Association of bitter and sweet taste gene receptor polymorphisms with dental caries formation

Abstract

Objectives: The aim of the study is to analyze the association of different bitter and sweet gene receptor polymorphisms and bitter and sweet food consumption on formation of dental caries in Turkish adult population.

Methods: This study included 205 adults whose detailed intraoral health examination was completed and decayed, missing, filled teeth (DMFT) index values were recorded. A mini questionnaire was applied to assess the consumption of bitter and sweet food. A venous blood sample from each participant was collected in Ethylenediamine tetraacetic acid (EDTA) tubes. Further, DNA samples were isolated from the blood samples by utilizing a DNA isolation kit, which were stored at +4 °C prior to the analysis. Taste receptor type 2 member 38 (TAS2R38; rs10246939, rs713598, rs1726866), Taste receptor type 1 member 2 (TAS1R2; rs35874116, rs9701796), and Taste receptor type 1 member 3 (TAS1R3; rs307355) gene polymorphisms were detected using real-time polymerase chain reaction (PCR).

Results: There was no statistically significant association between the TAS2R38, TAS1R2, and TAS1R3 gene polymorphism and the DMFT index (p>0.05). No significant difference was found between the consumption of bitter and sweet food and the DMFT index (p>0.05).

Conclusions: TAS2R38 (rs10246939, rs713598, rs1726866), TAS1R2 (rs35874116, rs9701796), and TAS1R3 (rs307355) gene polymorphism may not be associated with the formation of dental caries in the Turkish adult population.

Keywords: bitter taste; dental caries; gene polymorphisms; sweet taste; TAS1R2; TAS1R3; TAS2R38.
3 (TASIR3; rs307355) gen polymorphisms were determined using Real-Time PCR in twin studies. A significant association was found between the presence of these polymorphisms and the risk of dental caries [6]. Animal studies also strongly support the genetic contribution to the risk of developing caries [7–10]. Studies have demonstrated the importance of dietary habits, nutritional status, and chemically determined taste sensitivity in determining this risk [1, 4].

In the literature, there are a few studies that have profoundly covered the association of taste genes and dental caries formation across different age ranges: TASIR2 (ages 7–12) [7]; (ages 21–32) [21]; TASIR2, TASIR3, GNAT3 (guanine nucleotide binding protein, alpha transducing 3) (ages 1–42) [1]; CA6, TASIR1, TASIR3, TLR2, and TLR4 (ages 25–55) [19]; AMELX, CA6, DEFBI, and TAS2R38 (ages 20–60) [20]; TASIR2 and GLUT2 (glucose transporter genes) (ages 18–65) [22]; TASIR2 and GLUT2 (ages 11–13) [23]; and TASIR3, TASIR2, TASIR3, TAS2R16, TAS2R38, TAS2R50, SLC2A2, SLC2A4, GNAT3, SCN1B, and TRPV1 (ages 18–23) [24].

There is no study in the literature that has evaluated the association between dental caries formation with three taste genes simultaneously in the Turkish population: a bitter gene with three polymorphisms (TAS2R38 (rs10246939, rs713598, rs1726866)) and two sweet taste genes with three polymorphisms (TASIR2 (rs35874116, rs9701796) and TASIR3 (rs307355)). In this study, we aim to investigate the possible effect of bitter and sweet food consumption and different bitter and sweet gene receptor polymorphisms on the formation of dental caries.

Materials and methods

The study protocol was approved by the local Ethical Committee of Mersin University (date: 13/04/2017; number: 102), and the written informed consent was obtained from all participants prior to data collection.

This study included 205 adults (18–45 years old; 91 men, 114 women), who attended the Faculty of Dentistry clinic at Mersin University.

Table 1: Detailed information on the taste receptor gene polymorphisms.

| Gene  | Position          | SO term       | Alleles | MAF* |
|-------|-------------------|---------------|---------|------|
| TASIR2| rs35874116        | chr1:18854899 (GRCh38.p13) | Missense variant | T>C | 0.319942 |
|       | rs9701796         | chr1:18859635 (GRCh38.p13) | Missense variant | G>C | 0.780938 |
|       | rs307355          | chr1:1329774 (GRCh38.p12) | 2 KB upstream variant | T>C | 0.865808 |
| TASIR3| rs713598          | chr7:141973545 (GRCh38.p13) | Missense variant | C>G | 0.422175 |
|       | rs10246939        | chr7:141972804 (GRCh38.p13) | Missense variant | T>C | 0.464881 |
|       | rs1726866         | chr7:141972905 (GRCh38.p13) | Missense variant | G>A | 0.528597 |

*Global minor allel frequency data from dbSNP.
University for a routine dental examination. Clinical observations were made by two experienced dentists who had no knowledge of the individuals’ genotype. The patients were subjected to a detailed intraoral examination, and their decayed, missing and filled teeth (DMFT) scores were recorded after their teeth cleaning were performed with a brush and low abrasive polishing paste.

The DMFT index is used in accordance with the World Health Organization's tooth decay evaluation criteria, and it evaluates the oral dental health of the population. It is used for permanent teeth. Further, a similar index used for primary teeth is expressed in small letters and referred to as the dmft index. The sample was surveyed through this mini questionnaire regarding their bitter or sweet taste preferences at the same appointment:

1. If you consider your food consumption, which one do you prefer—bitter or sweet?
2. When you are hungry, which food do you prefer to consume—bitter or sweet?
3. If you consider your family’s food consumption, which one do you prefer—bitter or sweet?

Venous blood samples from each participant were drawn into EDTA tubes in the Medical Biochemistry Department. Furthermore, DNA samples were isolated from blood samples using a DNA isolation kit (Roche Diagnostics, GmbH, Germany), which were stored at +4 °C prior to the analysis. TAS2R38 (rs1026939, rs713598, rs1276866), TASIR2 (rs3587416, rs9701796), and TASIR3 (rs307355) gene polymorphisms were detected using real-time polymerase chain reaction (PCR) (LightCycler® 480 Instrument II – Diagnostics Roche GmbH, Germany) with a single nucleotide polymorphisms detection kit.

The inclusion and exclusion criteria for patient selection

This study included individuals aged between 18 and 45 years who visited Mersin University for a routine dental examination. Patients with general health problems such as anemia, diabetes mellitus, or any heart diseases; patients with amelogenesis imperfecta and dentinogenesis imperfecta; patients who do not have tooth brushing habits (those who brush less than twice a day); and patients with obesity were excluded from the study group.

Statistical analysis

Descriptive analyses were summarized as the mean ± standard deviation (SD). Additionally, categorical variables were summarized as counts and percentages. The chi-square test was used for categorical endpoints. An exact test was used when the probability of an expected count less than five was more than 25%. The Hardy-Weinberg equilibrium (HWE) of groups on account of genotypes was checked by the chi-square test and the frequencies of polymorphism were in HWE (p>0.05).

A p-value of less than 0.05 was considered statistically significant.

Results

The DMFT index values of 205 adults (aged 18–45 years (mean age 31.36 ± 8.25); 91 men, 114 women) were evaluated in the permanent dentition.

The basic description of participants is shown in Table 2. The education level of the samples is as follows: 47 participants (23%) had completed primary level, 79 of them (39.1%) secondary, and 76 of them (37.6%) graduate. The income level of the participants can be outlined as follows: 81 participants (39.5%) earn less than the minimum wage, 108 of them (52.7%) earn minimum wage, and 16 of them (7.8%) earn more than the minimum wage. There was no significant difference between the samples’ basic description. When the relationship between education or income and dental caries risk was analyzed, no statistically significant association was found.

In addition, according to the data obtained from the questionnaire completed by the participants, no significant association was found between the consumption of bitter and sweet food and DMFT index values (p>0.05).

Moreover, the taste gene receptor polymorphisms and DMFT index values were compared, and no statistically significant association was found between the individuals with TAS2R38, TASIR2, and TASIR3 gene polymorphisms and the DMFT index values (p>0.05) (Table 2).

The distribution of the major–minor allele in the population at stake is displayed in Table 3 and minor allele distribution is seen as TASIR2 (rs9701796) G 20%, TASIR3 (rs307355) C 88.05%, TAS2R38 (rs713598) G 48.29%, TAS2R38 (rs10246939) C 52.68%, TAS2R38 (rs1726866) A 47.56%.

| Table 2: Basic description of participants. |
|---------------------------------------------|
| Age, mean ± standard deviation, years       | 31.36 ± 8.25 |
| Sex                                         |              |
| Male                                        | 91 44.4      |
| Female                                      | 114 55.6     |
| Education                                   |              |
| Primary                                     | 47 23.3      |
| Secondary                                   | 79 39.1      |
| Licentiate                                  | 76 37.6      |
| Income                                      |              |
| <minimum wage                               | 81 39.5      |
| =minimum wage                               | 108 52.7     |
| >minimum wage                               | 16 7.8       |
| If you consider your food consumption, which one do you prefer, bitter or sweet? |              |
| Equal                                       | 73 35.6      |
| Bitter                                      | 60 29.3      |
| Sweet                                       | 72 35.1      |
| When you are hungry which food do you prefer to consume, bitter or sweet? |              |
| Bitter                                      | 43 21.0      |
| Sweet                                       | 75 36.6      |
| Not matter                                  | 87 42.4      |
| If you consider your family's food consumption, which one do you prefer, bitter or sweet? |              |
| Equal                                       | 86 42.0      |
| Bitter                                      | 61 29.8      |
| Sweet                                       | 58 28.3      |
The chi-square test was used for categorical endpoints. An exact test was used when the probability of an expected count less than five was more than 25%. Hardy-Weinberg equilibrium (HWE) of groups on account of genotypes was checked by chi-square test and the frequencies of polymorphism were in HWE (p>0.05).

### Discussion

Dental caries is a complex disease that is related to dietary habits, taste sensitivity, genetic factors, and teeth brushing habits [1–4, 25]. The evaluated literature states that 40–60% cases of dental caries are affected by genetic factors [1–6]. Evaluations of the relationships of dental caries, varying ages and genetic polymorphisms in different genes, such as *TAS1R1*, *TAS1R2*, *TAS1R3*, *TAS2R6*, *TAS2R8*, *CA6*, *TLR2*, *TLR4*, *TAS2R50*, *AMELX*, *DEFB*, *SLC2A2*, *SLC2A4*, *GNAT3*, *SCN1B*, *TRPV1*, and *GLUT2*, have been analyzed in various studies [1, 7, 19–24].

### Table 3: Association of taste gene receptor polymorphisms and caries risk groups within DMFT values and allele distribution.

|                | DMFT | p-Value |
|----------------|------|---------|
|                | Low risk (0–3) | Moderate risk (4–7) | High risk (>8) |
|                | n, % | n, % | n, % |
| **TAS1R3 (rs307355)** | | | |
| CC             | 43 (26.7%) | 56 (34.8%) | 62 (38.5%) | 0.548 |
| CT             | 9 (23.1%)  | 13 (33.3%) | 17 (43.6%) |
| TT             | 2 (40.0%)  | 0 (0.0%)   | 3 (60.0%)   |
| Total          | 54 (100.0%)| 69 (100.0%)| 82 (100.0%)|
| **TAS1R2 (rs35874116)** | | | |
| CC             | 2 (9.1%)   | 11 (50.0%) | 9 (40.9%)   | 0.055 |
| CT             | 20 (24.7%) | 32 (39.5%) | 29 (35.8%) |
| TT             | 31 (31.0%) | 25 (25.0%) | 44 (44.0%) |
| Total          | 53 (100.0%)| 68 (100.0%)| 82 (100.0%)|
| **TAS1R2 (rs9701796)** | | | |
| CC             | 36 (27.5%) | 44 (33.6%) | 51 (38.9%) | 0.986 |
| CG             | 16 (24.2%) | 22 (33.3%) | 28 (42.4%) |
| GG             | 2 (25.0%)  | 3 (37.5%)  | 3 (37.5%)  |
| Total          | 54 (100.0%)| 69 (100.0%)| 72 (100.0%)|
| **TAS2R38 (rs713598)** | | | |
| CC             | 15 (27.8%) | 15 (27.8%) | 24 (44.4%) | 0.661 |
| CG             | 28 (26.9%) | 39 (37.5%) | 37 (35.6%) |
| GG             | 11 (23.9%) | 14 (30.4%) | 21 (45.7%) |
| Total          | 54 (100.0%)| 68 (100.0%)| 82 (100.0%)|
| **TAS2R38 (rs10246939)** | | | |
| CC             | 14 (25.9%) | 17 (31.5%) | 23 (42.6%) | 0.987 |
| CT             | 29 (26.9%) | 38 (35.2%) | 41 (38.0%) |
| TT             | 11 (26.2%) | 14 (33.3%) | 17 (40.5%) |
| Total          | 54 (100.0%)| 69 (100.0%)| 81 (100.0%)|
| **TAS2R38 (rs1726866)** | | | |
| AA             | 11 (25.6%) | 14 (32.6%) | 18 (41.9%) | 0.879 |
| AG             | 29 (27.1%) | 39 (36.4%) | 39 (36.4%) |
| GG             | 14 (25.9%) | 16 (29.6%) | 24 (44.4%) |
| Total          | 54 (100.0%)| 69 (100.0%)| 81 (100.0%)|
| **TAS2R38 (rs411207)** | | | |
| AA             | 11 (25.6%) | 14 (32.6%) | 18 (41.9%) | 0.879 |
| AG             | 29 (27.1%) | 39 (36.4%) | 39 (36.4%) |
| GG             | 14 (25.9%) | 16 (29.6%) | 24 (44.4%) |
| Total          | 54 (100.0%)| 69 (100.0%)| 81 (100.0%)|
This study is the first to evaluate the relationship between dental caries formation and both TAS1R2 and TAS1R3 sweet taste genes and the TAS2R38 bitter taste gene with six polymorphisms and food consumption in a sample group of the Turkish adult population.

The perception of sweet, umami, and bitter tastes is mediated via G-coupled protein receptors encoded by the TAS1R1 and TAS1R2 taste receptor gene families. The area of bitterness sensitivity is the most extensively researched of all taste qualities. The bitter compounds phenylthiocarbamide (PTC) and propylthiouracil (PROP) were first noted in the early 1930s. The TAS2R38 gene was found to be responsible for the majority of the variation in bitter taste sensitivity [25] and included the study with three polymorphisms.

The sweet taste was found to be mediated by two genes, TAS1R3 [25] and TAS2R2 [25, 26]. The TAS2R38 bitter taste gene has three common SNPs (rs10246939, rs713598, rs17268663), and all of them were included in the study plan. Further, TAS2R2 (rs35874116, rs9701796) and TAS1R3 (rs307355) gene polymorphisms were selected, similar to Haznedaroğlu et al.’s study, which evaluated the same population with different age groups [7].

There is no analysis encountered in the literature that has explored allele and minor allele distribution gene polymorphisms in the Turkish population but the frequency of global minor allele distribution has been known (Table 1). When the minor allele distribution frequency of this study and frequency of global minor allele distribution were evaluated together, our findings appeared to be similar to the TAS1R2 (rs35874116) C allele, TAS1R3 (rs307355) C allele, and all polymorphisms that have been explored of TAS2R38 gene (rs713598 G allele, rs10246939 C allele, rs1726866 A allele); yet, they are lower than the TAS1R2 (rs9701796) C allele.

In the literature, there is no caries risk classification for adults that performs its evaluation with DMFT caries scores. On the contrary, with reference to children, there is caries risk group classification with DMFT/dmft scores [7]. In the studies that investigate adults, the results were analyzed among gene polymorphisms by median and standard deviation [21, 22]. We analyzed the results of both DMFT caries risk groups and median–standard deviation and found no significant results again (data not shown).

In a family study, Wendell et al. evaluated the relationship between TAS2R38 (rs10246939, rs713598, rs17268663) as a bitter taste gene and TAS1R2 (rs4920566, rs9701796) as a sweet taste gene in caries formation. No statistically significant results were found between the DMFT index values of the TAS2R38 (rs10246939, rs713598, rs17268663) and TAS1R2 (rs9701796) genes in the permanent dentition group, although a statistically significant difference was found in the mixed dentition group [1]. Our study, on the other hand, was different from this study as it included the evaluation of TAS1R2 (rs35874116) and TAS3R (rs307355) polymorphisms. This is also true since the TAS1R2 (rs4920566) polymorphism was not included in our study design. However, in permanent dentition, our results and Wendell et al.’s results were similar with regard to the TAS2R38 (rs10246939, rs713598, rs17268663) and TAS1R2 (rs9701796) gene polymorphisms.

Haznedaroğlu et al. found no correlation between the DMFT index and TAS1R2 (rs35874116, rs9701796) and TAS1R3 (rs307355) gene polymorphisms in permanent dentition but found significant results between TAS1R2 (rs35874116) when compared with DMFT + dmft in mixed dentition and, in different age groups, in C/C in the high caries risk group [7]. This data is similar to our study results within the same population in permanent dentition. In addition, Holla et al. showed that the same polymorphism in TAS1R2 (rs35874116) was associated with the risk of dental caries formation, defined as DMFT scores ≥4 in permanent dentition (11–13 years) with children in their study, which evaluated the TAS1R2 gene (rs35874116) and the GLUT2 gene [23]. The study conducted by Kulkarni et al. reported a direct effect of TAS1R2 (rs35874116) and GLUT2 taste genes on caries formation in adults. Kulkarni’s results were different from those of Haznedaroğlu et al. and Holla et al., which showed the lowest caries scores in the TAS1R2 (rs35874116) group and our study as finding TAS1R2 (rs35874116) ineffective on caries formation [21]. In another study, Robino et al. evaluated the relationship between TAS1R2 (rs3935570) and GLUT2 in dental caries formation. They found that both TAS1R2 and GLUT2 were associated with dental caries risk or protection in adults [22].

In addition, no significant difference was found between the consumption of bitter and sweet food and DMFT index values (p > 0.05) in this study. In the literature, another study evaluated bitter and sweet food preferences or intake and dental caries formation and similarly found no correlation with caries status and bitter and sweet food intake. However, it has also been reported that allelic variations in the GNAT3, SLC2A2, SLC2A4, TAS1R1, and TAS1R2 genes are associated with caries status [24].

In conclusion, we found that TAS2R38 (rs10246939, rs713598, rs17268663), TAS1R2 (rs9701796) and TAS1R3
(rs307355) gene polymorphisms have no effect on dental caries formation and bitter and sweet food consumption in the Turkish population.

It is known that candidate gene association studies have undergone various setbacks including false positives and poor control of population stratification [27]. Thus, the sample size was determined by power analysis where more participants were included in the study to overcome such a setback. The reasons for finding no association among polymorphisms as an outcome of the study might stem from the fact that this analysis was previously planned as a preliminary population study. It, thus, failed to impose control on all segments of the population, just like all genome-wide association studies (GWAS). Other reasons could be related with the fact that there is a lack of equal sampling on dental caries risk groups and that the patients involved have lower DMFT scores.

The results of this study show similarities and differences in the literature regarding the polymorphic changes by geographical region. The aim of this research was to investigate the taste receptors that have not been investigated in the Turkish population previously as a preliminary study. In the future, we aim to contribute to the literature with studies of different caries risk groups and different taste gene receptors and a more detailed analysis of the taste pathway mechanisms in relation with smoking and alcohol consumption.

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