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# DESCRIPTIVE ASSESSMENT OF GENETIC SENSITIVITY TO 6-N-PROPYLTHIOURACIL, DIETARY PREFERENCES, DIETARY PRACTICES AND CARIES EXPERIENCE AMONG TWINS AND SIBLING PAIRS

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## ABSTRACT:

**Aim:** Present study was conducted to observe Prop taster status, dietary preferences, dietary practices and dental caries status among monozygotic, dizygotic twins and sibling pairs. Studying inheritance of aforementioned factors using classic twin model and comparison with siblings provide evidence for genetic contribution of dental caries.

**Materials and methods:** A sample of 6-12 years old monozygotic (30 pairs) and dizygotic (30 pairs) twins served as the study group. 30 pairs of siblings of twins served as the control group. PROP sensitivity test was done to assess taste sensitivity. Dietary preference and practices was assessed using child eating behaviour questionnaire. DMFS, defs indices with the inclusion of incipient lesions [Nyvad's criteria] were measured. Pair wise concordance and correlations [Spearman's correlation coefficient] were measured.

**Results:** Monozygotic twins showed higher concordance for prop taster status, taste preference; dietary practices compared to dizygotic twins and sibling pairs. Monozygotic twins showed highly significant statistical correlation and concordance for decay score and incipient lesions compared to dizygotic twins and sibling pairs. Least concordance and intraclass correlations were shown by siblings. Higher correlations and concordance observed among monozygotic twins is suggestive of genetic contribution to dental caries.

**Conclusion:** Monozygotic twins showed high concordance and correlation for study variables followed by dizygotic twins and sibling pairs.

**Keywords:** Monozygotic and Dizygotic Twins, Caries, Siblings, Taste

## INTRODUCTION:

Twin research has made and will continue to make, important contributions to the understanding of pathology of dental caries. Identifying the relative roles of heredity and environmental factors in the pathogenesis of dental caries had been a prime concern for dental researchers for decades.[1] Until recently researchers concentrated on environmental factors that contribute to disease; little attention was paid to individual differences in disease susceptibility or traits. Understanding the genetic risk for caries helps dental practitioner in two ways. They can educate the patients that some form of decay has inherited risk. They could also provide a reason to why persons with similar behavioural risks (e.g. tooth brushing frequency or dietary habits) have different caries rates.

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Aggressive preventive programmes can be aimed at the susceptible genomes.

According to twin study model; identical (MZ) twins share all their genes, whereas fraternal (DZ) twins, like siblings, share on an average half their genes. It can be assumed that if a particular trait is highly correlated between MZ twins and less correlated between DZ twins, there is a genetic contribution to that particular trait. [2]

According to a recent systematic review, "Twin study designs can be developed that can accommodate important variables such as sucrose taste preference, enamel defects, tooth morphology, cariogenic organisms, salivary defence mechanisms, behavioural markers and salivary gland function to decipher the contributions of gene and environment to the phenotype".[2] Amongst them; role of dietary substrates particularly sucrose needs special mention in caries occurrence and progression. Sugar uptake is infact influenced by an individual's taste preference.[3] Certain genes can influence taste preference. According to some studies there are genetic influences on behavior too. This could again substantiate genetic influence on dietary preferences. Sensitivity to bitter taste is a heritable trait. This taste preference is elicited by assessing sensitivity to genetic taste marker Propylthiouracil. Recent studies have distinguished between prop non tasters, regular tasters, and supertasters based on the intensity of perceived bitter taste. New studies suggest that prop taster or supertaster status may influence taste preferences and even food acceptance. [4] Eating practices in children may also be genetic and may influence the caries occurrence and progression.[5] Studies are relatively rare, comparing genetic contribution of all aspects of problematic eating.

Hence the present study was conducted to see the intraclass correlations and concordance of these factors among monozygotic and dizygotic twins using classic twin model to decipher the genetic contribution of aforementioned factors. The taste perceptions, dietary habits and dental caries status were observed among monozygotic, dizygotic twins and their siblings. This study was conducted in twins reared together in a specific geographic area. This could provide apparently same environmental factors.

## MATERIALS AND METHODS:

A cross-sectional descriptive study was conducted in Kodinjhi village of Malappuram district, Kerala for a period of one month (June 2015-July 2015). Recently much attention was focused on this village because of increased incidence of twin births. It is a small village of 2,000 families comprising approximately 250 sets of twins. In the year 2008 (based on the available data) among 300 families who had children, 15 pairs were born. Apparently the reason for the twin births is unknown. In this unique village our study was conducted. A sample of 60 pairs of 6-12 years old monozygotic (30 pairs) and dizygotic (30 pairs) twins who met the inclusion criteria served as the study group. A sample of 30 sibling pairs of same age group; preferably siblings of twins served as control group. Sample size was determined based on 6% available error. Institutional ethical clearance was obtained. An informed consent was taken from all the subjects.

The diagnosis of twin zygosity was based upon factors such as sex, facial appearance, facial profile, hair color, eye color, iris color and medical records. Siblings pairs, monozygotic twins and dizygotic twins within the age group of 6-12 years who agreed to participate in study were included. Subjects who were un-cooperative, with underlying systemic disease or syndromes and under any medication and antibiotics three months before the study were excluded. Children who were participating in the study were subjected to PROP sensitivity test to assess taste sensitivity. Dietary preference, dietary habits were assessed using a questionnaire. Caries experience was measured. Examination was conducted by a single examiner and recording was done with the help of a trained assistant to avoid bias.

The Prop test was carried out by placing the prop strip on the dorsal surface of the anterior two-third region of the child's tongue for 30 seconds. Each strip contained about 1.6mg 6-n-propylthiouracil. The intensity of bitter taste was rated on a modified labeled magnitude scale (LMS).[7] Based on which, the children were broadly subdivided into two groups as PROP tasters and non-tasters. The tasters were further classified as super tasters and medium tasters.[8] Details of LMS were explained to the children verbally. Explanation was also rendered regarding the strongest imaginable bitter taste perception with suitable dietary examples. Method of preparation of Prop strips were similar to previously conducted studies.[8] Each twin was subjected to prop sensitivity test separately to avoid bias.

The food preference was assessed using a questionnaire. Sweet and sour taste preferences were established as mentioned in the previously conducted studies. A child frequently preferring two or more of the foods which were designated under the sweet category was considered as a sweet liker, while a child frequently preferring two or more of the foods under bitter/spicy/ pungent food group were considered a dis-liker. [9, 10] Child eating behavior questionnaire was used to assess dietary practices. The Caries experience was recorded using decay component of DMFS/dmfs, defs indices with the inclusion of incipient lesion based on Nyvad's criteria [11, 12, and 13] Only decayed component of dmfs/ defs/DMFS index was used to avoid over estimation of caries status. The data was tabulated and statistically analyzed using SPSS version 17 software package. Intra pair correlation using Spearman's correlation coefficient between dizygotic twins, monozygotic twins and siblings were observed regarding their caries experience. Pairwise concordance was observed for the aforementioned parameter. Intra pair prop sensitivity, taste preference and dietary practices were assessed using pairwise concordance. Association of prop status in the dizygotic, monozygotic and sibling group were compared using Mc-Nemar chi square test.

## RESULTS:

**Prop Taster status:**

The monozygotic twins [26 pairs (86.7%)] showed higher concordance compared to dizygotic twins [22 pairs (73.3%)] and sibling pairs [16 pairs (53.3%)] for prop taster status. Sibling pairs showed the least concordance rates [table:-1].

The difference in concordance between monozygotic twins, dizygotic twins and sibling pairs was tested by chi-square test on the basis of the pairwise concordance rates-that is, the proportion of concordance among all pairs affected. There was statistically significant difference between the groups (table 5, p-value-0.02*) for prop taster status.

Majority of the study subjects were prop non-tasters; 82(45.6%).

**Taste preference**

For taste preference twin groups showed similar concordance [25 pairs]. In the control group, 18 pairs were concordant for taste preference. 17(68%) pairs in the monozygotic group and 18(72%) pairs in the dizygotic group showed sweet taste preference. 8 (32%) pairs in the monozygotic group and 7 (28%) pairs in dizygotic group showed bitter/ spicy/ pungent taste preference (table 2). Sibling pairs showed lesser concordance ratios compared to twin groups. However there was no statistically significant difference between groups for taste preference (p-value-0.09).

Majority of them preferred sweet taste i.e.120 out of 180 (66.7%) [Table:-1, 2]

**Evaluation of dietary habits in relation to dental caries:-**

In our study for the ease of comparison out of five possible responses, only positive responses ‘often’ and ‘always’ were taken into account. When positive responses were evaluated monozygotic twins showed higher concordance compared to dizygotic twins and sibling pairs for majority of the questions. Sibling pairs showed least concordances. Questions and their responses are detailed in table:-3.

**Caries status:-**

In Monozygotic group, 16(53.3%) pairs were concordant for decay score. 24(80.0%) pairs were concordant for incipient lesions. Monozygotic twins showed high correlation for decay score and incipient lesions (P value-<0.001***, 0.002**respectively). In Dizygotic group, 11(36.7%) pairs were concordant for decay score. 18(60.0%) pairs were concordant for incipient lesions. Dizygotic twins showed high correlation for decay score and incipient lesions (P value-0.002**, 0.03* respectively). In Control group, 5(16.7%) pairs were concordant for decay score and 13(43.3%) pairs were concordant for incipient lesion. The difference was statistically significant (p-value-0.01*). They showed weak correlation for decay score (p-value -.04*) and incipient lesions (table 4, 5)

Overall monozygotic twins showed higher concordance and correlation followed by dizygotic groups. Least concordance was shown by control group.

## DISCUSSION:

The question of heritable nature of dental caries has intrigued the minds of dental investigators for decades. The twin study is one of the methods for a better understanding of the genetic basis of dental diseases. Monozygous twins result from a single fertilized ovum which after repeated divisions, develop into two distinct individuals. These individuals are genetically identical and always of the same sex. Two separately fertilized ova during the same pregnancy result in the birth of dizygotic twins. It can be male – female pairs or like sexed individuals. [13]

The simplest assumption that can be used for evaluation of twin paradigm is P = G + E, where P is a quantitative phenotype or underlying disease risk, G is the genotype, and E is the environment.

According to this model, phenotypic variance equals genotypic variance plus environmental variance. Environmental variance includes the variations that are shared by family members, that are inherent to the individual, and variation that occurs while measuring. If a quantitative trait is completely due to genetic predisposition (i.e., 100% heritability), then the correlation shown by the identical twins will be 1.0 and the fraternal twins would be 0.50; which is similar to any normal sibling pair. [13] According to the twin rule of pathology, proposed by Siemens: "any heritable disease will be more CONCORDANT in monozygotic twins than in dizygotic twins, and concordance will be even lower in non-siblings". [14] Even in the present study, concordance of Prop taster status, dietary preferences and dietary practices; concordance and correlations of dental caries status were explored in monozygotic twins, dizygotic twins and sibling pairs.

### Evaluation of genetic sensitivity to 6-n-Propylthiouracil

The sense of taste is a powerful predictor of food preferences. The sensitivity to bitter taste is a heritable trait. It can be elicited using genetic taste markers, phenylthiocarbamide (PTC) and 6-n-propylthiouracil (Prop). They taste bitter to some people but are tasteless to others. The ability to taste PTC and Prop is associated with increased predilection for bitter compounds. Taste sensitivity was evaluated using prop strips in our study. Based on the intensity of bitterness, prop taster status of the individuals was categorised into prop nontasters, medium tasters and supertasters.[3] It is a proven fact that most of the Supertasters had high density of fungiform papillae and fungiform taste buds. Hence this distinction is anatomical according to study conducted by Drewnoski et.al [4] Genomic studies revealed that bitter taste perception appears to be largely mediated by the TAS2R38 gene.[15] These genes in turn modify taste preference resulting in sensitivity or insensitivity to cariogenic foods. Even in the present study, the taste sensitivity and preference for food was evaluated.

In our study 86 % of the monozygotic twins showed concordance for prop taster status compared to 73.3 % in dizygotic twins and 16.7% of the sibling pairs. Monozygotic twins showed a higher concordance for prop taster status compared to dizygotic twins and siblings. Least concordance was seen in siblings. This is in accordance with study conducted by Krondl et al, where significant heritability was observed for PTC [phenylthiocarbamide] taste sensitivity. [16] In monozygotic group 14 % were discordant; this can be attributed to subjective nature of the scale that we have used. According to several studies, 'Labeled magnitude scale' uses just oral sensation for interpretation of perceived intensity. This limitation prohibits conclusions about the intensity of gustatory stimuli that had been actually perceived. [17]

Even though concordance for prop taster status was higher in monozygotic group, dizygotic group also showed a fairly good concordance. This can again be attributed to 50% heritability seen in dizygotic twins.

**Taste preference**

Taste preference was elicited using food frequency questionnaire in the present study. 25 pairs (83.3%) showed similar taste preference in both monozygotic and dizygotic group respectively. In the sibling group only 18(60.0%) showed similar taste preference, suggestive of both genetic and environmental effects as stated by Keskitalo et.al (2007).[18] Majority of children from our study preferred sweet taste, irrespective of their taster status. Even some children who were super tasters preferred sweet taste. This could be because they are exposed to sweetened foods at young age, as sweets and savouries are a mark of celebration in every culture and community.

**Evaluation of dietary practices**

Dietary habits were evaluated using a pre-structured questionnaire in our study. This was developed by Jane Wardle, Carol Ann Guthrie, Saskia Sanderson and Lorna Rapoport, 2001, University College, London.[19] The effects of defective eating behaviour including slowness in eating, pouching of food over a long period of time and selective eating has been considered etiological for the causation of caries according to Anandakrishna L et.al

(2014). [19] Hence they were evaluated in our study to see their genetic contribution. Here higher concordance was shown by monozygotic twins, compared to dizygotic twins and sibling group. It was evident from the results that the practices like taking more than 30 mts to finish meals, keeping food in mouth for more than 30 mts, pouching food on one side of the mouth, flavouring food with sugar and craving for his or her favourite food was observed to be higher among monozygotic group. These eating practices can modify caries occurrence and progression. The higher concordance in the monozygotic group suggests some amount of genetic influence as stated by Pados R et.al (1989)[5], Kestikalo et al (2007)[18] However we also observed other factors like drinking water after every meal, preferring juices instead of water and enjoy tasting new foods and soft smashed foods had concordance in the twin groups and sibling groups. This can be attributed to environmental factors; like observational learning as they are reared together. Environmental adaptation and learning, appear to exert a stronger influence on food-related behaviour according to study conducted by Krondl et. al. (1983) [16]

**Caries status**

The concordance and correlation for decayed surfaces and incipient lesions were highest in monozygotic twins compared to dizygotic twins and siblings. Difference between the groups was statistically significant (p-value <.01**). This is in accordance with study conducted by Conry et al, Fairpo et al, Lovelina et al, where monozygotic twins showed higher correlation compared to dizygotic twins.[20, 21, 22] As most of the etiological factors for caries are correlating among the study population, correlation is there for caries status in the present study.

Overall monozygotic twins showed higher concordance and correlation for the study variables, followed by dizygotic twins and siblings. This is in accordance with "twin rule of pathology" as mentioned earlier, according to which any heritable disease will be more concordant in monozygotic twins than in dizygotic twins and concordance will be even lower in non-siblings thus suggesting some amount of genetic influence. [14] Even pattern of correlation was $\gamma$ MZ> $\gamma$ DZ, $\gamma$ MZ<2$\gamma$DZ. This pattern of correlation is suggestive of shared environment plus additive genetic variance.

However the higher level of correlation among the various etiological factors of dental caries like eating practices and oral hygiene status can be attributed to the shared environmental influence as the twins and siblings are reared together. As per the literature, any environmental factors that are shared by family members can be attributed to the differences between families and this in turn make family members relatively more similar. Environmental factors can be parental or non-parental. Any influence of environmental factors of parents on offspring is called vertical cultural transmission. Non-parental factors are called sibling shared environments; factors that are shared between all types of offspring, twin or non-twin. [23]

## CONCLUSION:

Monozygotic twins showed higher concordance for variables like taste perception, taste preferences and dietary habits compared to dizygotic twins and siblings. Monozygotic twins showed higher concordance and correlation for dental caries status when compared to dizygotic twins and sibling pairs. However, in spite of scientific advancements, the role of genes remains essentially unexplored. Further genomic studies should be conducted to find the genes and their allelic variants that are attributed to caries process.

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## TABLES:

**Table 1:- Concordance values for prop taster status and taste preference.**

| | Monozygotic (n=30 pairs) | Dizygotic(n=30 pairs) | Control (30 pairs) | Total | p-value |
|---|---|---|---|---|---|
| Prop status | 26(86.7%) | 22(73.3%) | 16(53.3%) | 64(71.1%) | 0.02* |
| Taste preference | 25(83.3%) | 25(83.3%) | 18(60.0%) | 68(75.6%) | 0.09(NS) |
| #Chi square test; P>0.05 non significant, ns | | | | | |

**Table 2:- Distribution taste preference in the groups**

| Taste preference | category | | | Total |
|---|---|---|---|---|
| | MZ | DZ | SIBLING PAIRS | |
| Sweet taste | 39(65.0%) | 41(68.3%) | 40(66.7%) | 120(66.7%) |
| Bitter/ spicy/ pungent | 21(35.0%) | 19(31.7%) | 20(33.3%) | 60(33.3%) |
| Chi square value(df) = 0.15(2) , p= 0.93(NS) Chi square test<br>P>0.05 non significant, ns<br>MZ-Monozygotic, DZ- dizygotic | | | | |

Table 3; **Concordance for Dietary practices**

| | | Study groups:- | | | P-value |
|---|---|---|---|---|---|
| | | MZ(30 pairs) | DZ(30 pairs) | SB(30 pairs) | |
| My child takes more than 30 | *A | 23(76.7%) | 23(76.7%) | 19(63.3%) | 1.00(NS) |

| | | | | | |
|---|---|---|---|---|---|
| minutes to finish meal | *B | 4(13.3%) | - | - | |
| Takes more than 30 minutes to finish disliked food | A | 19(63.3%) | 19(63.3%) | 18(60.0%) | 0.91(NS) |
| | B | 5(16.7%) | 3(10%) | - | |
| Finishes meal quickly if favorite food is given | A | 25(83.3%) | 23(76.6%) | 20(66.6%) | 0.28(NS) |
| | B | 3(10.0%) | 1(3.3%) | - | |
| Keeps food in mouth for more than 30 minutes | A | 25(83.3%) | 20(66.6%) | 18(60.0%) | 0.04* |
| | B | 1(3.3%) | | | |
| Pouches food on one side of the mouth | A | 25(83.3%) | 23(76.6%) | 21(70%) | 0.6(NS) |
| | B | 2(6.6%) | | | |
| Leaves food on plate at the end of the meal | A | 17(56.6%) | 13(43.3%) | 16(53.3%) | 0.06(NS) |
| | B | 11(36.6%) | 7(23.3%) | 1(3.3%) | |
| Difficult to please with meals | A | 21(70.0%) | 19(63.3%) | 16(53.3%) | 0.19(NS) |
| | B | 3(10.0%) | 2 (6.7%) | 1(3.3%) | |
| Eating pattern depends on person feeding them | A | 24(90.0%) | 22(73.3%) | 17(56.6%) | 0.07(NS) |
| | B | - | - | 3(10%) | |

*All these questions had five scores: never, rarely, sometimes, often and always.
*A- response was Never/rarely/sometimes
*B-response was Often/always
There were total 18 questions in child eating behaviour questionnaire

Table: 3; Concordance for dietary practices continued…

| | | Study groups:- | | | P-value |
|---|---|---|---|---|---|
| | | MZ(30 pairs) | DZ(30 pairs) | SB(30 pairs) | |
| Child's food needs to be flavored | A | 12(30%) | 17(56.6%) | 17(56.6%) | 0.94(NS) |

| | | | | | |
|---|---|---|---|---|---|
| with sugar | B | 11(36.6%) | 3(10.0%) | 1(3.3%) | |
| Prefers soft mashed food to hard chewy foods | A | 25(83.3%) | 23(76.6%) | 21(70%) | 0.32(NS) |
| | B | | | 1(3.3%) | |
| Enjoys tasting new foods | A | 25(83.3 0%) | 22(73.3%) | 19(63.3%) | 0.15(NS) |
| | B | | | 1(3.3%) | |
| Craves for his or her favorite food | A | 17(56.6%) | 17(56.6%) | 13(43.3%) | 0.73(NS) |
| | B | 8(26.7%) | 6(20%) | 5(16.67%) | |
| Needs to be fed water after every morsel | A | 24(80.0%) | 19(63.3%) | 16(53.3%) | 0.02* |
| | B | | | | |
| Drinks water after every meal | A | 1(3.3%) | | | 0.37(NS) |
| | B | 24(80.0%) | 23(76.6%) | 21(70%) | |
| Prefers juices instead of water | A | 14(46.7%) | 13(43.3%) | 21(70%) | 0.12(NS) |
| | B | 12(40%) | 10(33.3%) | | |
| Prefers milk instead of food | A | 26(86.7%) | 23(76.6%) | 21(70%) | 0.15(NS) |
| | B | | | | |
| Eats more when upset or angry | A | 26(86.7%) | 22(73.3%) | 20(66.6%) | 0.23(NS) |
| | B | | | | |
| Eats less when upset or angry | A | 12(40%) | 12(40%) | 10(33.3%) | 0.14(NS) |
| | B | 12(40%) | 10(33.3%) | 8(26.7%) | |

*All these questions had five scores: never, rarely, sometimes, often and always.
*A- response was Never/rarely/sometimes
*B-response was Often/always

**Table 4: Concordance values for caries experience**

| | Monozygotic (n=30 pairs) | Dizygotic(n=30 pairs) | Control (30 pairs) | Total | p-value |
|---|---|---|---|---|---|
| Decay score | 16(53.3%) | 11(36.7%) | 5(16.7%) | 32(35.6%) | 0.01* |
| Incipient lesions | 24(80.0%) | 18(60.0%) | 13(43.3%) | 55(61.1%) | 0.01* |
| Chi square test | | | | | |

**Table 5: Correlation for caries experience**

| | Monozygotic(n=30 pairs) | | Dizygotic(n=30 pairs) | | Control | |
|---|---|---|---|---|---|---|
| | **Spearman's rho** | **p-value** | **Spearman's rho** | **p-value** | **Spearman's rho** | **p-value** |
| Decay score | 0.79 | <0.001*** | 0.55 | 0.002** | 0.36 | 0.04* |
| Incipient lesions | 0.55 | 0.002** | 0.40 | 0.03* | 0.19 | 0.29(NS) |