GLUT2 and TAS1R2 genotype and risk of dental caries

By

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To determine whether polymorphisms in the GLUT2 and TAS1R2 genes are associated with dental caries, 80 Caucasian individuals were recruited. A clinical and radiographic examination determined 3 caries scores: DMFT (decayed, missing and filled teeth), DMFT+radiographs and ICDAS (International Caries Detection and Assessment System). Associations between genotype(s) and caries scores were analysed. A significant increase in DMFT scores was shown in GLUT2 polymorphism carriers (i.e. GLUT2 risk). Carriers of the TAS1R2 gene polymorphism (i.e. TAS1R2 resistant) consistently demonstrated lower caries scores. Caries scores were significantly decreased in the GLUT 2 Resistant/ TAS1R2 Resistant combined group as compared to the GLUT2 Risk/ TAS1R2 Risk combined groups. Variations in GLUT2 and TAS1R2 genes are associated with risk of dental caries. A possible compounded effect of having both genetic polymorphisms is suggested.
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1. **Introduction**

1.1 **Caries – a silent epidemic**

Dental caries is an infectious transmissible disease in which bacterial by-products e.g. organic acids like lactic and pyruvic acid cause the pathological localized destruction of tooth tissue. Although dental caries is known to be a preventable condition, it has become a ‘silent epidemic’ of oral diseases. (The Surgeon General 2000 & U.S. Department of Health and Human Services. Rockville, Md, National Institute of Dental and Craniofacial Research, National Institutes of Health, 2000) However, as it often progresses silently in a non-life threatening nature, it is often considered an unimportant priority.

1.2 **Caries prevalence and trends**

Despite an overall decline in dental caries prevalence and severity, dental caries remains the single most common chronic childhood disease – 5 times more common than asthma and 7 times more common than hay fever. Over 50% of children aged 5 – 9 years old have at least one cavity or filling. This proportion swells to 78% among 17 year olds which demonstrates the inability to control the disease even as individuals approach adulthood. (The Surgeon General 2000 & U.S. Department of Health and human Services. Rockville, Md, U.S. Department of Health and Human services, National Institute of Dental and Craniofacial Research, National Institutes of Health, 2000) More recent statistics show that adults aged 20 years and over have 30.3% untreated dental decay with over 85% of adults having ever experienced decay. (National Center for Health Statistics, 2011)

The distribution of dental caries is skewed. 80% of permanent teeth affected by caries are found in approximately 25% of 5-17 yr old children. (Kaste et al., 1996) Parental caries status
has been shown to have a predictive effect on their child’s future caries status. (Mattila, et al., 2000; Mattila et al., 2005; Smith et al., 2002) This points to a possible genetic component which has not been studied to the same extent as other contributing factors.

Improvement in disease control is crucial. Goals for oral health improvement have been outlined in the Healthy People 2020 initiative. Beginning since 1979, Healthy People initiatives aim to improve the health of Americans, with the newly revised 2020 version recognizing the continual need to improve oral health and reduce the proportion of individuals afflicted with dental caries. (U.S. Department of Health and Human Services, 2011)

1.3 Implications of poor oral health

To be in perfect health, one must possess good oral health; oral health is an integral component of general health. However, the general popular perception is that oral disease has a minimal effect on a person’s life. Many give poor regards to a child’s dentition, often remarking that they are “just baby teeth”. However evidence suggests that children with dental caries have poorer quality of life. This puts them at a disadvantage for physical, psychological and social development.

Early childhood caries (ECC) can affect a child’s growth and development. Children with ECC weighed significantly less (14.7 ±2.3kg) than their caries-free counterparts (16.9±2.9kg). It is suggested that the impact of dental caries alters feeding and sleeping patterns, thus influencing growth velocity. However, these children experienced a period of ‘catch-up’ growth after undergoing dental rehabilitation, which affirms our understanding of the impact of ECC on growth and development. The growth acceleration observed in the
group with ECC negated any significant weight difference that had been noted in the beginning. (Acs et al., 1999)

Children aged 22 to 70 months were posed questions to assess their quality of life (QOL). This included the ability to speak clearly, chew, associated pain and discomfort as well as self-esteem issues. Examples of questions posed include “Does a hurting tooth wake you up at night?”, “Does a hurting tooth stop you from playing?” and “Do kids make fun of your teeth?” Children with early childhood caries had significantly lower QOL scores than children who were caries free. Similarly, parents perceived their child’s QOL to be diminished when affected by ECC. Furthermore, children who received dental treatment for ECC reported a significantly improved oral health-related QOL score 4 weeks after the treatment was completed. (Filstrup et al., 2003)

In an investigation into whether oral health affects the psychological aspect of quality of life, children and parental smile assessments were done. This considered the concerns of one’s appearance, self esteem, and whether oral health interfered with social interactions. Objective and subjective smile assessments showed that children with poorer oral health had significantly more negative evaluations of their own smiles. (Patel et al., 2007) It appears that poor oral health has a negative impact on a child’s self esteem, which impacts their ability to interact and communicate.

It was reported in 1992 that more than 51 million school hours are lost annually as a result of visits to a dentist or an oral problem, while more than 41 million restricted activity days were estimated for all ages at the national level in 1989. (Gift et al., 1992) This not only causes a disruption to school attendance, but interferes with family routines e.g. obtaining time off
from work. School performance is affected as well. Children in a North Carolina interview-based study with poor oral and general health were 2.3 times more likely to report poor school performance than their healthy counterparts. (Blumenshine et al., 2008)

An increase in attendance at the emergency department by patients with dental complaints related to dental caries (caries/abscess/cellulitis) has been noted. In a 5 year retrospective study in Texas Children’s Hospital, the number of caries related cases increased from 53 in 1997 to 138 in 2001 – a 260% increase. 68% of all dental admissions in 2001 were for caries related diagnoses. (Ladrillo et al., 2006) This sharp increase puts an unnecessary strain on health care resources.

Patients with severe dental infections who are admitted to the hospital have an average hospital stay of 3-5 days. (Y. T. Lin & Lu, 2006; Oliva et al., 2008) Ettelbrick and colleagues reported in 2000 that the average cost of care across 5 children’s hospitals for a single admission for odontogenic infection was $3,223. (Ettelbrick et al., 2000) Care in the emergency department is often more costly, time consuming, more taxing on the health care system but may not actually result in definitive care for the patient.

The bearing dental disease has over cost efficiency of the health care system cannot be disregarded. Control of dental disease has great potential to improve utilization of health care resources.

There is a good body of published literature that shows the multi-faceted implications of dental caries. As such, it is not hard to recognize the driving force behind improving the preventive modalities of dental caries.
2. **Preventive efforts**

In order to institute cost effective preventive recommendations, it is wise to evaluate the current preventive measures. It is comforting that these efforts have collectively shown promising results over the years. These may be population based approaches or approaches that target a select population at risk.

2.1 **Fluoride**

The ability of fluoride to inhibit and remineralize carious lesions is well documented. Widespread use of fluoride has been a major factor in the decline in the prevalence and severity of dental caries. Fluoride can be found in dentrifices like toothpaste, mouthrinses, topically applied gels and oral supplements. Community water fluoridation is another mode of delivering fluoride on a large scale. Moreover, it reduces the disparities in caries experience among the children of low socio-economic status (Jones et al., 1997; Slade et al., 1996) who have a considerably higher level of reported caries experience. (Christensen et al., 2010b; Gillcrsit et al., 2001) The cost-effectiveness of water fluoridation in the reduction of dental caries is clearly supported by the dental literature. (Center for Disease Control and Prevention, 2001; McDonagh et al., 2000; Spencer et al., 2008) Fluoride is well recognized by the dental profession as a major contributing factor to the decline in caries prevalence.

2.2 **Pit and Fissure Sealants**

Pits and fissures are surfaces susceptible to dental decay. Well retained dental sealants act as a physical barrier that prevents collection of micro-organisms and food in the pits and fissures, thus inhibiting the initiation of dental caries. The value of the sealant in caries reduction is shown in a long term study that showed a 76.3% reduction in caries in permanent 1st molars of children at 4 years and 65.4% at 9 years follow up. (Bravo et al., 2005) In
addition, evidence from Medicaid claims demonstrated a three-fold benefit. Children who received sealants on their permanent molars were less likely to receive restorative treatment, the time between receiving sealants and receiving restorative treatment was greater and the restorations were less extensive than unsealed molars. (Bhuridej et al., 2005) The advantage of dental sealant placements in caries reduction has been well affirmed in the scientific literature, (Ahovuo-Saloranta et al., 2008; Azarpazhooh & Main, 2008) with good evidence supporting it as a cost effective measure. (Quinonez et al., 2005; Weintraub et al., 2001)

2.3 Remineralisation therapy
Our understanding of enamel as a dynamic tooth material paved the way for research into other remineralisation therapies. These early interventions reduce the need for traditional restorative treatment. The organic acids produced by cariogenic bacteria diffuse through the plaque and into the tooth, leaching calcium and phosphate from enamel. Remineralization aims to create adequate concentration gradients of calcium, phosphate and fluoride at the tooth surface to facilitate recrystallization. Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) has been studied and included in sugar-free chewing gums and topically applied pastes. CPP-ACP binds readily to the surface of the tooth and bacteria in the plaque, buffering the free calcium and phosphate ions. This maintains a state of supersaturation, inhibits enamel demineralization and enhances remineralization, thus reducing the caries activity. (Llena et al., 2009; Morgan et al., 2008; Reynolds et al., 1995)

2.4 Education and access to care
Effective prevention is one that starts early. That is one of the reasons why medical professionals have been recruited in a continual bid to improve well child care. A survey of the Fellows of the American Academy of Pediatrics showed that more than 90% of
responders believed that they played a role in a child’s oral health, although only 54% of paediatricians reported examining the teeth of more than half of their youngest (0-3 years old) patients, with 4% placing fluoride varnish on children’s teeth. (Lewis et al., 2009) While the numbers may seem discouraging, this is essentially a huge initial step forward for preventive oral health care. Although lack of oral health knowledge is a barrier to medical professionals’ participation in oral-health related activities, a number of resources are available for this purpose (Douglass, & Krol, 2009) and evaluation of their effectiveness promising. (Kressin et al., 2009; Talib et al., 2010) Pediatricians’ uptake of their expanded role to oral health care has been heartening. (Close et al., 2010)

This responsibility is further shared with schools, via oral health education and school-based dental services that have the advantage of easing the issue of access to care. (Simmer-Beck et al., 2011) This allows an early and sustainable dissemination of important preventive dental health information, recognition of disease and referral for treatment. (Simmer-Beck et al., 2011)

Amidst the documented effectiveness of preventive regimens, dental caries has still managed to establish itself as a ‘silent epidemic’ today. Evidently, improvement needs to be made to the arsenal of preventive strategies.
3. Caries Detection

Detecting caries is a crucial step prior to managing the disease. Visual-tactile-radiographic procedures are fundamental to everyday dental practice, with cavitation traditionally being the hallmark of dental caries. However, with the advent of caries reversal modalities like remineralization, the ability to detect non-cavitated carious lesions early on becomes more important.

3.1 ICDAS – an overview

Dental caries is a dynamic disease process of demineralization and remineralization cycles. Sound enamel is translucent and micro-porous. Increased demineralization causes an increase in microporosity and a change in the refractive index of enamel, resulting in a decrease in its degree of translucency. Earlier lesions are only discernible when dried for a short period of time while more advanced lesions may be seen even when covered by saliva. These visual cues are important to our diagnosis.

The International Caries Detection and Assessment System (ICDAS) is a system designed to enhance the detection, assessment, diagnosis and monitoring of caries while measuring the disease process at different stages. The ICDAS committee is made up of a large team of cariologists from Europe and America, following an invitation to participate. (International Caries Detection and Assessment System Coordinating Committee, 2005a) ICDAS I criteria were developed after a meeting in Ann Arbor, Michigan, August 2002 and revised in 2005 at a Baltimore, MD, USA meeting.
3.1.1 The validity and reliability of ICDAS

A correlation exists between the macroscopic observations of carious lesions, stereomicroscopic findings and their respective histological depths. A strong linear relationship can be established between external and internal dental tissue changes. (Ekstrand et al., 1995) White spot lesions that are only visible on air-drying are most likely to be limited to the outer half of enamel, while the histological findings of white/brown spot lesions that are visible when enamel is wet show that demineralization extends between the inner half of enamel and the outer third of dentine. A lesion with enamel breakdown but without visible dentine and a lesion with a grey, brown or blue shadow of dentine shining through intact enamel extends to the middle third of dentine. Frank cavities with visible dentine indicate an encroachment into the inner third of dentine.

The validity of the ICDAS was measured during the ICDAS workshop by comparing clinical coding with histological findings. (International Caries Detection and Assessment System Coordinating Committee, 2005b) Participants examined the occlusal surfaces of 57 extracted teeth prior to sectioning and examination. Histological scores were compared with the scores determined clinically. The likelihood ratio (LR) scores tabulated ranged from 6.7 to 13 and were high compared to the LR of standard medical signs and symptoms. To put matters in perspective, an ST elevation on an electrocardiogram (ECG) has a LR of 11.2 while radiating pain to both arms has a LR of 7.1 for a myocardial infarction and a sharp or stabbing chest pain has a LR of 0.3. (Panju et al., 1998)

An in-vitro study (Ekstrand et al., 1997) using histological scores as the gold standard found that Spearman correlation coefficients for the visual ICDAS scores ranged from 0.87 to 0.93. Kappa values for inter and intra-examiner reproducibility ( $K = 0.54-0.69$, $K = 0.73-0.89$ )
showed a good level of agreement. In another study, 3 examiners used the ICDAS II examination system to analyse 50 extracted teeth. (Jablonski-Momeni et al., 2010) When measured against histological sections, at the D1 (first visual change in enamel when dry) threshold, sensitivity and specificity was 0.95 and 0.85 respectively, while at the D3 (localized enamel breakdown with no visible dentine or underlying shadow) threshold, sensitivity and specificity was 0.81 and 1.00 respectively. Examiners re-examined the same teeth on four separate occasions to test the reproducibility of their ICDAS II scores. Inter and intra-examiner kappa scores were in the good to very good range at 0.72 – 0.93.

Although the above studies demonstrate the accuracy and reliability of the ICDAS, other investigations have yielded different results. 2 experienced dentists who were new to the ICDAS II scheme used the ICDAS II visual scoring scheme to examine 163 permanent extracted molars. Their scores were measured against scores obtained via a histological examination of the same teeth, coloured with a dye. The relationship between the ICDAS II and histological classification systems was not strong (Spearman’s correlation coefficients 0.42 – 0.53). However, there was only fair intra-examiner agreement (unweighted K = 0.51) and intra-examiner agreement (unweighted K = 0.58, 0.59). This could be a result of the lack of familiarity with the ICDAS II system. (Diniz et al., 2009)

3.1.2 ICDAS – the system

ICDAS II uses a 2 digit coding system. The first digit is used to identify restorations / sealants. Table 1 demonstrates the differentiation between different types of restorations and the status of the fissure sealant. It further takes into account teeth that are missing for reasons other than caries and tooth surfaces that cannot be examined owing to access. (International Caries Detection and Assessment System Coordinating Committee, 2005a) The second digit
is the caries code which classifies the caries status on an ordinal scale. The ICDAS detection codes for coronal caries range from 0 to 6 according to the severity of the lesion and can be found in Appendix 1. The following flow-chart on the ICDAS e-Learning site is helpful for determining the respective codes. There are potentially 182 mutually exclusive tooth surfaces to score on the ICDAS adult chart form. (Appendix 2) Each tooth is divided into mesial, distal, facial, lingual and occlusal surfaces with further division on the tooth surface.

Table 1. Classification of the restoration, sealant or missing status in ICDAS

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Un-restored or unsealed</td>
</tr>
<tr>
<td>1</td>
<td>Sealant, partial – a sealant that does not cover all pits and fissures</td>
</tr>
<tr>
<td>2</td>
<td>Sealant, full – A sealant that covers all pits and fissure on a tooth surface</td>
</tr>
<tr>
<td>3</td>
<td>Tooth coloured restoration</td>
</tr>
<tr>
<td>4</td>
<td>Amalgam restoration</td>
</tr>
<tr>
<td>5</td>
<td>Stainless steel crown</td>
</tr>
<tr>
<td>6</td>
<td>Porcelain or gold or PFM crown or veneer</td>
</tr>
<tr>
<td>7</td>
<td>Lost or broken restoration</td>
</tr>
<tr>
<td>8</td>
<td>Temporary restoration</td>
</tr>
<tr>
<td>9</td>
<td>Tooth does not exist or other special cases</td>
</tr>
<tr>
<td></td>
<td>96 = tooth surface cannot be examined (surface excluded)</td>
</tr>
<tr>
<td></td>
<td>97 = tooth missing because of caries (all surfaces coded 97)</td>
</tr>
<tr>
<td></td>
<td>98 = Unerupted tooth (all surfaces coded 99)</td>
</tr>
</tbody>
</table>
3.1.3 ICDAS protocol

An examination using the ICDAS protocol requires the teeth to be cleaned and dried, either via a round of toothbrushing or a professional prophylaxis. A ball-ended explorer is used to remove any remaining plaque and debris and check for surface contour, minor cavitations or sealants. Tooth surfaces are examined wet before drying for a recommended duration of 5 seconds with an air syringe.

3.1.4 Limitations of ICDAS

ICDAS offers a wealth of information in terms of caries diagnosis. However, questions arose with regards to its clinical feasibility especially in reference to efficiency when used for epidemiological purposes. A study conducted by Braga et al. 2010 compared ICDAS II values with the WHO criteria. (Braga et al., 2010) There was no significant difference
between the dmf-s (decayed, missing, filled surfaces) and dmf-t (decayed, missing, filled teeth) values obtained with WHO and ICDAS score 3 cut-off. However, there was a fourfold increase in the dmf-s and dmf-t values when initial non-cavitated caries were included and a threefold increase when advanced non-cavitated caries lesions were included. While this demonstrates ICDAS II as more robust in detecting non-cavitated lesions than the WHO criteria, time spent on this detailed examination may potentially make it impractical. The average examination time spent on ICDAS II coding was reported to be twice as long as that for the WHO criteria, (3.7±1.8 min compared to 1.9±0.7 min), however the wealth of knowledge gained in the additional 2 minutes translates to better targeted preventive regimes and thus a better long term outcome. It seems well worth the extra 2 minutes.

3.2 Review of caries detection methods

Incorrect diagnoses result in incorrect and unnecessary treatment decisions with the risk of unnecessary restorations outweighing the benefits of detecting early decay. An ideal situation would be to diagnose dental caries with as much accuracy as our caries detection modalities, singly or combined, permit.

To evaluate the impact of employing various caries detection methods simultaneously on occlusal surfaces, examiners used electrical conductance measurement, visual examination, bitewing radiographs, quantitative light fluorescence and laser fluorescence to examine 96 extracted molar teeth and came up with a treatment decision. A month following the initial examination, all examiners re-examined the same sites and re-made their treatment decision. Information obtained from combining the diagnostic methods showed no significant improvement in diagnostic accuracy. No statistical differences were observed between the accuracy between the visual examination alone and the combination of visual examination
with other detection methods. Instead, analysis showed that more teeth were indicated for invasive treatments on the occlusal surface. It appears that having multiple detection methods result in overtreatment at 2 levels: more non-invasive treatment to sound teeth and more invasive treatment to teeth with lesions limited to enamel. (Pereira et al., 2009)

On top of the lack of added value these various caries detection modalities bear, dentists do not commonly possess these tools in their practices. This is a factor worth considering, because in reality, the most accurate tool constructed loses its clinical value if not used by practitioners. Currently, dental offices are most commonly equipped with radiographic examination facilities, making the visual-tactile-radiographic method of examination the most feasible, with the radiographic component especially important for interproximal caries diagnosis. The benefit of the ICDAS protocol is that it enhances caries detection but does not require additional equipment that practising dentists may be opposed to purchasing.

**Radiographic examination**

The visual examination of proximal surfaces is impeded by adjacent teeth. ICDAS II criteria has so far been studied in greater detail in diagnosing occlusal lesions and less emphasis has been placed on validating ICDAS II criteria in proximal lesions. Radiographic detection of proximal caries is highly specific and a positive diagnostic outcome is strongly indicative of a carious lesion. When the prevalence of caries is low, a more specific diagnostic modality (i.e. high positive predictive value) is preferred as it prevents unnecessary operative treatment.

J.H.G. Poorterman et al. 2000 looked at the additional value of the bite-wing radiograph compared to visual examination alone on an epidemiological scale. The population comprised of individuals in their permanent dentition, ranging from 14-54 years old. The number of unfilled surfaces in need of restorative treatment detected, based on additional radiographic findings was at least doubled (additional radiographic value of 206% - 700%) in all age
groups except the 14 yr olds. For the latter, bitewing examinations provided an additional radiographic value of 163%. Although on an epidemiological scale, the increased radiographic findings led to a rise of 5% in DMFS value only, the impact of the clinical findings that would have been missed otherwise (on an individual basis) cannot be ignored. (Poorterman, Aartman, Kieft, & Kalsbeek, 2000) Given the added value of bitewing radiographs, it would be logical for dentists to supplement their clinical examination with them.

There are several views available in dental radiography. These include dental panoramic, periapical and bitewing radiographs. Posterior bitewings have been the primary method for the diagnosis of proximal caries (Kidd & Pitts, 1990) and are the mainstay of recall examinations at the dental office. Full mouth series and panoramic with bitewing surveys showed similar accuracy for posterior approximal caries detection, but this was probably attributable to the existence of posterior bitewings in both surveys. (Akkaya, Kansu, Kansu, Cagirankaya, & Arslan, 2006) Other studies have shown the superiority of bitewings (Kidd & Pitts, 1990) over panoramic radiographs at proximal caries diagnosis. (Akarslan, Akdevelioglu, Gungor, & Erten, 2008; Kidd & Pitts, 1990; Scarfe et al., 1994)

Both conventional and digital radiograph modalities are used in today’s practice. Digital bitewings and conventional bitewings and micro-computed tomography were compared in an in-vitro study which looked at caries diagnosis performance. Micro-computed tomography for the use of proximal caries detection was studied as a possible alternative to histological examination as a validation method for cariology studies. No significant difference was found between the performance of the digital and conventional bitewings. (Mitropoulos et al., 2010)
Amidst the sophisticated advances in caries detection, visual examination with posterior bitewing radiographs appears to provide the most efficient combination to diagnosing caries. The design of this study is based on these principles.
4. **Caries Risk Factors**

An understanding of risk factors attributing to dental caries is crucial to effective prevention. The aetiology of dental caries is multi-factorial, ranging from dental to social to medical aspects. In addition, an individual’s potential of getting dental caries is dynamic, changing with age, lifestyle and dietary changes etc. Caries risk predictors vary slightly between a child and an adult, thus studies in cariology conducted in the pediatric population may be applicable to adults and vice-versa.

4.1 **Individual risk factors**

The level of oral hygiene and tooth pit and fissure morphology has been found to be a significant predictor of dental caries. (Disney et al., 1992; Sanchez-Perez, et al., 2009; Zhang & van Palenstein Helderman, 2006) One of the best predictor of future caries is the individual’s past positive caries experience. (Alaluusua et al., 1990; Disney et al., 1992; van Palenstein Helderman et al., 2001; Zero., 2001; Zhang & van Palenstein Helderman, 2006) However, it loses its usefulness if the goal is to prevent the manifestation of the disease itself.

4.1.1 **Microflora**

Caries is a microbial disease with mutans streptococci (MS) and lactobacilli (LB) being fingered as the culprits. (Beighton et al., 1996) The quantity of MS in saliva is both related to the number of colonized tooth surfaces (Lindquist et al., 1989) as well as the increment of new caries lesions. (Zickert et al., 1982) In contrast to MS, lactobacilli require retentive areas to proliferate, hence a high LB count is usually associated with frank cavitation. (Anders Thylstrup, 1986; Carounanidy & Sathyanarayanan, 2009) Lactobacilli are highly influenced by dietary carbohydrate content and intake frequency, and their presence is a reflection of an
acidogenic environment and denotes the presence of substrate for other bacteria like MS. (Anders Thylstrup, 1986)

A longitudinal study by Alaluusua S et al. (Alaluusua et al., 1990) found that results of bacterial tests (MS and LB) were significantly correlated to caries increment. However, conflicting results with regards to salivary tests do exist in the literature. (Sanchez-Perez et al., 2009)

4.1.2 Saliva

Navazesh et al. found that unstimulated flow rates have strong predictive validity for estimating caries risk. The normal unstimulated flow rate varies between 0.3-0.4 ml/min (Sreebny, 2000b) and values less than 0.1 ml/min should be considered abnormal. Most investigators consider a stimulated flow of <0.7 ml/min a low flow rate and an indicator of increased caries risk. (Anders Thylstrup, 1986; Holbrook, 1993; Pedersen et al., 1999) Although the literature is conflicting on the ability of salivary flow rate to predict future caries (Alaluusua et al., 1990), it is understood that with a reduced quantity of saliva, the oral clearance of the microorganisms and the food remnants is impaired. (Carounanidy & Sathyanarayanan, 2009) Consequently the pH and the buffering capacity are also reduced. This acidic environment created encourages the growth of aciduric and acidogenic microorganisms whilst reducing the remineralization capacity in the oral cavity.
4.2 Environmental risk factors

4.2.1 Diet

Sugar is a mainstay in everyone’s diet and its relationship with dental caries is well established. (Gustafsson et al., 1954; Harris, 1963; Moynihan & Petersen, 2004) The sense of taste gives us important information about the nature and quality of food, and of all the basic taste qualities, sweetness is the most universally liked. Studies in infants showed a preference for sweetened fluids even at birth. Characteristic facial expressions were noted when infants were offered the sweetened solution in contrast of other salty or bitter or sour solutions. Facial relaxation was noted with sweetened solutions. (Rosenstein & Oster, 1988)

Exclusively gastric tube-fed newborns offered sweetened solutions also showed a greater sucking response as compared to water alone. (Tatzer et al., 1985)

Sugar is a pleasant flavour and is a ready source of energy that can be used by the body. However, the propensity for sugar varies among individuals. Individuals vary in terms of sensitivity of taste, (Blakeslee & Salmon, 1935) preference and desire (Parker et al., 2003; Thompson et al., 1976; Zellner et al., 1999). Variation in perception and desires for sugar may change over time and may reflect a change in bodily requirements. Blood glucose regulation in the body is crucial for organs that make up the central nervous system (the brain and spinal cord). A constant delivery of glucose is needed at every moment of life in order that vital organs like the brain, the heart and muscles are adequately provided with their source of energy. When metabolic changes occur that reduce the availability of glucose in the blood, e.g. increases in plasma insulin concentration, then sweet preference increases. (Briese & Quijada, 1979) Genetic inheritance (Bretz et al., 2006; Keskitalo et al., 2007; Mennella et al., 2005) and both weight and weight fluctuations (Barkeling et al., 2001; Drewnowski., 1985) may alter the overall perception and preference for sweet tastes. Shifts in hormonal and
psychological states e.g. menstruation, (Barkeling et al., 2001) depression, emotional upset (Striegel-Moore et al., 1999) may affect the sensitivity and craving for sugar as well.

Many dietary studies have demonstrated the role of diet in the caries process, but the most prominent are the landmark studies set in the Hopewood House in Australia and the Vipeholm study in Sweden. The presence of refined carbohydrates in the diet (Harris, 1963) had great bearing on the prevalence of dental decay: the mean DMF of 13 year old children in the Hopewood House was 1.6, in comparison with a DMF of 10.7 in the general pediatric population of New South Wales. Increased frequency of sugar intake between meals led to a high caries incidence. The caries risk was further increased when sugar was consumed in a manner which had a strong tendency to be retained on tooth surfaces. (Gustafsson et al., 1954) Oral carbohydrate clearance is an important factor to consider, along with saliva flow rates. (Engvall & Birkhed, 1997; Hase, 1993) Different foods have varying clearance times: carbohydrates in fresh fruits, vegetables and drinks are eliminated within 5 minutes while sweets such as caramels, toffees, chocolates give high oral sucrose concentrations and clearance times of 15-20 minutes. These factors influence overall caries risk.

The importance of frequency as demonstrated by the above studies is exemplified by Stephan’s curve. (Robert M. Stephan, 1944) The importance of both the pH lowering activity by dental plaque and the frequency of sugar feeding event daily continues to be verified with more recent studies. (Beighton et al., 1996; Shimizu et al., 2008)

Dietary assessment in dental practice aims to evaluate the cariogenic challenges posed by the dietary choices and practices of the individual. A dietary analysis may come in the form of a twenty-four hour recall, a three to seven day dietary chart or a food frequency questionnaire.
In evaluating the dietary records, the nature of the diet and frequency of intake forms the basis for determining the cariogenic potential of the diet.

4.2.2 Fluoride

The widespread use of fluoride has reduced the prevalence of caries and reduced the rate of caries progression. Individuals may be exposed to fluoride on a community level, via home care dentrifices or professionally delivered mediums. Community exposure includes drinking fluoridated water, eating food that has been prepared with fluoridated water while home care dentrifices that deliver topical effects of fluoride include fluoridated toothpastes and mouthrinses. The American Academy of Pediatric Dentistry considers a child to be at high risk of dental caries if unexposed to fluoridated water, does not use fluoridated toothpaste and does not take fluoride supplements. (American Academy on Pediatric Dentistry Council on Clinical Affairs, 2008; Sreebny, 2000a)

4.3 Medical and Socioeconomic risk factors

The pattern of children with decayed and filled teeth is inversely related to income level. Children aged 2-5 years who fell in the lowest income category (0-100% of Federal Poverty Line) had 29% decay while those in the highest income category (301% and up of the FPL) had 6.0% decay in the primary dentition. (Vargas, Crall, & Schneider, 1998) The racial and income based disparity of dental disease and treatment needs is apparent, starting from childhood and persisting into early adulthood. (Edelstein & Chinn, 2009) Education level and socio-economic status conveys the potential attitude of the patient and family towards oral health and dental awareness. Individuals with less education and lower socio-economic status are more likely to have reduced access to care. A cross-sectional study conducted on 146
children showed that the level of dental caries had a significant inverse correlation to the educational level of the mother and the family income. (Ramos-Gomez et al., 2002) Other studies have shown similar findings. (Christensen et al., 2010a; Oliveira et al., 2008)

A consensus exists about the cariogenic potential of sugar-containing food. However, a less recognized source of cariogenic carbohydrates exists in sugar containing medications. Sucrose acts as a preservative, an anti-oxidant, a solvent, a demulcent and a bulking agent. A study by Kenny and Somaya determined the history of oral medication consumption by a hospital children population. Medications taken included Fer-in-sol, Tempra, Dilantin. (Kenny & Somaya, 1989) Chronically ill children examined at a mean age of 31 months consumed an additional 17g of sugar from oral medication daily, which totalled up to a mean additional 8.7kg of sugar by the time of examination. In-vivo testing with commonly prescribed pediatric medications revealed the acidogenic potential of these drugs. (Bigeard, 2000) Plaque pH post-drug consumption was significantly lower than their sucrose-free controls.

Other medications like antihistamines, anticholinergics, hypnotics, antipsychotics and antihypertensives reduce the flow rate of saliva. (Roberts & Roberts, 1979) Diseases such as Sjögren’s syndrome and prior irradiation to the head and neck area are related to decreases in salivary flow rate. This increases the risk of dental caries. (Mathews et al., 2008; National Institute of Health Consensus Development Panel, 2001; Pedersen, Bardow, & Nauntofte, 2005) Physical disabilities or other special health care needs that impact motor coordination or cooperation are also considered factors that increase one’s caries risk. (American Academy on Pediatric Dentistry Council on Clinical Affairs, 2008; National Institute of Health Consensus Development Panel, 2001)
Dentistry has been largely focused on restoring the after-effects of this transmissible bacterial infection. This meant that we were always one step behind the disease. Armed with an understanding of caries risk factors and the knowledge that the surgical intervention of dental caries is insufficient in stopping the disease process, dental professionals have shifted their focus onto prevention. This change in mindset from restoration to prevention is evident: “Preventing the initial cavity... is preferable to restoring the tooth after the disease has occurred” (Healthy People 2010 & 2nd ed. U.S. Department of Health and Human Services. With Understanding and Improving Health and Objectives for Improving Health. 2 vols. Washington, DC, 2000) A tool that takes into account known caries risk factors will allow for identification of the high caries risk population for a more individualized approach in terms of patient education and preventive treatment planning. (Fontana & Zero, 2006)
5. Caries risk assessment

Caries risk assessment determines the likelihood of the incidence of caries over a certain time period. Identifying patients at a high caries risk permits fabrication of individualized target-oriented treatment plans which can optimize the patient’s compliance and the overall outcome. This would not only improve the efficiency of dental care delivery systems on both the population and individual level, but serve as a monitoring aid for the success of treatment. (Reich et al., 1999)

Some of the caries risk assessment tools available today include the Cariogram, (Bratthall & Hansel Petersson, 2005), American Academy of Pediatric Dentistry (AAPD) Caries Assessment Tool (American Academy on Pediatric Dentistry Council on Clinical Affairs) and the American Dental Association Caries Assessment Form. (American Dental Association) The Cariogram uses a graphical model to illustrate the individual’s chance of avoiding caries, based on various weighted risk factors. The risk of dental caries can be inversely deduced. The Cariogram is designed for both children and adults, while the other 2 are formulated for children, with further division based on age. At a quick glance of these caries assessment tools, one can appreciate the agreement that the disease is multifactorial.

These tools appear to encompass a broad range of factors. However, upon closer examination, a discerning observer would be cognizant of the lack of emphasis on genetics. The only factor faintly reminiscent of a hereditary contribution is the consideration of the caries experience of either mother or caregiver in the AAPD Caries Assessment Tool as well as that of siblings in the ADA tool. This shortcoming is revealing of a gap in the dental literature. However, an extensive review of the available literature on this subject gives insight into the difficulty of inclusion of specific genetic risk factors into these tools.
6. Genetics of diseases

6.1 Why do we look at genetics?

It is apparent that many traits or characteristics are inherited. Researchers have been paying attention to the genetic inheritance of diseases in a preventive mode of health care in order to improve early screening and overall outcomes with early and personalized intervention. Early screening can take the form of screening for genetic markers. Management of breast and ovarian cancer is a good illustration of this principle in the medical field.

Two genes, \textit{BRCA1} and \textit{BRCA2}, have been identified as breast cancer susceptibility genes, and clinically significant mutations are estimated to occur in about 1 in 300 to 500 of the general population. Women with these gene mutations are at a 45-80\% (Antoniou et al., 2003; Ford et al., 1998) increased risk of developing breast cancer and have up to a 40\% lifetime risk of ovarian cancer. (Antoniou et al., 2003) Women known to have an increased risk of breast and ovarian cancers are advised to consider risk-reduction strategies which include surveillance (breast self-exam, clinical breast exam, mammography and breast magnetic resonanace imaging), chemoprevention, prophylactic oopherectomy and prophylactic mastectomy. (Narod & Offit, 2005) These treatment options vary greatly in terms of invasiveness and side effects.

The importance of genetic testing (\textit{BRCA1} and \textit{BRCA2}) in disease management is highlighted in a study by Uyei A et al. 2006 where results of the test had a significant bearing on the treatment adopted. (Uyei et al., 2006) The need for genetic counselling has been echoed by numerous researchers. (Lux et al., 2006; Tinelli et al., 2010)
Evidence of genetic susceptibility to disease is mounting in the periodontal disease literature. Individuals with interleukin 1 polymorphisms have been associated with severity of periodontitis (Kornman et al., 1997; McDevitt et al., 2000) and increased bleeding on probing. (Lang et al., 2000) Several other polymorphisms related to inflammatory cytokines are also being investigated for its anti-inflammatory activities e.g. interleukin 6, (Nibali et al., 2010) interleukin 8, (Kim et al., 2010) interleukin 13, (Wu et al., 2010) tumour necrosis factor (Costa et al., 2010; Menezes & Colombo, 2008) to varying degrees of success.

There is a significant body of evidence that genetic factors are important determinants of diseases. As shown in the literature regarding breast and ovarian cancer, information gained from genetic testing is being well used to reduce the disease prevalence. Dental caries remains a significant problem and identification of links between genetics and dental caries will be crucial to new therapeutic intervention strategies which may include a caries risk assessment tool with a high level of predictive ability, risk counselling early on in life, even before the eruption of teeth, and consequent strategic management.

6.2 Approaches to genetic research

Our early understanding in the genetic contribution to food intake behavior stemmed from studies that looked at family units or compared monozygotic to dizygotic twins. The candidate gene approach is a newer alternative. Candidate genes are identified on the basis of their perceived role in mechanisms related to food intake, which may occur at multiple points along the entire food intake process, even before food is consumed. (Eny, KM and El-Sohemy, A, 2010) Genetic variations in these genes such as single nucleotide polymorphisms (SNPs) or copy number variants (CNVs) can be examined to determine the role of selected genes in food intake behaviours. (Alfredo Martinez et al., 2007; Kowalski, 2004)
Polymorphisms refer to differences in DNA sequences and a SNP (pronounced “snip”) is a type of polymorphism involving a single base pair. (National Institutes of Health. National Human Genome Research Institute) SNPs are the simplest form of genetic polymorphism in the human genome. The prevalence of SNPs is approximately 0.1-0.15%, translating to a single base difference per 1000-1500 base pairs. (Li & Sadler, 1991; Sachidanandam et al., 2001) SNPs are widely but not uniformly distributed over the entire human genome and may occur in coding or non-coding regions. (Li & Sadler, 1991) SNPs have a low rate of recurrent mutation and this genomic variation contributes to the diversity in the human species. The tools available today allow the determination of gene polymorphisms from a blood sample. Mapping of individual SNPs on candidate genes may serve as markers for disease genes and is of great value towards improving health care through discovery of new and early diagnostic tools and pharmacogenomics. However, selection of candidate genes is reliant on prior knowledge of their biological functions, which may be unknown. The genome-wide association study approach resolves this issue.

A genome-wide association study (GWAS) is a powerful, hypothesis free, unbiased approach used to identify specific genetic variation with diseases of interest. It involves scanning the whole genome of a large population of individuals and looking for genetic markers that can be used to predict the disease. (National Institutes of Health. National Human Genome Research Institute) This is more rapid and cost effective as compared to the candidate gene method because unlike the latter, previous knowledge of the trait aetiology is not required. Currently, there are only two published genome-wide scans in this area.

Results of both the candidate gene approach and GWAS are of great value towards improving health care through a better understanding of diseases as well as to facilitate the development of better prevention and treatment strategies.
6.3 Genetics of caries

Kurihara induced dental caries in inbred strains of mice via inoculation with Streptococcus mutans serotype c. Results showed difference in susceptibility to dental caries across the different mice strains and a genetic predisposition was suggested. (Kurihara et al., 1991)

Specific regions on the mice chromosomes linked to dental caries susceptibility have also been studied. The H-2 region on chromosome 17 was studied in 2 strains of inbred mice: BALB/cJ (H-2^{d/d} caries prone) and C3H/HeJ (H-2^{k/k} caries resistant). After 90 days of exposure to a caries-inducing diet, both groups had significantly different caries scores (Table below). When the MHC (H-2) region derived from the caries resistant strain C3H/HeJ was introduced into the caries prone strain BALB/cJ, reported caries scores (57.6 ± 7.8) were significantly lower than caries prone BALB/cJ strains. This demonstrates the influence of genetic factors on dental caries. (Suzuki, Kurihara, & Kurihara, 1998)

Table 1. Mean caries scores of different mouse strains (mean ± standard error) (Suzuki et al., 1998)

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Caries score</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cJ (caries prone)</td>
<td>286.5 ± 16.4</td>
</tr>
<tr>
<td>C3H/HeJ (caries resistant)</td>
<td>8.8 ± 2.4</td>
</tr>
<tr>
<td>BALB/cJ + MHC (H-2) region from caries resistant C3H/HeJ strain</td>
<td>57.6 ± 7.8</td>
</tr>
</tbody>
</table>

Although caries studies in humans are complex, evidence for a genetic contribution to dental caries has existed for years with twin studies being conducted in the late 1950s.

Monozygotic (MZ) twins share 100% of their genes while dizygotic (DZ) twins share on average 50% of their genes, as do ordinary siblings. Comparisons will thus allow hereditary
variability to be ascertained. However, there are limitations as the twin method assumes both prenatal and postnatal environments for MZ and DZ twins are similar. Only when environmental influence is controlled, can the observed similarities be attributed to genetic inheritance. Assuming 100% heritability, the correlation for fraternal twins should be 0.50 while that for identical twins would be 1.0.

Monozygotic twins showed a greater concordance in prevalence of dental caries as compared to dizygotic twins in both the deciduous and permanent dentitions. (Shuler, 2001) Results of an examination of 224 pairs of twins concurred with previous findings but concluded that environmental factors had an influence on dental caries as well. (Mansbridge, 1959)

Further understanding in the role of inheritance and incidence of dental caries came from the Minnesota Study of Twins Reared Apart study. (Boraas et al., 1988; Conry et al., 1993) Such studies, where genetically similar individuals are separated at birth and brought up under differing environmental influences bear the strongest evidence to date of the influence of genetics on caries incidence. Monozygotic but not dizygotic adult twins who were separated early in life and reared apart showed a highly significant relationship in terms of the numbers of teeth present and the percentage of teeth/surfaces restored. Similarities in dental caries experience between monozygotic twins held true for both an increase and decrease in dental caries. A statistically significant resemblance in dental caries status, despite being exposed to different environmental factors was observed, and thus ascribed to their shared genetic information. Many twin studies that followed demonstrated similar findings, (Bretz et al., 2005; Bretz et al., 2006; Rintakoski et al., 2010) solidifying our understanding of the genetic contribution to dental caries.
The question of whether genetics affect children and adults similarly has been addressed in recent studies. (Wang et al., 2010) Both primary and permanent caries scores were found to be significantly related to genetic inheritance, with a higher heritability estimate (54-70%) in the primary dentition and a lower but significant heritability in the permanent dentition (35-55%). Inclusion of non-cavitated white spot lesions further increased the strength of heritability.

The above research exemplifies the role of genetics as a whole. However, with technological advances, we have moved from reaffirming the genetic component in dental caries to questioning which specific genes are involved. (Wang et al., 2010)

Amelogenesis takes direction from genetic programming. Thus, variations of genes that result in an altered enamel structure may increase an individual’s caries susceptibility. The dental literature has verified this relationship between enamel defects and dental caries. (Milgrom et al., 2000; Targino et al., 2010) The AMELX gene that codes for amelogenin, which is important for enamel formation, has been shown to be a significant factor in caries susceptibility in both the primary and permanent dentition. (Deeley et al., 2008; Kang et al., 2010; Patir et al., 2008) Evidence also suggests that tuftelin, another gene involved in amelogenesis, in combination with high mutans streptococci levels, results in increased caries susceptibility in a pediatric population. (Slayton et al., 2005)

Functional polymorphisms of DEFB1 (beta defensin 1) (Ozturk et al., 2010) and Lactotransferrin A/G (exon 2, Lys/Arg) polymorphism (Azevedo et al., 2010) have also been shown to be associated with caries susceptibility. Other genes contributing to caries susceptibility include the carbonic anhydrase 6 gene involved in salivary buffering capacity
(Peres et al., 2010) and acidic proline-rich proteins (PRH 1) that affect biofilm colonization. (Zakhary et al., 2007) Human leukocyte antigen Class II alleles (Altun et al., 2008) which are involved in host microbial responses have also been studied.

Genome wide association studies on caries risk are limited in the literature but one published in 2008 showed that loci 5q13.3, 14q11.2, and Xq27.1 were suggestive of low caries susceptibility while 13q31.1 and 14q24.3 were suggestive of high caries susceptibility. (Vieira et al., 2008) A more recent publication in 2011 (Shaffer et al., 2011) studied populations from the Center for Oral Health Research in Appalachia, the Iowa Fluoride Study, the Iowa Head Start Study and an independent sample from the Denmark National Birth Cohort. Carrying out a GWAS for childhood dental caries, many genomic regions whilst failing to meet genome wide significance thresholds ( p value < 10E-7), showed suggestive evidence for association. Different SNPs were also found in association with dental caries based on varying levels of fluoride exposure. Previously studied candidate genes e.g. AMBN (Deeley et al., 2008), TUFT1 (Slayton et al., 2005), TAS1R2, TAS2R38, GNAT3 (Wendell et al., 2010) did not meet genome wide significance but met nominal significance (i.e. p value <0.05). Table 2 below summarizes their findings, as well as lists novel candidate genes with possible contributions to the dental status. A more detailed discussion can be found in Shaffer et al. 2011’s paper.
Table 2. Nominated genes of interest (Shaffer et al., 2011)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>SNP</th>
<th>Functional expression of associated gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTN2</td>
<td>1</td>
<td>rs7556238</td>
<td>ACTN2 – actinin alpha 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Ameloblast organization</td>
</tr>
<tr>
<td>MTR</td>
<td>1</td>
<td>rs7556238</td>
<td>MTR – methionine synthase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Maternal MTR possibly associated with non-syndromic cleft lip and palate</td>
</tr>
<tr>
<td>EDARADD</td>
<td>1</td>
<td>rs10489788</td>
<td>EDARADD – ectodysplasin-A receptor-associated adapter protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Causes hypohidrotic ectodermal dysplasia</td>
</tr>
<tr>
<td>MPPED2</td>
<td>11</td>
<td>rs538811</td>
<td>MPPED2 – metallophosphoesterase domain containing 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Possible role in response to oral bacterial colonization</td>
</tr>
<tr>
<td>LPO</td>
<td>17</td>
<td>rs3744103</td>
<td>LPO – lactoperoxidase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Salivary enzyme important in oral bacterial metabolism, plaque formation and gingivitis</td>
</tr>
<tr>
<td>TFIP11</td>
<td>22</td>
<td>rs3752523</td>
<td>TFIP11 – tuftelin-interacting protein 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Secretory ameloblasts and odontoblasts</td>
</tr>
<tr>
<td>EPHA7</td>
<td>6</td>
<td>rs1983722</td>
<td>EPHA7 – ephrin type-A receptor 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Development of tooth and supporting tissues in mouse model</td>
</tr>
<tr>
<td>ZMPSTE24</td>
<td>1</td>
<td>rs10489431</td>
<td>ZMPSTE24 – zinc metallopeptidase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Associated with mandibuloacral dysplasia</td>
</tr>
</tbody>
</table>

The available studies highlight that several genetically variable factors contribute to the observed correlation. All these can exert an effect on caries incidence. However, many of these mentioned genes have limited modifiability currently. The dietary component is a modifiable caries risk factor but its inheritability has not been thoroughly explored. This begs the question— does a genetically predetermined dietary preference exist? And if so, does it contribute to caries risk? Preliminary research appears to suggest so. (Wendell et al., 2010; Wright, 2010)
7. **Genes of Interest**

Researchers have identified numerous candidate genes associated with ingestive behaviour, which can be largely classified into sensory, energy homeostatic and reward circuits of food intake. (Eny, K.M, El-Soehemy, A, 2010) The TAS1R2 gene (Taste receptor, Type 1, Member 2) has been found to influence taste perception (i.e. the sensory aspect) (Nelson et al., 2001; Zhao et al., 2003) while the GLUT 2 gene (Glucose transporter Type 2) partakes in energy homeostatic pathways. (Brown, 2000)

7.1 **GLUT2 studies**

The first step in glucose induced insulin secretion involves the entry of glucose in the pancreatic β-cell via transport proteins. (Marty et al., 2007) The GLUT2 gene, coded by the SLC2a2 gene (solute carrier family 2 (facilitated glucose transporter) member 2), is found on chromosome 3 in humans. GLUT2 is a member of the facilitative glucose transport protein family and is expressed in the pancreas, liver, small intestine, kidney and brain (Arluisin et al., 2004; Brown, 2000; Leloup et al., 1994; Roncero et al., 2004) and is primarily involved in glucose homeostasis. (Brown, 2000) Owing to its expression in regions of the brain involved in food intake regulation, in both humans and rodents, GLUT2 may be involved in the central glucose sensor apparatus.

A common single nucleotide polymorphism (rs5400) in the GLUT2 gene (SLC2a2) involves a substitution of threonine for isoleucine amino acid at codon 110 (Thr110Ile). Deletion of the GLUT2 gene was shown to impair the regulation of normal food intake in rodents. (Bady et al., 2006) A study in 20-29 yr college aged students found that 18% of the population studied possessed the GLUT2 polymorphism, with the highest prevalence seen in Caucasians. (K.M. Eny et al., 2008) Individuals with the Thr110Ile GLUT2 gene polymorphism displayed
a higher habitual intake of sugar, consuming 131g/day of sugar, as compared with the Thr/Thr genotype who consumed an average of 115 g/day of sugar. Further analysis of type of sugars consumed showed that carriers of the Ile allele consumed significantly more sucrose, fructose and glucose. (Table 3) No statistical significance was observed for the other macronutrients measured e.g. fat, protein. This daily difference in sugar consumption is equal to the amount of sugar in a typical can of sweetened beverage like a cola.

Table3. Comparison between individuals homozygous for the Thr allele and carriers of the Ile allele for 1 month average daily intakes of macronutrients for population 2 (excerpt)

<table>
<thead>
<tr>
<th>Sugars, g/day</th>
<th>Thr/Thr (n=478)</th>
<th>Thr/Ile + Ile/Ile (n=109)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>47.0 ± 1</td>
<td>55.0 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactose</td>
<td>17.0 ± 0.7</td>
<td>19.7 ± 1.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Maltose</td>
<td>2.20 ± 0.06</td>
<td>2.36 ± 0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>Fructose</td>
<td>25.4 ± 0.7</td>
<td>28.0 ± 1.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>23.7 ± 0.6</td>
<td>26.0 ± 1.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

(K. M. Eny et al., 2008)

These results suggest that the genetic variation in the GLUT2 gene may be responsible for individual variations in preference for sugar-containing foods.
7.2 TAS1R2 studies

TAS1R2 gene is located on human chromosome 1 and encodes one of the 2 protein subunits, which makes up the sweet taste receptor. (Liao & Schultz, 2003) T1R2 contributes to sweet taste perception. Furthermore, TAS1R2 and TAS1R3 (taste receptor, type 1, member 3) double knockout mice studies have shown a complete loss of response to all sugars. (Zhao et al., 2003) TAS1R2 has a diverse tissue distribution, affecting beyond the tongue and the palate (Liao & Schultz, 2003; Nelson et al., 2001) to include the gastrointestinal tract (Mace et al., 2007; Young et al., 2009), pancreas (Nakagawa et al., 2009) and hypothalamus (Ren et al., 2009), which are tissues important in regulation of metabolic and energy homeostasis. (Zheng & Berthoud, 2008)

2 of the common polymorphisms of the TAS1R2 gene that result in amino acid substitutions are Ile191Val (rs35874116), and Ser9Cys (rs9701796). Based on a population of young men and women, aged 20-29 years old, recruited via the Toronto Nutrigenomics and Health Study, the minor allele frequency for Ile191Val was 25% for the Val allele. The Ser allele was present in 21% of the population examined. (K.M. Eny et al., 2010) This group of individuals was examined to determine whether the genetic variants of TAS1R2 influenced sugar consumption. Results of a self-administered food frequency questionnaire revealed no effect of carriers of Val allele in lean individuals (Body Mass Index < 25 kg/m²). However, in overweight individuals, (BMI≥25kg/m²) Val carriers consumed a significantly lower amount of total sugars, specifically sucrose, fructose and glucose. No differences were observed for protein, fat or alcohol consumption in both lean and overweight individuals. No differences in macronutrient consumption were observed for the Ser9Cys polymorphism in lean or overweight individuals. (results not shown here)
Table 4: Comparison between individuals homozygous for the Ile allele and carriers of the minor Val allele (Ile191Val) for 1 month average daily intakes of macronutrients in lean (BMI<25kg/m²) subjects

<table>
<thead>
<tr>
<th>Carbohydrates (g/day)</th>
<th>Ile/Ile</th>
<th>Val carriers</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>120 ± 3</td>
<td>120 ± 4</td>
<td>0.62</td>
</tr>
<tr>
<td>Sucrose</td>
<td>49 ± 2</td>
<td>49 ± 2</td>
<td>0.55</td>
</tr>
<tr>
<td>Fructose</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
<td>0.74</td>
</tr>
<tr>
<td>Glucose</td>
<td>25 ± 1</td>
<td>26 ± 1</td>
<td>0.51</td>
</tr>
</tbody>
</table>

(K. M. Eny et al., 2010)

Table 5: Comparison between individuals homozygous for the Ile allele and carriers of the minor Val allele (Ile191Val) for 1 month average daily intakes of macronutrients in overweight (BMI≥25kg/m²) subjects

<table>
<thead>
<tr>
<th>Carbohydrates (g/day)</th>
<th>Ile/Ile</th>
<th>Val carriers</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>122 ± 6</td>
<td>103 ± 6</td>
<td>0.01</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50 ± 3</td>
<td>43 ± 3</td>
<td>0.05</td>
</tr>
<tr>
<td>Fructose</td>
<td>27 ± 2</td>
<td>22 ± 2</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>25 ± 1</td>
<td>20 ± 2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

(K. M. Eny et al., 2010)

TAS1R2 may affect the consumption of sugars by differences in detection of taste intensity but this may also be related to postingestive mechanisms owing to its tissue distribution. Overall, these results show that Val carriers consume significantly less sugar on a daily basis. This is another demonstration of the role of genetic variation in ingestive behaviours.
7.3 Taste genes may predict dental caries risk

To date, the possible linkage between the genetics of taste and dental caries has not been well established. An electronic search of the PUBMED database (up to July 2011) showed no previously published data in English on the association of GLUT 2 gene polymorphisms with oral health status. There is a relative paucity of studies in the literature with regard to taste and dental caries risk. The search revealed a small number of papers that looked at 2 broad categories – sweet and bitter taste sensitivities.

Wendell et al 2010, looked into taste genes TAS1R2, TAS2R38 (taste receptor, type-2, member38) and GNAT3 (guanine nucleotide binding protein, alpha transducing-3) in a large cohort of families from Appalachia. Dental records of 3 separate groups: primary, mixed and permanent dentition were analysed. (Wendell et al., 2010) The population was dichotomized based on caries status: individuals with caries and individual free of caries. They successfully demonstrated significant associations between variations in the TAS2R38 and TAS1R2 genes in the primary and the mixed dentition respectively. No associations were found in the group in permanent dentition. These results firstly demonstrate the possible genetic associations between taste and dental caries risk and secondly raise the question that perhaps different genes affect the caries risk at different ages.

Genetic sensitivity to the bitter taste of 6-n-propylthiouracil (PROP) is an inherited trait. (Drewnowski et al., 2001) Studies examining the genetically determined taste sensitivity to PROP in a pediatric population showed that a decline in taste sensitivity was significantly associated with an increase in overall caries experience. (Rupesh & Nayak, 2006a) This relationship has been affirmed by other similar studies. (Hedge & Sharma, 2008a; B. P. Lin, 2003; Rupesh & Nayak, 2006b)
Table 6. Taste candidate genes studied by S. Wendell et al. 2011

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference SNP (rs)</th>
<th>Significant effect(s) on caries risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste Receptor, Type-1, Member 2 (TAS1R2)</td>
<td>rs4920566, rs9701796</td>
<td>Protective + Risk</td>
</tr>
<tr>
<td>Taste Receptor, Type-2, Member 38 (TAS2R38)</td>
<td>rs713598, rs1726866, rs10246939</td>
<td>Protective, Protective, Protective</td>
</tr>
<tr>
<td>Guanine Nucleotide Binding Protein, Alpha transducing-3 (GNAT3)</td>
<td>rs2074674, rs6962693</td>
<td></td>
</tr>
</tbody>
</table>

After reviewing the available publications on dental caries and gene associations, the gap in the literature is apparent, in particular the contribution of genes involved in nutrition.
8. **Aims**

The broad aim of the study is to deepen our understanding of the genetic basis of dental caries. The specific aim of the study is to determine whether common polymorphisms in the TAS1R2 and GLUT2 genes is associated with dental caries.
9. **Hypothesis and novelty of the project**

Our hypothesis was that common polymorphisms in the GLUT2 and TAS1R2 genes are associated with dental caries. Based on previous studies, we hypothesized that carriers of the GLUT2 gene polymorphism (Ile carriers) and carriers of the TAS1R2 gene polymorphism (Val carriers) would have more extensive and less extensive dental caries respectively.

Our results will demonstrate the influence of the GLUT2 and TAS1R2 gene over dietary choices and whether this translates into an increased risk of dental caries. While this initial study was conducted on an adult cohort, future studies might examine how early in life these genetic influences set in and affect dietary habits, predisposing children to dental caries. Results of this study could suggest a novel way of detecting patients at risk of developing caries as early as birth, enhancing the implementation of preventive regimes.
10. **Methods and Materials**

Subjects who participated in the Toronto Nutrigenomics and Health Study were contacted randomly by email for the purpose of the study. (K. M. Eny et al., 2008) Only Caucasian participants were contacted, based on the high prevalence of GLUT2 gene in this ethnocultural group. Informed consent to take part in the study was obtained and any individuals with any significant medical conditions or special diets were excluded. University of Toronto Research Ethics Board approval was obtained prior to commencement of this study (Appendix 3). Participants were paid a fee for participation.

Participants’ body mass indices (BMI) and genotyping results were obtained from the Toronto Nutrigenomics and Health Study and hence not repeated. The DNA was extracted from a venous blood sample and polymorphisms were detected by using a TaqMan allelic discrimination assay. A more detailed description of the genotyping process has been described in a publication by Eny et al. 2008. (K. M. Eny et al., 2008)

A clinical examination was conducted by 1 examiner who was blinded to the individual’s genotype. Salivary tests conducted collected information regarding stimulated salivary flow and bacteriological counts. The latter was obtained via cultures on bacteria specific, sugar containing agar (MSB media and Rugosa agar from VWR Canada) to quantify lactobacillus (LB) and mutans streptococci (MS) counts. To calculate stimulated salivary flow (volume/min), subjects were given a standard paraffin wax tablet to chew on for six minutes. Only saliva collected in the last five minutes was collected. The volume of saliva expectorated was averaged over five minutes to give the desired stimulated salivary flow rate. Samples were incubated at 37°C for 48 hours and then compared with a visual chart for scoring purposes.
Plaque and gingivitis scores were recorded using Loe and Silness indices. (Loe & Silness, 1963; Silness & Loe, 1964) A rubber-cup prophylaxis was done and teeth were re-examined under a clinical operatory light source with aid of an air syringe, mouth mirror and probe. A second examination was conducted based on the International Caries Detection and Assessment System (ICDAS) protocol. Details about this system are available for download on the ICDAS website [http://www.icdas.org/index.html](http://www.icdas.org/index.html). 2 bitewing radiographs were taken using DENTSPLY Rinn's XCP horizontal bitewing holder and size 2 films. Bitewing radiographs were taken to detect proximal dental caries and secondary caries.

3 caries scores were tabulated. A DMFT (decayed, missing, filled) score was calculated for each patient using results from the clinical examination and a 2\textsuperscript{nd} score (DMFT+Xray) tabulated after combining clinical and radiographic findings. Only teeth missing due to caries were included; teeth congenitally missing or extracted for orthodontic purposes were excluded. The ICDAS score was the 3\textsuperscript{rd} score, calculated based on the ICDAS recommended scoring system.

All subjects filled in a questionnaire that collected information regarding their current oral hygiene practices including the frequency of brushing and oral hygiene aids used and existing caries risk factors (e.g. Fluoride exposure). A prospective 3 day dietary record was collected. The frequency of daily sugar exposure was calculated based on exposure to any sugar containing foods at or between meals. A caries risk assessment score was tabulated per individual, based on a caries risk questionnaire, incorporating the data on daily sugar exposure. All forms for data collection can be found in Appendix 4.
11. **Statistics**

Student’s t test was used to analyze differences in caries scores between the genotypes. Student’s t tests were also used to analyze the differences in age, number of teeth present, body mass index, frequency of sugar exposure, caries risk assessment scores, DMFT, DMFT+Xray and ICDAS scores. Chi square analysis for gender distribution was conducted separately.

Assuming each genotype analysed had a caries-resistant and caries-risk variant, as a secondary analysis, subjects were divided into 4 groups based on combining both genotypes. This analysis was aimed at finding the possible compounded protective effect of the various SNPs. Table 7 below shows the possible permutations of genotypes. Mean caries scores were calculated and differences analyzed using ANOVA tests.

Table 7. Table of genotype permutations (GLUT2 and TAS1R2)

<table>
<thead>
<tr>
<th></th>
<th>TAS1R2 polymorphism (risk)</th>
<th>TAS1R2 polymorphism (resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT2 polymorphism (resistant)</td>
<td>Resistant/risk</td>
<td>Resistant/resistant</td>
</tr>
<tr>
<td>GLUT2 polymorphism (risk)</td>
<td>Risk/risk</td>
<td>Risk/resistant</td>
</tr>
</tbody>
</table>
12. Results

90 Caucasian adults responded with interest and 10 were further excluded from analysis. Reasons for exclusion included recent antibiotic course (n=2), medical conditions (n=2), born and raised outside of Canada, (n=5) and incomplete genotyping data (n=1). There were more females (n=56) than males (n=24), with a mean age of 26.4±2.6 years old (range: 21-31yrs). Demographic information and the distribution of genotypes are shown in Tables 8 and 9. Val carriers of the TAS1R2 genotype represent both Ile/Val and Val/Val variants.

Distribution of study population

![Distribution of study population](image)

Figure 2. Distribution of study population and excluded participants
Age and Gender Distribution

Age range: 21-32 yrs old    Mean age: 26.4 ± 2.8 yrs old

Figure 3. Demographic data of study population

Distribution of genotypes

No polymorphism    Gene polymorphism present

Figure 4. Distribution of GLUT2 and TAS1R2 genotypes
 Analysis of the data showed a significant increase in DMFT scores (mean ± SEM) (6.1±1.24 vs. 4.3±0.43, p=0.04) in carriers of the Ile allele of GLUT2. (Table 8 and Figure 5) Carriers of the TAS1R2 polymorphism consistently demonstrated lower caries scores: DMFT (4.1±0.47 vs. 5.8±0.89, p=0.05), DMFT+radiographs (4.9±0.57 vs. 7.5±0.91, p=0.01), and ICDAS (19.5±2.15 vs. 26.1±2.82, p=0.03). (Table 9 and Figure 6) There was no significant difference in BMI in the GLUT2 group but a significant difference was detected in the TAS1R2 group. (Table 8 and Table 9) No statistically significant results were found in the other variables. (age, number of teeth, gender, caries risk assessment scores and frequency of daily sugar exposure)

Based on our findings, possessing the TAS1R2 polymorphism, and lacking the GLUT2 polymorphism had a caries-resistant effect on caries scores separately. We explored the possibility that possessing the 2 separate resistant SNPs would have an added protective effect over caries scores. Based on genotype stratification (Table7), caries scores of the combined Risk/Risk group exceeded the mean caries scores of the single at-risk genotypes alone (i.e. GLUT2 – Thr/Ile or TAS1R2 – Ile/Ile). The disparity in caries scores was greater when the Risk/Risk group was compared to the Resistant/Resistant group, demonstrating a possible compounded protective effect of having both resistant polymorphisms. All groups had significantly lower caries scores when compared to the Risk/Risk group. Only 1 caries score remained statistically insignificant (ICDAS scores of Resistant/Risk against Risk/Risk). These results are displayed graphically in Figure 7.
Table 8. Comparison of subject characteristics by GLUT 2 genotype

<table>
<thead>
<tr>
<th></th>
<th>GLUT 2 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thr/Thr</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>56</td>
</tr>
<tr>
<td>Age</td>
<td>26.2 ± 0.35 yrs</td>
</tr>
<tr>
<td>Gender – Female</td>
<td>36</td>
</tr>
<tr>
<td>– Male</td>
<td>20</td>
</tr>
<tr>
<td>No. of teeth</td>
<td>28.8 ± 0.19</td>
</tr>
<tr>
<td>Frequency of daily sugar exposure</td>
<td>1.8 ± 0.13</td>
</tr>
<tr>
<td>Caries Risk Assessment score</td>
<td>10.3 ± 0.40</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>23.6 ± 1.80</td>
</tr>
<tr>
<td>DMFT</td>
<td>4.3 ± 0.43</td>
</tr>
<tr>
<td>DMFT + Xray</td>
<td>5.7 ± 0.54</td>
</tr>
<tr>
<td>ICDAS score</td>
<td>21.6 ± 1.75</td>
</tr>
</tbody>
</table>

Mean±SEM (for all such values)

Comparison of caries risk scores

Fig 5. Comparison of caries scores of participants based on GLUT2 polymorphism
<table>
<thead>
<tr>
<th>No. of subjects</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender – Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of teeth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of daily sugar exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caries Risk Assessment score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMFT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMFT + Xray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICDAS score</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SEM (for all such values)

**Comparison of caries risk scores**

<table>
<thead>
<tr>
<th>Caries risk scores</th>
<th>No TAS1R2 polymorphism</th>
<th>TAS1R2 Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMFT</td>
<td>5.8 ± 0.87</td>
<td>7.5 ± 0.91</td>
</tr>
<tr>
<td>DMFT + Xray</td>
<td>4.1 ± 0.75</td>
<td>4.9 ± 0.87</td>
</tr>
<tr>
<td>ICDAS</td>
<td>26.1 ± 2.82</td>
<td>19.5 ± 2.15</td>
</tr>
</tbody>
</table>

*p values*  

<table>
<thead>
<tr>
<th>p values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>0.05</em></td>
<td></td>
</tr>
<tr>
<td><em>0.01</em></td>
<td></td>
</tr>
<tr>
<td><em>0.03</em></td>
<td></td>
</tr>
</tbody>
</table>

Fig 6. Comparison of caries scores of participants based on TAS1R2 polymorphism
Table 10. Table of genotype permutations (GLUT2 and TAS1R2)

<table>
<thead>
<tr>
<th></th>
<th>No TAS1R2 polymorphism (risk)</th>
<th>TAS1R2 polymorphism (resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No GLUT2 polymorphism (resistant)</td>
<td>Resistant/risk</td>
<td>Resistant/Resistant</td>
</tr>
<tr>
<td>GLUT2 polymorphism (risk)</td>
<td>Risk/risk</td>
<td>Risk/Resistant</td>
</tr>
</tbody>
</table>

Table 11. Comparison of subject characteristic by genotype-combinations

<table>
<thead>
<tr>
<th>Genotypes (GLUT2 genotype, TAS1R2 genotype)</th>
<th>GLUT2 Thr/Thr carriers</th>
<th>GLUT2 – Thr/Ile carriers</th>
<th>GLUT2 – Thr/Thr carriers</th>
<th>GLUT2 – Thr/Ile carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects</td>
<td>30</td>
<td>14</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>DMFT</td>
<td>4.2 ±0.01 a</td>
<td>4.0 ± 0.02 f</td>
<td>4.5 ± 0.01 g</td>
<td>9.1 ± 0.08 a,f,g</td>
</tr>
<tr>
<td>DMFT+Xray</td>
<td>5.2 ± 0.01 b</td>
<td>4.4 ± 0.02 e</td>
<td>6.3 ± 0.01 h</td>
<td>10.5 ± 0.07 b,e,h</td>
</tr>
<tr>
<td>ICDAS</td>
<td>19.9 ± 0.01 c</td>
<td>18.7 ± 0.10 d</td>
<td>23.5 ± 0.02</td>
<td>32.9 ± 0.22 c,d</td>
</tr>
</tbody>
</table>

Mean±SEM (for all such values)
Note: each matched alphabet shows a significant difference in the pairs (p<0.05)

Comparison of caries risk scores – genotypes combined

* represents a significant difference when compared to GLUT2=Thr/Ile, TAS1R2=Ile/Ile

Fig 7. Comparison of caries risk scores of participants based on combined genotypes
13. Discussion

This study is among the first to demonstrate evidence for the association of taste genes with dental caries in a young adult population. Participants with the Thr/Ile variation in GLUT 2 had a significantly higher DMFT caries score while all 3 caries scores of individuals with the Ile/Val variation in TAS1R2 were significantly lower. Previous nutritional studies provide a possible biological explanation for our results.

The subset of the population who rate bitter compounds such as 6-n-propylthiouracil (PROP) as intensely bitter are referred to as supertasters. Studies show that supertasters dislike sweet and fatty foods and even in childhood, register a significantly lower body mass index (BMI) as compared to their nontaster counterparts. (Hedge & Sharma, 2008b) This was further reflected in caries scores, with supertasters having a significantly lower caries score. (Hedge & Sharma, 2008b; B. P. Lin, 2003; Rupesh & Nayak, 2006b) It is believed that supertasters are able to better perceive the sweet or bitter taste of foods, and hence, may have a selective preference for foods containing less sugar as compared to nontasters. (B. P. Lin, 2003; Rupesh & Nayak, 2006b) This explanation has been verified anatomically – supertasters have a higher density of fungiform papillae and taste receptors on the anterior portion of the tongue than nontasters. (Bartoshuk et al., 1994)

The GLUT2 gene is involved in regulation of postprandial glucose levels. GLUT 2 nutritional studies have shown the association of this polymorphism with a significant daily increase in sugar consumption. (K. M. Eny et al., 2008) With reduced function and impaired glucose sensing, carriers of the GLUT2 polymorphism may require this increased daily sugar intake to signal fullness. (K. M. Eny et al., 2008) This genetically predetermined ingestive behavior may hence translate to a possible altered selective preference for and increased
consumption of sugar containing foods, contributing to the increased caries scores demonstrated in our study.

TAS1R2 is responsible for one’s sensitivity to the sweet taste. Available nutritional research points to a decrease in daily sugar consumption in overweight individuals possessing the TAS1R2 polymorphism but not in lean individuals. (K. M. Eny et al., 2010) It is probable that overweight individuals with the TAS1R2 polymorphism have a lower sensitivity to the sweet taste and hence a less acquired preference for sugar-containing foods. A post-ingestive mechanism has been proposed as well (K. M. Eny et al., 2010) due to their expression in the gastrointestinal tract, pancreas and hypothalamus. (K. M. Eny et al., 2010) In our study, we demonstrated the caries resistant effect of the TAS1R2 polymorphism over dental caries. Given the available evidence, it is plausible that this genetically predetermined dietary preference could result in decreased caries scores.

An interesting relationship we explored was whether an added caries-resistant effect exists when both resistant GLUT2 and TAS1R2 variants are present. Our preliminary analysis suggests that possessing both caries-resistant genotypic variants leads to a decreased caries experience. Similarly, possessing both caries-risk genotypic variants led to an increased caries experience. This reveals a possible compounding effect of specific taste genes and how they collectively contribute to an individual’s overall genetic caries susceptibility. This finding has important implications for future research into genetic susceptibility and integration of combined genetic predispositions into potential caries prediction models.

Body mass index (BMI) was statistically increased in the group with the TAS1R2 polymorphism (mean ± SEM) ( 24.0 ± 0.51 versus 22.6 ± 0.64, p=0.05). Health Canada’s
guidelines classify a BMI of 18.5-24.9 under the normal weight category. (Health Canada, 2005) Hence, this statistically significant difference has little clinical significance. There was no statistically significant difference in BMI with regards to the GLUT2 groups, with the mean BMI of our study population standing at 23.4 ± 0.40 (mean ± SEM) We thus concluded that our study population was a lean cohort. Eny et al. 2010 (K. M. Eny et al., 2010) demonstrated a difference in daily sugar exposure in the overweight group only. Amidst being a lean cohort and a lack of difference in daily sugar consumption, there was still a significant difference in caries scores in the TAS1R2 group. This suggests that the TAS1R2 genotype remained an independent variable significantly related to caries scores. This was not sufficiently substantiated by a dietary assessment, pointing to the disadvantage and inadequacy of relying on dietary records alone to detect at risk groups. Consequently, it highlights the sensitivity of the test for the TAS1R2 genetic predispositions.

A study by Wendell et al 2010 (Wendell et al., 2010) demonstrated no significant difference in caries scores based on TAS1R2 genotype in the permanent dentition. The TAS1R2 SNPs they studied were rs4920566 and rs9701796, which differed from the TAS1R2 SNP selected for this study (rs35874116). Hence, we are not able to confirm their specific findings.

Amidst very promising results, our study has a few limitations.

1. Non random sample
2. Limited sample size
3. Unequal gender distribution
4. Narrow age range
Our study was designed in a Caucasian only sample even though it limits the applicability of our results to the general population. However, based on the varying frequencies of SNPs across populations and the knowledge of the increased prevalence of the GLUT 2 polymorphism in Caucasians, limiting the study to a single race would limit false results. Sampling all the races at this early stage may dilute the effect under investigation. The predominantly female population was a function of the study population of the Toronto Nutrigenomics and Health Study, which we obtained our sample from. Although all participants were contacted, only data from 80 individuals were available for analysis. These drawbacks led to an underpowered study, whose power could only be ascertained retrospectively given the lack of preceding published data on this topic. (See below table 12)

<table>
<thead>
<tr>
<th>Power</th>
<th>Ideal sample (n)</th>
<th>Power</th>
<th>Ideal sample(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on GLUT 2</td>
<td></td>
<td>Based on TAS1R2</td>
<td></td>
</tr>
<tr>
<td>- DMFT</td>
<td>53%</td>
<td>- DMFT</td>
<td>52%</td>
</tr>
<tr>
<td>- DMFT+xray</td>
<td>25%</td>
<td>- DMFT+xray</td>
<td>80%</td>
</tr>
<tr>
<td>- ICDAS</td>
<td>18%</td>
<td>- ICDAS</td>
<td>59%</td>
</tr>
</tbody>
</table>

*Ideal sample calculated based on assuming 80% power, α=0.05

Table 12. Post-hoc power and sample size analysis (IBM SPSS Sample Power v3.0)

Using IBM SPSS Sample Power v3.0, we found that the power of our study varied depending on the type of caries score. For example, the power of detecting a significant difference in caries experience was 53%, based on DMFT values in the GLUT2 group, while the power reached 80% based on DMFT+Xray values in the TAS1R2 group. This was in part due to the differing caries scores and distribution of genotype in each category. In our study, 30% of the individuals possessed the GLUT2 polymorphism (an uneven distribution) while 55% possessed the TAS1R2 polymorphism which may be a contributing factor to the lower powered GLUT2 analysis.
Calibration of the single examiner was omitted in the study design. This could have led to variability and error in the recorded caries scores. However, this examiner was blinded to the genotype of all participants during the investigation thus reducing the chances of bias and systematic errors. Moreover, there was a difficulty in recruiting a suitable candidate well versed in the ICDAS system to act as the gold standard. Nomination of a gold standard examiner for calibration would have had little added benefit as studies have shown the deficiency in inter-examiner reliability when both examiners are inexperienced in ICDAS. (Diniz et al., 2009) However, to capitalize on the ICDAS’ classification of the whole caries spectrum, cavitated and non-cavitated, it was decided to supplement the study with the classic DMFT index. A measure of internal consistency of the single examiner could be reflected in the agreement in caries risk scores by the 3 distinct methods that yielded similar inferences in our study.

In our study, we found a significant difference in all caries scores in the TAS1R2 group and only DMFT values in the GLUT2 group. No significant differences were found in the GLUT2 group based on DMFT+Xray and ICDAS values, however this could be attributed to the low power of these 2 calculations (Power : DMFT+Xray 25%, ICDAS 18%).

It is clear that genetic association studies ideally involve a large sample size to obtain representative data of a given population. However, this study importantly verifies the specific genetic contributions to dental caries experience in an adult Caucasian population and facilitates the estimate of a desired sample size for a future adequately powered study (80% power, \( \alpha=0.05 \)) to verify our findings.
An ideal construct for a study of genetic association with disease is reflected in a current large scale GWAS study that is part of the Gene Environment Association Studies initiative (GENEVA), funded by the National Institute of Health. The availability of funding for greater sampling as well as the collaboration of multiple centres greatly add to the robustness of the results. Amidst the limitations of this study, our results play an important role in providing a novel insight into the functional significance of these genetic variants in young adults. Further studies targeting children with early childhood caries and validation of the current results in a pediatric population are needed.

The global interest in microbiology is palpable. The Human Microbiome Project is a National Institutes of Health initiative aimed at characterizing the various microbial communities in the human body e.g. nasal passages, gastrointestinal tract and the oral cavity. (Division of Program Coordination, Planning, and Strategic Initiatives, National Institutes of Health, 2011) Once characterized, it will be interesting to use our knowledge of individual genetic diversity to understand the genetic predispositions to oral microbial colonization and the effect on dental caries development.

Admittedly, we have a good understanding of the multitude of factors contributing to dental caries. As we continue to deepen our understanding of the genetic basis of dental caries, we ought to look at how this information can translate to improvement in patient care. At present, we are unable to control or change tooth formation genes e.g. AMELX. Fortunately, diet is considered a modifiable risk factor and the suggestion that genetic variation may account for individual ingestive behaviours makes personalised nutritional counselling an integral part of the preventive dental approach.
In our study, a 3 day dietary record was used to record frequencies of sugar exposure. This prospective record has an advantage of independence of memory recall, where people may tend to over or underestimate their food intake. The disadvantage is its main focus is on the frequency of sugar exposure and not quantitative intake. The food frequency questionnaire (FFQ) on the other hand, is better able to provide a detailed breakdown of the individual’s caloric intake or nutrient (e.g. protein, fat, carbohydrates) profile. This may explain why previous GLUT2 and TAS1R2 studies (K. M. Eny et al., 2008; K. M. Eny et al., 2010) that utilised a food frequency questionnaire (FFQ) demonstrated a significant difference in quantitative sugar intake, but this relationship was not apparent in this study. This may suggest that a FFQ may provide an added advantage in initial dietary assessment in dentistry, when attempting to identify areas that warrant improvement.

In addition, current dietary counselling done in dentistry has a tendency to focus on the cariogenicity of foods. Substituting sugary snacks with foods containing artificial sweeteners to solve the issue may sound like a logical solution, however, research has shown that a biphasic sustained increased hunger may ensue instead.(Tordoff & Alleva, 1990) When tailoring dietary changes to each individual, the counsellor needs to consider the myriad of factors that come into dietary choices. These include cultural factors, socioeconomic factors, energy demands and now genetic factors. In particular, genetic variants may affect the ability of an individual to adopt dietary changes. Only by taking all these into account can initial modifications be feasible and maintainable on the long term. Notably, these are more applicable to the adult population as they can make concerted efforts to make dietary modifications. Children, however, are less cognisant of their dietary choices (McClain et al., 2009) and hence genotypic effects may have a bearing on success of diet counselling. In addition, parental practices and early exposure to flavours do appear to shape infants’
acceptability and preferences (Birch, 1998; Mennella et al., 2001) although studies conducted over a short experimental period have shown otherwise. (Liem & Mennella, 2002; Liem & de Graaf, 2004; Wurtman & Wurtman, 1979) This is an area worth exploring clinically—whether the early modification of the child’s “Sweet Tooth” can modulate the lifelong sweet preference.
14. Conclusions

Our study demonstrates the influence of specific genetic variations in the GLUT2 and TAS1R2 genes on overall caries risk in an adult population and suggests a possible compounded protective effect of having both resistant genetic polymorphisms. These findings have important implications for future research into genetic susceptibility and integration of combined genetic predispositions into caries prediction models. Future studies using the genome-wide association study (GWAS) approach to identify other polymorphisms associated with caries risk and validation of the current results in a pediatric population afflicted with early childhood caries are still needed.
15. **Appendices**

15.1 Appendix 1 ICDAS Decision Number 2

15.2 Appendix 2 ICDAS chart

15.3 Appendix 3 Forms for data collection

15.4 Appendix 4 Research Ethics Board Approval
International Caries Detection and Assessment System
Decision Number 2: classification of the carious status based on ICDAS

Sound tooth surface: Code 0
There should be no evidence of caries (either no or questionable change in enamel translucency after prolonged air drying (suggested drying time 5s)). Surfaces with developmental defects such as enamel hypoplasias; fluorosis; tooth wear (attrition, abrasion and erosin), and extrinsic or intrinsic stains will be recorded as sound. The examiner should also score as sound a surface with multiple stained fissures if such a condition is seen in other pits and fissures, a condition which is consistent with noncarious bumbs (e.g. frequent tea drinking).

First visual change in enamel: Code 1
Code 1: Pits and fissures
When seen wet there is no evidence of any change in colour attributable to carious activity, but after prolonged air drying (approximately 5s is suggested to adequately dehydrate a carious lesion in enamel) a carious opacity or discoloration (white or brown lesion) is visible that is not consistent with the clinical appearance of sound enamel.

Or
When there is a change in colour because of caries which is not consistent with the clinical appearance of sound enamel and is limited to the confines of the pit and fissure area (whether seen wet or dry). The appearance of these carious areas is not consistent with that of stained pits and fissures as defined in code 0.

Code 1: Smooth tooth surfaces
When seen wet there is no evidence of any change in colour attributable to carious activity, but after prolonged air drying a carious opacity (white or brown lesion) is visible that is not consistent with the clinical appearance of sound enamel. This will be seen from the buccal or lingual surface.

Distinct visual change in enamel: Code 2
The tooth must be viewed wet. When wet, there is a (i) carious opacity (white spot lesion) and/or (ii) brown carious discoloration which is wider than the natural fissure/fossa that is not consistent with the clinical appearance of sound enamel. (Note: the lesion must still be visible when dry)

Localized enamel breakdown because of caries with no visible dentine or underlying shadow: Code 3
The tooth viewed wet may have a clear carious opacity (white spot lesion) and/or brown carious discoloration which is wider than the natural fissure/fossa that is not consistent with the clinical appearance of sound enamel. Once dried for approximately 5s there is carious loss of tooth structure at the entrance to, or within, the pit of fissure/fossa. This will be seen visually as evidence of demineralization [opaque (white), brown or dark brown walls] at the entrance to or within the fissure or pit, and although the pit or fissure may appear substantially and unnaturally wider than normal, the dentine is not visible in the walls or base of the cavity/discontinuity.

If in doubt, or to confirm the visual assessment, the WHO/CPI/PSR probe can be used gently across a tooth surface to confirm the presence of a cavity apparently confined to the enamel. This is achieved by sliding the ball end along the suspect pit or fissure and a limited discontinuity is detected if the ball drops into the surface of the enamel cavity/discontinuity.

Underlying dark shadow from dentine with or without localized enamel breakdown: Code 4
This lesion appears as a shadow of localized breakdown (loss of continuity of the surface that is not showing the dentine). The shadow appearance is often seen more easily when the tooth is wet. The darkened area is an intrinsic shadow which may appear as grey, blue or brown in color. The shadow must clearly represent caries that started on the tooth surface being evaluated. If in the opinion of the examiner, the carious lesion started on an adjacent surface and there is no evidence of any caries on the surface being scored than the surface should be coded ‘0’.

Distinct cavity with visible dentine: Code 5
Cavitation in opaque or discoloured enamel exposing the dentine beneath. The tooth viewed wet may have darkening of the dentine visible through the enamel. Once dried for 5s there is visual evidence of loss of tooth structure at the entrance to or within the pit and fissure – frank cavitation. There is visual evidence of demineralization [opaque (white), brown or dark brown walls] at the entrance to or within the pit and fissure and in the examiner judgement dentine is exposed.

The WHO/CPI/PSR probe can be used to confirm the presence of a cavity apparently in dentine. This is achieved by sliding the ball end along the suspect pit or fissure and a dentine cavity is detected if the ball enters the opening of the cavity and in the opinion of the examiner the base is in dentine. (In pits and fissures the thickness of the enamel is between 0.5 and 1.0mm. Note the deep pulpal dentine should not be probed.)

Extensive distinct cavity with visible dentine: Code 6
Obvious loss of tooth structure, the cavity is both deep and wide and dentine is clearly visible on the walls and at the base. An extensive cavity involves at least half of a tooth surface or possibly reaching the pulp.
Clinical investigation:
A retrospective clinical study to investigate the genetic basis of dental caries based on the existence of a genetic polymorphism.

Principal Investigator:
Dr Gajanan Kulkarni, Associate Professor, Pediatric and Preventive Dentistry. Tel: 416-979-4929 x4460

Student investigator:
Dr. Tabitha Chng, M.Sc.-Pediatric Dentistry candidate

Co-investigators:
Dr. Hardy Limeback, Professor, Preventive Dentistry
Dr. Ahmed El-Sohemy, Associate Professor, Nutritional Sciences

Purpose:
To investigate the association of a genetic polymorphism with the manifestation of dental caries in the permanent dentition.

Description:
Results of your genotype (whether or not you have the genetic polymorphism) will be obtained from the earlier study you participated in. The genotyping study will not be repeated.

A dental examination similar to a regular check-up at the dentist will be conducted. We will examine your teeth, do a cleaning for you and take 2 radiographs (X-rays). In addition, a salivary test will be conducted. You will be required to chew on paraffin pellets for 5 minutes to aid in the collection of the saliva that will be used to count the levels of two types of bacteria in your mouth. Lastly, you will need to fill out a questionnaire and a diet history record.

Potential harms, injuries or inconvenience

There is no foreseeable harm or injury as a result of participating in this study. The inconvenience lies in having to come to the Faculty of Dentistry for the examination to be conducted and the time spent in collecting the needed information.

Potential benefits:

A dental examination has the potential benefit of early detection of dental disease which may be managed thereafter at the Faculty of Dentistry or any other dental clinic. You will get copies of the X-rays if you wish. Should any oral or dental problems be identified by the examiner, you will be notified of your options regarding follow up and treatments.
Duration of study:

Only a one-time participation is required to complete the clinical examination which should not take more than 30 minutes.

Confidentiality:

Confidentiality will be respected and no information that discloses your identity will be released or published without consent unless required by law. For your information, the research consent form will be inserted in the patient health record.

Participation:

Participation in this study is completely voluntary. You may choose to withdraw from this study at any point of time without provision of a reason.

Consent:

I acknowledge that the research procedures described above have been explained to me and that any questions that I have asked have been answered to my satisfaction.

I have been informed of the right not to participate and the right to withdraw from this study at any given point of time without provision of a reason.

The potential harms and discomforts have been explained to me. I understand the benefits (if any) of participating in the research study. I know that I may ask now, or in the future, any questions I have about the study or the research procedures.

I have been assured that records collected in this study will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law.

I, __________________________ hereby consent to participate in the above mentioned study

_____________________________________

Signature / Date

_____________________________________

Name / Signature of person who obtained consent
Invitation to participate
Uncovering the genetic link of dental caries

Dear

I am a graduate student in the Faculty of Dentistry at the University of Toronto. I am conducting a research study to investigate the genetic basis of dental caries, based on an earlier study which you have participated in.

Results from the first study will be obtained. In addition, a dental examination similar to a regular check up at the dentist will be conducted. You will be provided a free professional dental cleaning followed by a free examination of your teeth. We will obtain 2 radiographs (X-rays). In addition, a simple salivary (spit) test will be conducted, which requires chewing on paraffin pellets for 5 minutes. The saliva collected will allow us to count the levels of bacteria present. Lastly, you will need to fill out a short 1-2 minute questionnaire and a diet history record. Only one visit is required.

There is no foreseeable harm or injury that could result from participation in this study.

A dental examination has the potential benefits of early detection of any dental disease which may be managed thereafter at the Faculty of Dentistry or any other dental clinic. Copies of the radiographs taken will be given to you if you wish to have them. Should any oral or dental problems be identified, you will be notified at the same time.

Participation is strictly confidential. All study information will be kept in a secure location at the University of Toronto, Faculty of Dentistry. The results of the study may be published or presented at meetings. We respect your privacy and your identity will not be revealed.

You will receive $20 to reimburse you for your time and travel expenses. The dental examination, X-rays and cleaning is free but valued at approximately $150.

Participation in this study is completely voluntary. You may choose to withdraw from this study at any given time without provision of a reason.

If you have any questions about this study, you may contact me at tabitha.chng@utoronto.ca or my faculty advisor Dr Gajanan Kulkarni, 416-979-4929 ext 4460, G.Kulkarni@dentistry.utoronto.ca .

Your participation is kindly appreciated. If you would like to participate, please contact our research coordinator Erica Day Tasevski, 416-978-6461, nutrition.genetics@utoronto.ca .

Regards,
Taby
Dr Tabitha Chng
M.Sc Pediatric Dentistry candidate
tabitha.chng@utoronto.ca
# Caries risk assessment

## History (determined by interviewing the patient)

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Risk indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>High (=2)</td>
</tr>
<tr>
<td></td>
<td>Moderate (=1)</td>
</tr>
<tr>
<td></td>
<td>Low (=0)</td>
</tr>
<tr>
<td>Last dental visit</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Never smoked before</td>
</tr>
<tr>
<td></td>
<td>Never or only when symptomatic</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>Regular, within the past year</td>
</tr>
<tr>
<td>Presence of dental decay</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Presence of intra-oral appliances</td>
<td>Yes (What appliance is it?)</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Daily between meal exposures to sugars/</td>
<td>≥3</td>
</tr>
<tr>
<td>carbohydrates</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td>Meal time only</td>
</tr>
<tr>
<td>Daily exposure to fluoride</td>
<td>Does not use fluoridated toothpaste; drinking water</td>
</tr>
<tr>
<td></td>
<td>is not fluoridated and is not taking fluoride</td>
</tr>
<tr>
<td></td>
<td>supplements</td>
</tr>
<tr>
<td></td>
<td>Uses fluoridated toothpaste; usually does not drink</td>
</tr>
<tr>
<td></td>
<td>fluoridated water and does not take fluoride</td>
</tr>
<tr>
<td></td>
<td>supplements</td>
</tr>
<tr>
<td></td>
<td>Uses fluoridated toothpaste; drinks fluoridated</td>
</tr>
<tr>
<td></td>
<td>water or take fluoride supplements</td>
</tr>
<tr>
<td>Daily brushing frequency</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Daily flossing frequency</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
</tr>
<tr>
<td></td>
<td>≥1</td>
</tr>
</tbody>
</table>

## Clinical evaluation (determined by clinical examination)

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingivitis</td>
<td>Severe (&gt;50%)</td>
<td>Moderate (30-50%)</td>
</tr>
<tr>
<td>Decalcification spots</td>
<td>&gt;1</td>
<td>1</td>
</tr>
</tbody>
</table>

## Supplemental professional assessment

<table>
<thead>
<tr>
<th>Radiographic assessment of caries</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated salivary flow rate</td>
<td>&lt;0.5ml/min</td>
<td>0.5-0.7ml/min</td>
</tr>
<tr>
<td>Mutans Streptococci CFU/ml</td>
<td>&gt;1x 10⁶</td>
<td>1x 10⁵-1x 10⁶</td>
</tr>
<tr>
<td>Lactobacilli CFU/ml</td>
<td>&gt;1x 10⁴</td>
<td>1x 10³-1x 10⁴</td>
</tr>
</tbody>
</table>

*adapted from AAPD caries assessment tool 2009 and University of Toronto - Preventive Dentistry caries risk and preventive needs assessment form*
No. _____________

<table>
<thead>
<tr>
<th>Country of Origin</th>
<th>Ethnicity</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Are you currently in good health?</th>
<th>Y / N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever been hospitalized?</td>
<td>Y / N</td>
</tr>
<tr>
<td>Do you take any medication?</td>
<td>Y / N</td>
</tr>
<tr>
<td>Have you taken any antibiotics in the last 2 weeks?</td>
<td>Y / N</td>
</tr>
<tr>
<td>Have you ever received radiotherapy to the head and neck region?</td>
<td>Y / N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you have any problems related to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular system</td>
</tr>
<tr>
<td>Respiratory system</td>
</tr>
<tr>
<td>Central nervous system</td>
</tr>
<tr>
<td>Immune system</td>
</tr>
<tr>
<td>Gastrointestinal system</td>
</tr>
<tr>
<td>Endocrine system</td>
</tr>
<tr>
<td>Hematology</td>
</tr>
<tr>
<td>Genitourinary system</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is the source of your drinking water?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap in Ontario / bottled water / others</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Last visit to the dentist ( reason )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brushing frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>___1x / 2x / 3x / &gt;3x</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flossing</th>
<th>Mouthwash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y / N</td>
<td>Y / N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Others dental aids</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Dental Insurance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y / N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Any known dental defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y / N</td>
</tr>
</tbody>
</table>
Loe and Silness Plaque Index 1964

0 = no plaque in the gingival area
1 = a film of plaque adhering to free gingival margin and adjacent area of tooth. Plaque seen w probe
2 = moderate accumulation soft deposits within gingival pocket and on margin, tooth with naked eye
3 = abundance of soft matter within gingival pocket and/or gingival margin and adj tooth

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Buccal</th>
<th>Lingual</th>
<th>Mesial</th>
<th>Distal</th>
<th>Average / tooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12</td>
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<td>36</td>
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<tr>
<td>32</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>44</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Average PI score for patient

Loe and Silness Gingival Index 1963

Score 0 = Absence of inflammation
Score 1 = Mild inflammation; slight change in colour and little change in texture.
Score 2 = Moderate inflammation; moderate glazing, redness, edema and hypertrophy; bleeding on pressure.
Score 3 = Severe inflammation; marked redness and hypertrophy; tendency towards spontaneous bleeding; ulceration.

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Mesio-buccal papillae</th>
<th>Buccal</th>
<th>Disto-buccal</th>
<th>Lingual</th>
<th>Average / tooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12</td>
<td></td>
<td></td>
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<td>24</td>
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<td>36</td>
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<td>32</td>
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<tr>
<td>44</td>
<td></td>
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</tr>
</tbody>
</table>

Average GI score for patient
Appendix 4

PROTOCOL REFERENCE #: 25621

October 26, 2010

Dr. Gajanan Kulkarni
Faculty of Dentistry
University of Toronto
124 Edward St.
Toronto, ON M5G 1G6

Dr. Tabitha Chng
Faculty of Dentistry
University of Toronto
124 Edward St.
Toronto, ON M5G 1G6

Dear Dr. Kulkarni and Dr. Chng:

Re: Your research protocol entitled, "Are individuals with certain "Sweet Tooth" gene polymorphisms more prone to dental caries?"

ETHICS APPROVAL

Original Approval Date: October 26, 2010
Expiry Date: October 25, 2011
Continuing Review Level: 1

We are writing to advise you that a member of the Health Sciences Research Ethics Board has granted approval to the above-named research study, for a period of one year. Ongoing projects must be renewed prior to the expiry date.

All your most recently submitted documents have been approved for use in this study.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events should be reported to the Office of Research Ethics as soon as possible.

Please ensure that you submit an Annual Renewal Form or a Study Completion Report 15 to 30 days prior to the expiry date of your study. Note that annual renewals for studies cannot be accepted more than 30 days prior to the date of expiry, as per federal and international policies.

If your research has funding attached, please contact the relevant Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your project.

Yours sincerely,

Daniel Gyewu
Research Ethics Board Manager - Health Sciences
16. References


Caries Research, 27(5), 431-437.


International Caries Detection and Assessment System Coordinating Committee. (2005b). Rationale and evidence for the international caries detection and assessment system (ICDAS II).


