Original Article

Bitter receptor gene (TAS2R38) P49A genotypes and their associations with aversion to vegetables and sweet/fat foods in Malaysian subjects

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Recently, the bitter receptor gene (TAS2R38) was identified to be responsible for phenylthiocarbamide (PTC) bitter sensitivity. Its two predominant haplotypes at three Single Nucleotide Polymorphisms (SNPs) are found to be definitive for the PTC status, which the ProAlaVal and AlaValIle haplotypes are associated with tasters and non-tasters, respectively. TAS2R38 haplotypes have been reported to influence food preferences (like cruciferous vegetables and fat foods) and cardiovascular disease risk factors. We examined, in 215 Malaysian subjects (100 males, 115 females), the association of the P49A SNP of TAS2R38 with anthropometric measurements and aversion to a list of 36 vegetables, 4 soy products, green tea and 37 sweet/fat foods. The subjects were successfully genotyped as 110 PA, 81 PP and 24 AA (with the A49 allelic frequency of 0.37), by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Ethnicity (Malay, Chinese or Indian), but not gender, was associated with the P49A TAS2R38 genotypes (p<0.001). However, no significant differences in terms of Body Mass Index, Total Body Fat, waist circumference and Waist-Hip Ratio were found between the genotypes (p<0.05). Only aversions to green tea, mayonnaise and whipped cream, but not soy products, vegetables, and other sweet/fat foods, were associated with the P49A genotypes (p<0.05). Therefore, the P49A SNP of the bitter receptor gene TAS2R38 could not serve as a predictor of anthropometric measurements and aversion to vegetables or sweet/fat foods in the sampled Malaysian subjects, and this suggests the existence of other possible factors influencing food selection among Malaysians.

Key Words: bitter, TAS2R38, single nucleotide polymorphism, Malaysia, nutrigenomics

INTRODUCTION

Bitter taste is the most complex out of the five taste modalities which may influence food preferences and nutritional status of humans. Taste blindness to the N-C=S moiety-containing bitter chemical phenylthiocarbamide (PTC) was discovered by Fox in 1931. PTC sensitivity is a classical Mendelian recessive trait; non-tasters have two recessive alleles (tt) while tasters have at least one dominant allele (T). The variation in the distribution of this Mendelian recessive traits across populations with taste blindness ranges from 3% in West Africa, 6-23% in China, 40% in India and around 30% in North American Caucasian populations. In 2003, Kim et al. had identified the bitter receptor TAS2R38 as the gene which is responsible for the ability to taste PTC. This gene is located on chromosome 7p35 in the human genome and contains a single coding exon of 1002 base pairs in length, coding for the TAS2R38 7-transmembrane domain G-Protein Coupled Receptor protein. Three common Single Nucleotide Polymorphisms (SNPs) were identified in this gene, which are at base pairs 145 (CÆG), 785 (CÆT) and 886 (GÆA), resulting in 3 amino acid substitutions at codons P49A, A262V and V296I. The PAV haplotype associates with tasting whereas AVI associates with non-tasting. A study showed that in a small isolated village in Talana, eastern Sardinia, only three genotypes were found which were AVI/AVI as homozygous non-tasters, AVI/PAV as heterozygous tasters, and PAV/PAV as homozygous tasters. Bitter compounds at high concentrations generally elicit food rejection, a behaviour critical to avoid ingesting the many toxic compounds found in foods, such as rancid fat, hydrolyzed protein and plant alkaloids. Increased bitter taste sensitivity among individuals may cause avoidance of vegetables rich in anti-tumour and anti-oxidant compounds because of their perceived bitterness, and instead encouraged the consumption of sweet and fatty foods, potentially increasing the risk of cardiovascular diseases, obesity, and cancer. A variety of studies have been conducted to study the association of the ability to taste PTC with eating behaviour. For example, in young women, there was an inverse relationship between bitter sensitivity and acceptance of tart citrus, Brassica vegetables, spinach, Corresponding Author: Dr Yee-How Say, Department of Biomedical Science, Faculty of Science, UTAR Perak Campus, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia. Tel: +605-4668888; Fax: +605-4661676 Email: sayyh@utar.edu.my; howsyh@gmail.com Manuscript received 9 November 2009. Initial review completed 10 June 2010. Revision accepted 21 July 2010.
and coffee. Female bitter tasters also showed a decreased acceptance of sweet and fatty foods. Taster genotypes have also been observed to associate with a preference for sweet-tasting foods in children, but not in adults. Bitter tasters also had a lower intake of soy products and green tea and rated these foods to be more bitter than non-tasters.

This study therefore sought to determine whether there is a taste genetic component in influencing the food selection among Malaysians. We evaluated frequencies of the first variant site of the TAS2R38 bitter haplotype PAV, denoted P49A, in 3 ethnicities (Malays, Chinese and Indians) for the first time to determine the association of this SNP with anthropometric measurements and aversion to a list of vegetables, soy products, green tea, sweet and fat foods.

MATERIALS AND METHODS

Subjects

Random convenience sampling was used in this study. Booths were set up at Universiti Tunku Abdul Rahman campuses, a primary and a secondary school, around Klang Valley, from October to December, 2008. The ranges of age, sex and race of the samples were not limited. A short introduction of this study was given to subjects who passed by the booth. Subjects were categorized into 3 major races, Malays, Chinese and Indians. The institutional board approved this study, all individuals participating in this study signed informed consent forms and all samples were taken in accordance with the 1995 Declaration of Helsinki (as revised in Edinburgh 2000).

Questionnaire

A six-page questionnaire was carried out to evaluate the demographic data and dietary habits (Supplementary Data 1). The demographic data section delivered information of age, gender and ethnicity. Subjects were asked to indicate how much they liked or disliked a list of 36 mostly local Asian vegetables, 4 soy products and 37 sweet or fat foods, using a ten-point hedonic preference scale which ranged from one (extremely like) to ten (extremely dislike), with a neutral point at five (neither like nor dislike). Subjects were also asked whether they liked to drink green tea or not.

Anthropometric measurements

Blood pressure was taken using an automated blood pressure monitor (Omron SEM-1) after the subjects had rested for 5 minutes. Height (in cm), waist and hip circumference (in inc.) of the subjects were measured using a measuring tape and their Waist-Hip Ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. A bio-impedance body fat weighing scale (Salter Body Analyser & Scale, UK) was used to determine the body mass index (BMI) and total body fat (TBF). A BMI of ≥27 kg/m² was considered obese.

DNA sampling and extraction

Subjects were asked to rinse their mouths thoroughly using mineral water before DNA sampling of buccal cells. A 5 ml of 3% sucrose was then given to each subject and they were asked to use their tongues to rub the inner part of cheeks. After 1 minute of gargling, the mouthwash was collected in a clean paper cup and was immediately poured into a 15 ml centrifuge tube containing 3 ml of TNE buffer [17 mM Tris / HCL (pH 8.0), 50 mM NaCl and 7 mM EDTA] diluted in 66% ethanol, and stored at 4°C until further use. Genomic DNA was extracted using the isopropanol-ethanol precipitation method as described by Aidar and Line, 2007.

TAS2R38 P49A Genotyping

The TAS2R38 P49A polymorphism was amplified by a set of forward and reverse primers with the following sequences - forward primer: 5’–CTTTGTTTTGCAGTGAAGAGGCGG–3’; reverse primer: 5’- AGGTTGCTTGGTTGCAATCATC -3’. The amplification reaction for an individual PCR tube was performed in a total volume of 20 µl reaction mixture, containing 150 ng DNA template, 500 nM forward primer, 500 nM reverse primer, 200 µM dNTP, 1.5 mM MgCl₂, 1x Taq polymerase buffer (Fermentas), and 1U of Taq polymerase (Fermentas). PCR was performed for 30 cycles with the denaturing temperature of 94°C for 30 seconds, followed by the annealing temperature of 64°C for 45 seconds and extension temperature at 72°C for 45 seconds, before finishing with an extension step of 72°C for 5 minutes. Ten microlitres of the PCR product was added with 1U HaeIII restriction enzyme and incubated at 37°C for 1 to 8 hours. RFLP products were resolved by 3% agarose gel electrophoresis before staining with ethidium bromide and viewed under an UV transilluminator. The AA homozygote yielded the 221 bp uncut fragment only, the PA heterozygote yielded the 221, 177 and 44 bp fragments, while the PP homozygote yielded two bands of 177 and 44 bp (Supplementary Data 2). The homozygous AA subjects were considered as bitter non-tasters while heterozygous PA and homozygous PP subjects were assumed as bitter tasters.

Statistical analysis

The statistical analysis was carried out using SPSS for Windows V 16.0. Descriptive statistics were used to analyze the variables for socio-demographic data and means were compared by one-way ANOVA. The association between TAS2R38 P49A genotypes with various socio-demographic factors and food aversion was analysed by Pearson chi-square test. Mean, confidence interval of means and p-value of less than 0.05 was considered statistically significant.

RESULTS

Among 215 subjects in this study, the males and females were almost equally balanced with males comprising 46.5%. The subjects ranged from 10 to 76 years old with the mean age of 21.3 ± 10.4 years old. Nearly half of the total subjects were Chinese (48.9%), followed by Malays (27.5%) and Indians (22.7%) (Table 1). There was no significant difference in the P49A genotypes between the males and females. However, a previous study found that females showed higher sensitivity towards bitter-tasting compounds compared to males, due to the high number of...
fungiform taste buds and density of the fungiform papillae present.17

Five anthropometric measurements including blood pressure (BP), BMI, TBF, WC and WHR were taken to investigate whether they were associated with the TAS2R38 P49A genotypes. From the results obtained, while the mean BMI of the Malaysian female subjects was in the normal-weight category, the males slightly exceeded the cut-off point for overweight (23 kg/m²) (Table 1). The TAS2R38 genotypes showed significant differences in both Systolic BP (SBP) and Diastolic BP (DBP) mean values (F=4.339, p=0.014 and F=4.697, p=0.010, respectively; by One-Way ANOVA) (Figure 1). However, no significant differences of BMI, TBF, WC and WHR were found between the genotypes (p<0.05).

Among the 110 PA, 81 PP and 24 AA subjects (with the A49 allelic frequency of 0.37), the percentage of AA subjects was interestingly high in Indians (n= 17, 70.8%),

| Table 1. Socio-demographics, TAS2R38 P49A genotypes and anthropometric measurements of the Malaysian subjects |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Male (n=100)                                    | Female (n=115)  |
| Age (mean±SD); years                           | 21.2±12.4       | 21.4±8.4        |
| Age group, years                                |                 |                 |
| 10 to 19                                        | 45 (20.9%)      | 42 (19.5%)      |
| 20 to 29                                        | 44 (20.5%)      | 61 (28.4%)      |
| 30 to 39                                        | 2 (0.9%)        | 4 (1.9%)        |
| 40 to 49                                        | 2 (0.9%)        | 7 (3.3%)        |
| Over 50                                         | 7 (3.3%)        | 1 (0.50%)       |
| Ethnicity                                       |                 |                 |
| Malay                                          | 27 (12.6%)      | 32 (14.9%)      |
| Chinese                                        | 55 (25.6%)      | 50 (23.3%)      |
| Indian                                         | 16 (7.4%)       | 33 (15.3%)      |
| Others                                         | 2 (0.9%)        | 0 (0%)          |
| TAS2R38 P49A Genotype                          |                 |                 |
| AA                                             | 10 (4.7%)       | 14 (6.5%)       |
| PA                                             | 58 (27%)        | 52 (24.2%)      |
| PP                                             | 32 (14.9%)      | 49 (22.8%)      |
| BMI (mean±SD); kg/m²                           | 23.2±5.8        | 22.7±5.6        |
| BMI Categories                                  |                 |                 |
| Underweight (<18.5 kg/m²)                      | 21 (9.7%)       | 28 (13%)        |
| Normal (18.5–22.9 kg/m²)                       | 30 (14%)        | 39 (18.1%)      |
| Overweight (23.0–26.9 kg/m²)                   | 26 (12.1%)      | 24 (11.2%)      |
| Obese (≥27.0 kg/m²)                            | 23 (10.7%)      | 24 (11.2%)      |
| Total Body Fat (mean±SD; %)                    | 19.9±13.1       | 25.3±12.5       |
| Waist circumference (mean±SD), in              | 31.5±6.1        | 29.1±5.0        |
| Waist-hip ratio (mean±SD)                      | 0.9±0.06        | 0.85±0.08       |
| Systolic blood pressure (mean±SD), mmHg        | 123.6±15.9      | 117.9±18.2      |
| Diastolic blood pressure (mean±SD); mmHg       | 76.1±10.0       | 72.8±9.9        |
Table 2. Distribution of TAS2R38 P49A genotypes among the ethnic groups of the Malaysian subjects

<table>
<thead>
<tr>
<th>Race</th>
<th>AA</th>
<th>PA</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay</td>
<td>3</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td>Chinese</td>
<td>4</td>
<td>53</td>
<td>48</td>
</tr>
<tr>
<td>Indian</td>
<td>17</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

\( \chi^2 (df) \) 39.607 (6)  
\( p \geq 0.0001 \)  
95% CI 0.000-0.014

due to the fact that almost half of the 215 subjects were Chinese. Ethnicity was significantly associated with the P49A genotypes among the Malaysian subjects, with a low frequency of non-tasters AA (11.2%). (Table 2)

The types of vegetables in this study can be divided into 2 categories: 25 bitter vegetables and 11 sweet vegetables (Table 3). We assumed that vegetables like bak choy, broccoli, cabbage, cauliflower and Chinese kale as cruciferous vegetables from the family Brassicaceae (or Cruciferae), which are less likely to be eaten by bitertasters due to the fact that almost half of the 215 subjects were Chinese. Ethnicity was significantly associated with the P49A genotypes among the Malaysian subjects, with a low frequency of non-tasters AA (11.2%). (Table 2)

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rot, corn and pumpkin may still taste ‘sweet’, although they have flavonoids. Based on a 10-point hedonic scale, a higher score indicates a higher aversion for a vegetable. In AA subjects, 11 out of 25 bitter vegetables (44%) had mean aversion scores of less than 4, while 7 out of 11 sweet vegetables (63.6%) had mean aversion scores of higher than 4. In contrast, in PP subjects, 18 bitter vegetables (72%) had mean aversion scores of higher than 4 and all the sweet vegetables had mean aversion scores of lower than 4. These indicate that PP tasters had a higher aversion of bitter vegetables and preferred sweet vegetables more compared to other genotypes. However, not a single vegetable or soy product had significant association with TAS2R38 and vegetables/fat foods aversion.

In AA subjects, 11 out of 25 bitter vegetables (44%) had mean aversion scores of less than 4, while 7 out of 11 sweet vegetables (63.6%) had mean aversion scores of higher than 4. Lower than 4. These indicate that PP tasters had a higher aversion of bitter vegetables and preferred sweet vegetables more compared to other genotypes. However, not a single vegetable or soy product had significant association with TAS2R38 and vegetables/fat foods aversion. Green tea preference was found to be significantly associated with TAS2R38 P49A genotypes (p = 0.037) and the non-taster AA subjects interestingly seemed to dislike green tea drinking more compared to tasters PA or PP.

Besides vegetables, soy products and green tea, we also examined if the aversion of sweet and fat foods is associated with TAS2R38 P49A - using a list of 37 sweet and fat foods. From Table 4, only 10 out of the 37 sweet/fat foods (27%) had mean aversion scores of more than 4 in AA subjects, while this was almost the same for the PP subjects at 32.4%. This indicates that the preference of sweet/fat foods was almost similar for all the subjects and the aversion of sweet/fat foods among the genotypes was low. Only the aversion of regular mayonnaise and whipped cream had significant association with the genotypes of the subjects (p = 0.02 and p = 0.037, respectively). Non-taster AA seemed to dislike mayonnaise more compared to taster PP (but not taster PA), while tasters PP and PA disliked whipped cream more compared to non-taster AA.

**DISCUSSION**

Taken together, the bitter receptor gene TAS2R38 P49A SNP is not associated with the anthropometric measure-
ments and aversions to majority of vegetables, soy products and sweet/fat foods. Therefore, there might not be a taste genetic component in influencing the selection of food among Malaysians of different ethnicities; although the P49A genotypes were significantly different among the 3 races in this study. Nevertheless, the non-tasters AA were found to be less common among the Malaysian subjects (11.2%, with the A49 allelic frequency of 0.37).

This supports previous research which showed that the non-tasting gene is less commonly presented in Asians, where only 6-10% had the non-tasting gene.18

The non-association between TAS2R38 P49A genotypes and BMI is supported by previous studies.19,20 Timpson et al.19 concluded that TAS2R38 status was not important in determining the related risk factors in the British Women’s Heart and Health Study sample. Consistently, the study of Tepper et al.20 done on Carlantino, Southern Italy samples also found no relationship between TAS2R38 haplotypes and BMI. However, a contrasting result was shown by Tepper et al.20 at the same time. They reported that the PTC phenotypes alone instead of genotypes were found to be significantly associated with BMI and waist circumference in females. Besides, studies which supported the association between lower PTC phenotypic responsiveness with higher CVD risk factors,21-24 also opposed the results obtained in this study.

In the present study, aversion to bitter or sweet vegetables is not significantly different in TAS2R38 genotypes. It is different from a review which found that bitter Brassica vegetables, such as Brussels sprouts, cabbage, spinach, and kale were rejected by most of bitter tasters.7 Reviews have shown that vegetables and fruits consumption among adults relied on familiarity and habit, social interactions, cost, availability, time constraints, media, advertising, and health.26,27 Apart from that, biological, environmental and socio-cultural factors affect the indexes of dietary intake.27 For example, pre-school children from low-income family, who were tasters for bitter tasting compounds, were associated with the low intake of vegetables compared to pre-school children from high-income family.28

A study showed that respondents who were non-tasters had high acceptance of soy products such as tofu and soymilk and rated these soy products less bitter compared to tasters.13 However, aversions to soy products did not show any significant association with TAS2R38 genotypes among Malaysians. Only green tea preference had significant difference in TAS2R38 genotypes and the non-taster AA interestingly seemed to dislike drinking green tea. The reason for this is yet to be elucidated.

Bitterness of bitter-tasting compounds showed positive associations with creamy or oiliness of high-fat milks and foods,29 salad dressings,30 and corn oil.31 In another study, it was found that bitter-tasting compounds, sex and creaminess were significant predictors of preference for sampled high-fat foods.32 Men who were bitter non-tasters reported the sampled foods as creamier and preferred these foods most. On the other hand, females who were bitter-tasters strongly reported that greater creaminess was less pleasant in the same study.32 Out of the 37 sweet/fat foods listed in this study, only mayonnaise and whipped cream - which both elicit a creamy sensation to the tongue, had significant associations with the genotypes of the subjects. Non-taster AA seemed to dislike mayonnaise more but preferred whipped cream more as compared to tasters. However, it is still inconclusive to make an interpretation that bitter sensitivity is a predictor for the preference of creamy/fat food, and further studies should be conducted to shed light on this.

While there seems to be an association between bitter sensitivity and dietary selection, the potential interaction between bitter sensitivity and actual dietary intake has yet to be fully elucidated. A study carried out among preschool children revealed that the low-income group that were bitter tasters had lower intake of vegetables and consequently had higher BMIs.28 A similar link between increased bitterness sensitivity and decreased vegetable consumption was found in an Italian population.29 Among men undergoing endoscopic screenings for colon polyps, those with the highest bitter sensitivity ate fewer vegetables.34 It is interesting to note that bitter sensitivity also correlated positively with polyp number, suggesting a possible link between bitter sensitivity and colon cancer.34 In contrast, in a group of young women, bitter sensitivity was not associated with the consumption frequency of 22 bitter foods.35 In the future, a food frequency questionnaire could also be conducted beside the aversion scores for the list of vegetables, soy products, green tea and sweet/fat foods.

The number of subjects recruited in the study was rather small, limiting the power for statistical analysis of the results. Caution is required when interpreting our findings. The TAS2R38 genotyping could be improved if the gene haplotypes (PAV or AVI) instead of the single P49A SNP are examined; the former would give a more profound prediction on bitter perception. The bitter genotypes or haplotypes, bitter phenotypes could be determined using the 6-n-Propylthiouracil (PROP) bitter strip.36 PROP is one of the members of the bitter-tasting compounds known as thioureas, which are responsible for bitter taste. It lacks the sulphurous odour of PTC and is less toxic – therefore safer and more acceptable to respondents. The distribution of fungiform papillae could also be used to determine the bitter phenotypes. Papillae counting could be observed using videomicroscopy under lower magnification. Density of fungiform papillae is different among bitter tasters and non-tasters.37 Bitter sensitivity can also be done on different cohorts to explore its influence towards human daily live and susceptibility to certain diseases. Since many previous studies have been done on the influence of bitter sensitivity on aversion of cruciferous vegetables which contain vital cancer-fighting components, this study could be further extended to cancer patients.34,35 Instead of random convenience sampling, a case control study could be done to have a better comparison of bitter sensitivity among cancer patients and healthy individuals.

In summary, our results show that the frequency of TAS2R38 A49A non-tasters is very low among the 3 major ethnic groups of the Malaysian population, and that the TAS2R38 P49A SNP is not a suitable predictor of body indices and food selection for the population. This suggests the existence of other possible factors influencing the selection of foods that may have adverse or bene-
ficial effects towards health, such as biological (other genes besides the TAS2R38 bitter receptor gene), environmental and socio-cultural factors.

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AUTHOR DISCLOSURES
Shee-Xuen Ooi, Pui-Leng Lee, Huey-Yi Law and Yee-How Say, have no conflicts of interest.

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苦味受体基因（\textit{TAS2R38}）P49A 基因型与马来西亚受试者对蔬菜和甜/脂肪食物厌恶感的关联

苦味受体基因（\textit{TAS2R38}）被确认为负责苯基硫脲（\textit{PTC}）苦味的敏感性。它在三个单核苷酸多形性（SNPs）的两个主要单倍型被确定与 PTC 感受性有关，而 ProAlaVal 和 AlaValle 单倍型分别对应味感者和味盲者。\textit{TAS2R38} 单倍型已被报道能影响对食物的喜好（如十字花科蔬菜和脂肪食品）和心血管疾病的危险因素。我们检验马来西亚 215 名受试者（100 名男性，115 名女性）之 \textit{TAS2R38} 的 P49A 单核苷酸多态性，并测试它们与受试者的体位测量及对 36 种蔬菜、4 项黄豆制品、绿茶和 37 项甜/脂肪食物的厌恶感之关联性。通过聚合酶链反应限制性片段长度多性（PCR-RFLP）技术，受试者成功地被鉴定为 110PA，81PP 和 24AA 基因型（A49 等位基因频率为 0.37）。种族特点（马来人，华人或印度人），而不是性别，与 \textit{TAS2R38} 之 P49A 基因型有关联（\textit{p}<0.001）。然而，在各基因型中，身体质量指数，身体总脂肪，腰围和腰臀围比例并无显著差异（\textit{p}<0.05）。只有对绿茶，蛋黄酱和鲜奶油，而不是对黄豆制品，蔬菜和其他甜/脂肪食物的厌恶感，与 P49A 基因型有关联（\textit{p}<0.05）。因此，苦味受体基因 \textit{TAS2R38} 的单核苷酸多态性 P49A 不能作为马来西亚受试者体位测量和对蔬菜或甜/脂肪食物厌恶的预测。这表明，可能存在其他因素影响马来西亚人对食物的选择。

关键词：苦味、\textit{TAS2R38}、单核苷酸多态性、马来西亚、营养基因组学