauce	With cheese and meat sauce, 1 plate	440	1.32

Sources: Malaysia Food Composition Database http://www.nutriweb.org.my/food-db/index.php and USDA Food Composition Databases https://ndb.nal.usda.gov/ndb/search/list.

Supplementary table 2. Factor loadings for perceived oiliness, creaminess and fat contents of custards and commercial foods by PCA

Factors	Fast food prefer-ence (1)	Starchy foods craving (2)	*Mamak*-food craving & fre-quency (3)	*Mamak* food prefer-ence (4)	Fast food frequency (5)	Soup FreCrav Like (6)	Choco-late FreCrav Like (7)	Ice-cream FreCrav Like (8)	Soup Noodles FreCrav Like (9)	Curry Noodles FreCrav Like (10)	Fried Noodles FreCrav Like (11)	Spa-ghetti FreCrav Like (12)	Fried Rice FreCrav Like (13)	Cheese Cake FreCrav Like (14)	Fried Squid FreCrav Like (15)	Fried Fish FreCrav Like (16)
Initial eigenval-ues (% of variance)	18.9	9.7	5.9	5.8	4.1	3.9	3.5	3.4	30	2.8	2.8	2.4	2.2	2.1	2.0	1.9
Like chicken chop	0.87†	0.24	0.02	-0.01	0.09	-0.04	0.00	0.03	-0.07	0.14	0.02	0.03	0.04	0.11	0.14	0.01
Like fried chicken meal	0.87†	0.02	0.07	0.06	0.13	0.03	0.05	-0.00	-0.07	0.08	0.02	0.00	0.08	0.13	0.07	0.12
Like fried nuggets/balls	0.66†	0.01	0.01	0.34	0.10	0.11	0.04	0.04	0.10	0.00	0.03	-0.04	0.25	0.08	-0.03	0.09
Like French fries	0.66†	0.31	-0.12	0.22	0.14	-0.03	0.06	0.10	-0.07	0.20	0.05	-0.09	-0.17	-0.06	0.06	0.04
Like burger	0.61†	0.20	-0.03	0.15	0.09	0.20	0.14	0.07	0.00	-0.07	0.15	0.00	-0.03	0.05	-0.06	-0.07
Like hot dog	0.56†	0.05	-0.16	0.28	0.01	0.13	-0.02	0.15	0.22	-0.16	0.08	0.03	0.13	-0.19	0.07	-0.07
Craving potato chips	0.15	0.69†	0.17	0.02	0.13	-0.06	0.13	0.14	0.12	0.05	0.01	0.10	0.01	0.06	0.14	0.06
Craving *nasilemak*	-0.01	0.66†	0.08	0.22	0.12	0.12	-0.03	-0.03	0.01	0.09	0.24	0.12	0.31	0.06	-0.04	0.02
Craving French fries	0.27	0.66†	0.14	0.06	0.28	0.06	0.09	0.12	-0.01	0.16	0.06	0.04	-0.11	0.00	0.11	0.14
Frequency *nasibriyani*	-0.09	-0.05	0.70†	0.06	0.21	0.09	0.04	0.14	0.04	-0.01	0.15	0.13	0.01	0.06	0.05	0.06
Frequency *rojak*	-0.06	0.11	0.68†	0.07	0.31	-0.11	0.14	-0.03	0.12	0.08	0.11	0.20	-0.09	-0.04	0.00	-0.06
Craving *rojak*	0.01	0.38	0.65†	0.09	-0.12	0.01	0.11	-0.01	0.20	0.05	0.12	0.05	0.11	0.01	0.01	0.02
Craving *nasibriyani*	-0.08	0.31	0.63†	0.11	0.06	0.27	-0.07	0.08	0.03	0.04	0.12	-0.07	0.16	0.13	0.16	0.14
Like *nasilemak*	0.23	0.25	-0.02	0.72†	0.14	0.08	0.06	-0.08	-0.08	0.11	0.18	0.04	0.13	0.09	-0.06	0.01
Like *roti telur*	0.19	0.02	0.12	0.65†	-0.01	0.05	-0.01	0.13	0.12	-0.07	-0.04	0.09	0.12	0.10	0.11	-0.13
Like *nasibriyani*	0.19	-0.09	0.50	0.60†	-0.10	0.08	0.03	0.06	-0.04	0.08	0.07	-0.17	0.09	0.11	0.03	0.18
Like *rojak*	0.17	0.06	0.45	0.59†	-0.13	-0.03	0.03	0.04	0.12	0.14	-0.02	0.02	-0.12	0.00	0.07	0.06
Frequency French fries	0.15	0.24	0.00	0.09	0.79†	0.07	0.08	0.11	0.03	0.10	0.10	0.04	-0.09	0.02	0.09	0.09
Frequency fried chicken chop	0.15	0.09	0.14	-0.08	0.78†	-0.00	-0.00	0.04	0.08	0.03	-0.03	0.02	0.19	0.09	0.12	0.06
Frequency fried chicken meal	0.20	0.08	0.12	-0.04	0.70†	0.09	0.07	0.05	-0.01	-0.04	0.09	-0.03	0.19	0.07	0.09	0.28
Frequency creamed soup	-0.01	-0.06	0.11	-0.01	0.15	0.77†	0.10	-0.03	-0.10	0.14	0.09	0.22	-0.10	0.10	0.14	-0.01

†The values are loaded high into 16 components, which are numbered in parenthesis. The names of the components were derived based on the commonality of the variables in them.

Supplementary table 2. Factor loadings for perceived oiliness, creaminess and fat contents of custards and commercial foods by PCA (cont.)

Factors	Fast food prefer-ence (1)	Starchy foods craving (2)	*Mamak*-food craving & fre-quency (3)	*Mamak* food prefer-ence (4)	Fast food frequency (5)	Soup FreCrav Like (6)	Choco-late FreCrav Like (7)	Ice-cream FreCrav Like (8)	Soup Noodles FreCrav Like (9)	Curry Noodles FreCrav Like (10)	Fried Noodles FreCrav Like (11)	Spa-ghetti FreCrav Like (12)	Fried Rice FreCrav Like (13)	Cheese Cake FreCrav Like (14)	Fried Squid FreCrav Like (15)	Fried Fish FreCrav Like (16)
Craving creamed soup	0.02	0.30	0.09	-0.00	0.04	**0.74**†	0.10	0.11	0.10	0.14	0.05	0.11	0.05	0.15	0.11	0.05
Like creamed soup	0.28	-0.09	-0.04	0.20	-0.03	**0.74**†	0.14	0.09	0.06	0.05	-0.03	0.08	-0.03	0.21	0.03	0.00
Frequency chocolate	-0.01	0.01	0.13	-0.04	0.17	0.04	**0.82**†	0.05	-0.01	0.10	0.02	0.14	-0.02	0.05	0.01	0.06
Like chocolate	0.36	-0.02	0.01	0.19	-0.04	0.14	**0.76**†	0.10	-0.01	-0.06	-0.01	-0.09	-0.04	0.11	0.01	-0.00
Crave chocolate	0.01	0.25	0.05	-0.05	0.02	0.21	**0.73**†	0.30	0.10	-0.01	-0.03	-0.03	0.06	0.18	0.11	0.10
Frequency ice-cream	-0.00	0.02	0.12	-0.03	0.27	-0.00	0.13	**0.79**†	0.03	0.06	0.08	0.18	0.05	0.01	0.05	-0.05
Like ice-cream	0.28	-0.02	-0.08	0.27	-0.04	-0.01	0.16	**0.75**†	0.09	-0.04	0.06	-0.03	0.07	0.08	0.03	-0.04
Craving ice-cream	0.01	0.29	0.15	-0.04	-0.03	0.20	0.08	**0.74**†	0.03	0.00	0.14	0.05	0.08	0.12	0.04	0.16
Frequency soup noodles	-0.09	0.00	0.10	-0.05	0.16	-0.05	0.07	0.06	**0.78**†	0.09	0.10	0.21	0.17	0.09	-0.04	-0.06
Like soup noodles	0.10	-0.06	0.02	0.33	-0.02	0.06	0.04	0.08	**0.76**†	0.16	0.16	-0.05	-0.10	0.02	-0.02	0.10
Craving soup noodles	-0.03	0.37	0.28	-0.07	-0.05	0.06	-0.07	0.00	**0.71**†	0.07	0.18	0.12	0.10	0.03	0.04	0.05
Like curry noodles	0.31	0.01	0.05	0.16	-0.18	0.08	-0.04	0.04	0.08	**0.80**†	0.06	-0.09	0.00	0.04	0.01	0.06
Craving curry noodles	0.04	0.30	0.02	-0.00	0.06	0.14	0.01	0.09	0.16	**0.75**†	0.02	-0.10	0.12	0.05	0.09	0.16
Frequency curry noodles	-0.06	0.03	0.11	-0.05	0.31	0.11	0.10	-0.11	0.08	**0.70**†	0.17	0.14	0.11	-0.00	0.02	-0.08
Frequency fried noodles	-0.01	0.07	0.22	-0.05	0.18	0.07	0.08	0.04	0.19	0.09	**0.78**†	0.11	0.07	0.08	0.05	-0.03
Like fried noodles	0.26	-0.01	-0.00	0.36	-0.02	0.00	-0.05	0.15	0.12	0.10	**0.74**†	-0.05	-0.03	0.03	0.05	0.11
Craving fried noodles	0.09	0.34	0.28	-0.06	0.01	0.04	-0.05	0.14	0.13	0.06	**0.73**†	0.03	0.12	0.09	0.09	0.07
Frequency spaghetti	-0.09	0.01	0.19	-0.10	0.18	0.21	0.08	0.01	-0.00	0.01	0.10	**0.75**†	-0.02	0.08	0.09	0.05
Craving spaghetti	-0.04	0.27	0.14	-0.05	-0.05	0.16	-0.06	0.14	0.22	-0.00	0.03	**0.74**†	0.10	0.12	0.06	0.12
Like spaghetti	0.17	0.03	-0.10	0.43	-0.09	0.06	0.04	0.11	0.12	-0.09	0.04	**0.69**†	-0.03	0.17	0.014	0.00
Frequency fried rice	0.02	-0.10	0.04	0.07	0.24	-0.03	-0.01	0.16	0.05	0.08	0.04	0.02	**0.78**†	0.03	0.02	0.08

The values in bold are loaded high into 16 components, which are numbered in parenthesis. The names of the components were derived based on the commonality of the variables in them.

Supplementary table 2. Factor loadings for perceived oiliness, creaminess and fat contents of custards and commercial foods by PCA (cont.)

Factors	Fast food prefer-ence (1)	Starchy foods craving (2)	*Mamak*-food craving & fre-quency (3)	*Mamak* food prefer-ence (4)	Fast food frequen-cy (5)	Soup FreCrav Like (6)	Choco-late FreCrav Like (7)	Ice-cream FreCrav Like (8)	Soup Noodles FreCrav Like (9)	Curry Noodles FreCrav Like (10)	Fried Noodles FreCrav Like (11)	Spa-ghetti FreCrav Like (12)	Fried Rice FreCrav Like (13)	Cheese Cake FreCrav Like (14)	Fried Squid FreCrav Like (15)	Fried Fish FreCrav Like (16)
Craving fried rice	0.06	0.37	0.08	0.01	0.02	-0.04	-0.05	0.02	0.06	0.15	0.09	0.06	0.71†	-0.02	0.07	0.13
Like fried rice	0.37	0.04	-0.05	0.43	-0.07	-0.03	0.09	-0.02	0.08	-0.05	0.01	-0.07	0.58†	-0.11	0.02	0.01
Like cheese cake	0.25	0.03	-0.07	0.21	-0.02	0.19	0.14	0.04	0.08	0.01	0.01	0.07	0.04	0.80†	-0.02	-0.03
Craving cheese cake	-0.04	0.25	0.06	0.03	0.08	0.26	0.09	0.16	0.14	-0.04	0.07	0.11	0.05	0.76†	0.11	0.04
Frequency cheese cake	-0.11	-0.04	0.30	-0.03	0.18	0.05	0.13	0.02	-0.10	0.15	0.17	0.22	-0.18	0.62†	0.11	-0.03
Like fried squid	0.27	-0.00	-0.07	0.23	0.06	0.03	0.05	0.04	-0.00	0.01	0.05	0.02	0.06	0.10	0.82†	0.09
Craving fried squid	0.07	0.34	0.20	-0.11	0.11	0.16	-0.01	0.10	0.03	0.05	0.10	0.05	0.07	0.10	0.74†	0.13
Frequency fried squid	-0.14	-0.00	0.18	-0.02	0.42	0.21	0.12	-0.01	-0.06	0.11	0.05	0.16	-0.04	-0.09	0.64†	0.14
Craving fried fish	0.10	0.30	0.05	-0.03	0.18	0.10	-0.03	0.10	0.05	0.10	0.05	0.02	0.07	-0.03	0.13	0.77†
Frequency fried fish	-0.08	-0.00	0.12	-0.01	0.35	-0.03	0.19	-0.06	0.01	-0.05	0.06	0.14	0.08	-0.01	0.14	0.70†
Like fried fish	0.58	-0.10	0.02	0.10	-0.07	-0.09	0.05	-0.01	0.31	0.18	0.00	0.04	0.04	0.05	0.06	0.58†
Like cheese cake	0.25	0.03	-0.07	0.21	-0.02	0.19	0.14	0.04	0.08	0.01	0.01	0.07	0.04	0.80†	-0.02	-0.03
Craving cheese cake	-0.04	0.25	0.06	0.03	0.08	0.26	0.09	0.16	0.14	-0.04	0.07	0.11	0.05	0.76†	0.11	0.04
Frequency cheese cake	-0.11	-0.04	0.30	-0.03	0.18	0.05	0.13	0.02	-0.10	0.15	0.17	0.22	-0.18	0.62†	0.11	-0.03
Like fried squid	0.27	-0.00	-0.07	0.23	0.06	0.03	0.05	0.04	-0.00	0.01	0.05	0.02	0.06	0.10	0.82†	0.09
Craving fried squid	0.07	0.34	0.20	-0.11	0.11	0.16	-0.01	0.10	0.03	0.05	0.10	0.05	0.07	0.10	0.74†	0.13
Frequency fried squid	-0.14	-0.00	0.18	-0.02	0.42	0.21	0.12	-0.01	-0.06	0.11	0.05	0.16	-0.04	-0.09	0.64†	0.14
Craving fried fish	0.10	0.30	0.05	-0.03	0.18	0.10	-0.03	0.10	0.05	0.10	0.05	0.02	0.07	-0.03	0.13†	0.77
Frequency fried fish	-0.08	-0.00	0.12	-0.01	0.35	-0.03	0.19	-0.06	0.01	-0.05	0.06	0.14	0.08	-0.01	0.14†	0.70
Like fried fish	0.58	-0.10	0.02	0.10	-0.07	-0.09	0.05	-0.01	0.31	0.18	0.00	0.04	0.04	0.05	0.06†	0.58

The values in bold are loaded high into 16 components, which are numbered in parenthesis. The names of the components were derived based on the commonality of the variables in them.