RESEARCH ARTICLE

THE ASSOCIATION BETWEEN THE RS35874116 GENE *TAS1R2* POLYMORPHISM WITH CARIES INDEX

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ABSTRACT

Background: Dental caries is a multifactorial disease influenced by complex genetic and environmental factors. At least 27 gene polymorphisms have been identified that are associated with caries risk. Polymorphisms of genes encoding taste receptors, such as the TAS1R2 gene, can cause individual differences in perception and sensitivity to sweet tastes. Research shows that the TAS1R2 gene polymorphism is associated with the consumption of sweet foods, which affects increasing caries risk. This study aims to determine the relationship between the TAS1R2 gene rs35874116 polymorphism and the caries index in Tangerang Selatan population.

Methods: This study is observational with a cross-sectional method involving 266 research subjects (45 males and 221 females; 20-55 years). DNA was extracted from venous blood using the Genomic DNA Mini Kit from Geneaid and the genotyping process using the rhAmp-SNP assay kit, which was analyzed using RT-PCR. Dental caries was assessed by calculating D (decayed), M (missing), and F (filled) on permanent teeth. The hypothesis between the TAS1R2 genotype and the DMF-T index was tested using the Mann-Whitney test, while the comparison between the TAS1R2 genotype and caries risk was tested using Kruskal-Wallis' test. The results of the study were considered significant if $p<0.05$.

Results: The minor allele frequency was 16.5%. The proportion of TT, TC, and CC genotypes were 71.%, 24.1%, and 4.5%, respectively. According to WHO classification, the DMF-T index of all research subjects was 5.62, a high category. The group of subjects with the CC genotype had a higher DMF-T index (7.08; very high) than the group of subjects with the CT genotype (6.05; high) and TT (5.38; high). CT and TT genotypes with high risk were significantly lower than those with low-moderate caries risk.

Conclusion: There is no significant association between the TAS1R2 gene polymorphism rs35874116 with the caries index value in the people in South Tangerang.

Keywords: Caries, polymorphism, rs35874116, TAS1R2, sweet taste

INTRODUCTION

Caries is the most dominant oral health problem globally, both in developed and developing countries.[1] Data from the Global Burden of Disease Study in 2016 estimated that dental and oral diseases affect at least 3.58 billion people worldwide, with dental caries the most common permanent. The 2018 Basic Health Research Report (Riskesdas) shows an increase in the prevalence of dental caries in Indonesia. In Indonesia, dental caries sufferers increased by 35.6%, from 53.2% in 2013 to 88.8% in 2018. The average DMF-T index also increased by 2.5 from 4.6 in 2013 to 7.1 in 2018.[2,3]

A complex interaction between genetic and environmental factors influences the incidence and development of caries.[4] Heritability studies have confirmed that genetic factors account for 50-70% of caries prevalence in primary teeth and 35-55% in permanent teeth. [5,6] Several recent studies state that there are 27 gene polymorphisms associated with caries risks, such as genes related to teeth morphology, enamel formation, salivary flow and composition, and gene polymorphisms encoding sweet taste receptors.[7-9] Environmental factors that can influence the development of caries are dental plaque, cariogenic diet, fluoride deficiency, poor oral hygiene, the number of cariogenic bacteria, and inadequate salivary flow.[10]

Polymorphisms of genes encoding taste receptors can cause differences in perception and sensitivity between individuals to taste. Sweet taste receptors are heterodimers

of two protein subunits, T1R2 (taste receptor, type 1, member 2) and T1R3 (taste receptor, type 1, member 3) encoded by the TAS1R2 and TAS1R3 genes.[9,11] Genetics on the taste of sweetness associated with consuming sweet foods behavior.[11-13] Research by Han et al.[13] and Lopez et al.[14] found that individuals with the TT genotype consumed lower sugar than those carrying the C allele. Consumption of large amounts of sugar will increase the risk of caries.[15-17]

Research conducted by Eriksson et al.[12] and Wendell et al.[6] stated that TAS1R2 gene polymorphisms could affect the risk of caries through dietary and taste preferences. The research of Holla et al.[9] stated that the presence of the C allele in the TAS1R2 gene rs35874116 increased the risk of caries, thus getting the results that individuals with the T allele had lower caries scores than individuals with the C allele. Research conducted by Haznedaroglu et al.[10] on 184 Turkish children showed different results; 45.7% of subjects with the TT genotype had a high risk of caries (>8 teeth), while 58.3% of the CC genotype and 42% of the CT genotype had a moderate risk of caries (4-7 tooth).

Research on the role of genetic polymorphisms in the etiology of caries, especially the TAS1R2 rs35874116 gene, has not been widely carried out in Indonesia. This study aimed to determine the association between the TAS1R2 gene polymorphism rs35874116 and the caries index value in the Indonesian population.

METHODS

This study is an observational study with a cross-sectional method. This research was conducted in January-March 2020. The population of this research is the people in South Tangerang. The population covered by this research are people who live around the campus of FK UIN Syarif Hidayatullah Jakarta, Pisangan Health Center, Rawabuntu Health Center, Reni Jaya KPKM, and Buaran KPKM. The sample size in this study was calculated based on the Lemeshow formula.[18] The research subjects consisted of 266 subjects taken by simple random sampling from 650 subjects of nutrigenomics research FK UIN Syarif Hidayatullah Jakarta using the randomization feature in Microsoft Excel software.

The tools and materials used in this study were mouth rinses, gloves, masks, mouth mirrors, sonde, examination forms, and stationery. Two examiners have carried out caries assessment with inter-examiner and intra-examiner consistency of 88% and 98% of 25 randomly selected respondents. The DMF-T index examination was carried out by instructing the respondent to open his mouth and check the condition of his teeth with a mouth mirror and a probe. The examination results will be recorded on the examination form that has been prepared. The records used in this study were D (decayed) for permanent teeth affected by caries but not yet filled, M (missing) for missing or extracted teeth due to caries, and (filled) for filling teeth. The criteria for assessing D (decayed) are discolored teeth, and there is entanglement, the presence of broken enamel, cavitation, and a soft base surface. The criteria for assessing M (missing) were missing teeth or had been extracted due to caries and carious teeth that had indications for extraction. The criteria for assessing F (filled) are teeth that have been filled.[19] Criteria D, M, and F follow the manual for the basic method of oral health surveys issued by WHO.[20] The caries risk categories are divided into low (0-3 teeth), moderate (4 - 7 teeth), and height (>8 teeth) according to the categories created by Haznedaroglu et al.[10]

The genotype data is secondary data taken from the nutrigenomic research of the Syarif Hidayatullah State Islamic University Jakarta. DNA extraction and genotyping were carried out by the main researcher in the laboratory of the Faculty of Medicine, Syarif Hidayatullah State Islamic University Jakarta. DNA was extracted from whole blood using a commercial Genomic DNA Mini Kit from Geneaid. The isolated DNA was quantified by spectrophotometric techniques using the Envoi DS-11 Spectrophotometer. Samples for DNA testing were amplified by PCR according to the manufacturer's instructions or procedures. Genotyping of TAS1R2 rs35874116 was performed using rhAmp™ SNP genotyping.

The data obtained were tested for normality using the Kolmogorov-Smirnov test. In addition, data were analyzed by non-parametric test using Mann-Whitney test to determine the relationship between genotype rs35874116 TAS1R2 with a caries index value and Kruskal-Wallis test to determine the relationship between genotype rs35874116 TAS1R2 and caries risk. Hypothesis testing was considered significant if it p-value <0.05.

ETHICAL APPROVAL

This study was obtaining ethical approval from the Research Ethics Commission of the Faculty of Medicine, Syarif Hidayatullah State Islamic University Jakarta (Number: B-001/F12/KEPK/TL.00/1/2019).

RESULTS

Data was collected on 266 research subjects (45 males and 221 females; ages 20-55 years) who were taken randomly using the simple random sampling method from 650 subjects in nutrigenomics research FK UIN Syarif Hidayatullah Jakarta. Table 1 shows the Demographic characteristics and genotypes of subjects.

Table 1. Subjects characteristics

Characteristics	n	%
Gender		
Male	45	16.9%
Female	221	83.1%
Age, years		
20-29	28	10.5%
30-39	55	20.7%
40-49	142	53.4%
50-55	41	15.4%
Education		
Low	51	19.2%
Moderate	176	66.2%
High	39	14.7%
Profession		
Housewife	183	68.8%
Office worker	30	11.3%
Lecturer/teacher/student	12	4.5%
Entrepreneur/service/freelancer	14	5.3%
Domestic worker/laborer/ unskilled worker	20	7.5%
Driver	6	2.3%
Army/police	1	0.4%
***TAS1R2* rs35874116 Genotype**		
CC	12	4.5%
CT	64	24.1%
TT	190	71.4%

Table 1 shows that of the 266 subjects involved, the highest number of subjects was female (83.1%). The majority of subjects belonging to the age group 40-49 years (53.4%) with a median of 43 years (20-55 years), have a moderate level of education (66.2%), and work as housewives (68.8%). Most subjects had the TT genotype (71.4%), and only 12 people (4.5%) had the CC genotype.

Table 2. DMF-T index

All participants (n)	Total			Total D+M+F	DMF-T index	Categories according to WHO
	D (%)	M (%)	F (%)			
266	951 (63.6%)	513 (34.3%)	31 (0.21%)	1495	5.62	High

Table 2 shows the number of teeth affected by caries D (decayed) as many as 951 teeth, missing or extracted due to caries M (missing) as many as 513 teeth, and filling with F (filled) as many as 31 teeth so that the DMF-T index is 5.62. According to WHO, the DMF-T index in this study was in the high category.

Table 3. DMF-T index category based on gender, age group, and *TAS1R2* gene polymorphism rs35874116

	DMF-T index	Categories according to WHO
Gender		
Male	5.6	High
Female	5.62	High
Age, years		
20-29	4.43	Moderate
30-39	4.64	High
40-49	5.73	High
50-55	7.39	Very high
***TAS1R2* rs35874116 genotype**		
CC	7.08	Very high
CT	6.05	High
TT	5.38	High

Table 3 shows that the results of the DMF-T index, both male and female, are in the high category. Subjects in the 50-55 year age group had the highest DMF-T index, 7.39. The results of the DMF-T index in subjects who have the CC genotype are in the very high category.

Table 4. The association between the *TAS1R2* rs35874116 genotype and DMF-T index

Genotype	DMF-T Index	*p-Value*
CC	7.08 ± 4.400	0.111*
CT + TT	5.55 ± 4.764	

**Mann-Whitney test*

Table 4 shows the relationship between the TAS1R2 genotype and the DMF-T index using a recessive model approach, namely the CC genotype compared to CT+TT. Statistically, there was no significant difference (p=0.111) in the DMF-T index in the CC and CT+TT genotype groups, but clinically there was a significant difference in the median DMF-T index, which was 3.

Genetic factors contribute 50-70% to caries risk. Haznedaroglu et al.[10] classified the caries risk as low risk if the individual had 0-3 caries, moderate risk if the individual had 4-7 caries, and high if the individual had more than 8 caries. Table 5 shows the proportion of caries risk in each TAS1R2 rs35874116 genotype.

Table 5. Caries risk distribution based on *TAS1R2* rs35874116 genotypes

TAS1R2 rs35874116 genotype	Caries risk (%)			Total (n)	*p*-Value
	Low risk (0-3 caries)	Moderate risk (4-7 caries)	High risk (>8 caries)		
CC	3 (25%)	4 (33.3%)	5 (41.7%)	12	0.171*
CT	21 (32.8%)	31 (48.4%)	12 (18.8%)	64	
TT	76 (40%)	82 (43.2%)	32 (16.8%)	190	
Total	100 (37.6%)	117 (44%)	49 (18.4%)	266	

**Kruskal-Wallis test*

Table 5 shows that the largest proportion of CC genotypes had a high caries risk 41.7%), while the largest proportion of CT and TT genotypes had moderate caries risk (48.4% and 43.2%). There was no significant relationship (p=0.171) between the TAS1R2 rs35874116 genotype and caries risk.

DISCUSSION

Data collection was carried out on working days and hours so that table 1 shows that most of the research subjects are female and work as housewives. The majority of the education level of the research subjects is moderate (having the latest education graduating from junior high school and senior high school). This result is in line with the results of the survey on the level of education in Indonesia by the Central Bureau of Statistics (BPS) in 2020 that the education level of the Indonesian population is mostly high school graduates, namely 50.88% (junior high school graduates: 21.78%, high school graduates: 29.10%).[21] This study cannot be generalized to all Indonesian populations because the subjects were taken from the population of South Tangerang, who were the subjects in the nutrigenomic research of the Syarif Hidayatullah State Islamic University Jakarta.

The frequency of the minor allele (C allele) in the TAS1R2 rs35874116 gene obtained in this study was 16.5%. The results of the minor allele frequency (C allele) in this study are similar to those obtained in the study of Choi et al.[22] in the Korean population, which is 14%. The minor allele frequency in this study is lower than the studies conducted on the Caucasian population by Haznedaroglu et al.[10], who obtained the minor allele frequency (C allele) of 27.4%, and Holla et al.[9], Who obtained the minor allele frequency (C allele) by 34.3%. The difference in the frequency of minor alleles between studies was caused by racial differences in the study population. The meta-analysis study conducted by Chisini et al.[23] showed differences in the TAS1R2 gene rs35874116 in various populations; East and South Asian populations have lower minor allele frequencies than European African populations. The major allele of a gene in one population can even become a minor allele in another population; for example, the G allele on the GLUT2 gene rs5398 in African populations becomes the minor allele, while in Asian, European and American populations, it becomes the major allele.[23]

The DMF-T index in all subjects was 5.62, which means high based on the WHO category (Table 2). The most significant contributor to the high DMF-T index is component D (decayed), covering 63.6% of the total caries types. The minor contributor to the high DMF-T index is component F (filling) because it only covers 2% of the total D, M, and F. These results indicate that most subjects do not or have not done good treatment for their caries. The high caries rate in this study is in line with the results of the Riskesdas in 2018, which stated that the proportion of tooth decay/cavities in Indonesia was 45.3%, while the proportion of teeth that had been treated was only 4.1%. This result could indicate low patient visits to the dentist to treat

cavities/caries. Based on the 2018 Riskesdas data, 95.5% of Indonesians have never gone to the dentist for treatment at all.[3]

Table 3 shows the DMF-T index categories in more detail by gender, age group, and TAS1R2 rs35874116 genotype. There is no difference in the DMF-T index category between the male and female groups because both are in the high category. The DMF-T index category is based on the results of the 2018 Riskesdas report, which shows that the DMF-T index of the Indonesian people, both male and female, is in the very high category according to WHO.[3] The DMF-T index category appears to increase in line with age. The aging process causes various physiological changes that increase the risk of caries, including morphological changes in enamel, dentin, pulp, and cementum and reduced sensitivity of nerve cells to pain stimuli.[24,25] Other risks in line with increasing age are reduced saliva production, decreased oral hygiene efforts, and the presence of systemic disease factors.[26-28]

Table 3 also shows that the group of study subjects with the CC genotype had a very high DMF-T index, while the other genotypes had a high category. Table 4 shows no significant relationship between the TAS1R2 gene polymorphism rs35874116 and the DMF-T index statistically. The small number of subjects can cause the absence of statistical differences. Table 4 also shows a relatively significant difference in the median DMF-T index, which is three points and has different categories according to WHO. These results indicate the effect of the TAS1R2 gene polymorphism on caries. The CC genotype group had a DMF-T index of 7.08, while the T allele carrier group had a DMF-T index of 5.55. The results of this study are in line with research by Holla et al.[9], who found that carriers of the T allele had lower DMF-T scores than those of the T allele.

The perception of taste plays an essential role in a person's preference for food and eating habits. The perception of sweetness is mediated by the sweet taste receptor, a heterodimer of G-protein-coupled-receptors encoded by the TAS1R2 and TAS1R3 genes. The TAS1R2 gene is located on chromosome 1. Genetic variations in the TAS1R2 gene can contribute to high daily sugar intake. Han[13] and Lopez[14] studies found that carriers of the T allele in the TAS1R2 gene rs35874116 consume less sugar than the CC genotype.

Excessive sugar consumption can increase the risk of caries. 15–17 Data from the 2014 Total Dietary Survey (SDT) show that 5.7% of Indonesians aged 19-55 years consume excess sugar (>50 grams/day).[29] Ministry of Health RI has recommended daily sugar intake not to exceed 10% of total caloric needs or less than 50 grams of sugar/day.[29] A systematic review conducted by Moniyhan and Kelly[30] and Sheiham and James[31] found that the recommended sugar intake of less than 10% was inadequate for reducing the risk of caries. The risk of caries will decrease if the daily sugar intake is less than 5%. This study has limitations; there is no assessment of sugar intake in subjects, so that it cannot be concluded that there is a relationship between sugar intake, TAS1R2 gene polymorphisms, and caries.

Caries are influenced by genetic and environmental factors, including eating habits. The important role of genetics in caries is supported by the results of studies of individual twins who found that genetic factors determine 40-60% of caries susceptibility. Among environmental factors, food intake/eating habits are considered to be the most potent etiological factor for caries.[10]

Table 5 shows that the largest proportion (41.7%) of the CC genotype had a high caries risk (>8 caries), while the largest proportion of the CT and TT genotypes had a moderate caries risk (4-7 caries). This result is not in line with the research of Haznedaroglu10 which found the opposite; the largest proportion of subjects with the TT genotype had a high caries risk (>8 caries). There was no significant relationship (p=0.171) between the TAS1R2 rs35874116 genotype and caries risk.

The limitation of this study is that the research results cannot be generalized to the Indonesian population because the subjects were taken only from the people of South Tangerang who were involved in nutrigenomic research at the Syarif Hidayatullah State Islamic University Jakarta. However, in this study, the subjects came from various ethnic groups, not only the natives of South Tangerang. Furthermore, considering that caries is a disease caused by multi factors, further research should also consider factors other than genetic factors, including measuring food intake, especially simple carbohydrate/sugar intake, conducting oral hygiene checks, and assessing the behavior of respondents in terms of maintaining oral hygiene.

CONCLUSION

It can be concluded that there is no significant association between the TAS1R2 gene polymorphism rs35874116 with the caries index value in the people in South Tangerang. The CC genotype has a DMF-T index in the very high category, while the other genotypes are in the high category.

CONFLICT OF INTEREST
None declared.

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