

**PUBLIC HEALTH GENETICS AND DENTAL CARIES:
A SUMMARY OF CURRENT RESEARCH AND A PROPOSED PUBLIC HEALTH
INTERVENTION**

by

Kathleen Deeley

BS, University of Pittsburgh 2004

Submitted to the Graduate Faculty of
the Department of Human Genetics,
Graduate School of Public Health in partial fulfillment
of the requirements for the degree of
Master of Public Health

University of Pittsburgh

2013

UNIVERSITY OF PITTSBURGH
GRADUATE SCHOOL OF PUBLIC HEALTH

This essay is submitted

by

Kathleen Deeley

on

August 10th 2013

and approved by

Essay Advisor:

Candace M. Kammerer, BS, PhD

Associate Professor

Human Genetics

Graduate School of Public Health

University of Pittsburgh

Essay Reader:

Alexandre R. Vieira, DDS, MS, PhD

Associate Professor

Oral Biology

School of Dental Medicine

University of Pittsburgh

Copyright © by Kathleen Deeley

2013

PUBLIC HEALTH GENETICS AND DENTAL CARIES:
A SUMMARY OF CURRENT RESEARCH AND A PROPOSED PUBLIC HEALTH INTERVENTION

Kathleen Deeley, MPH
University of Pittsburgh 2013

ABSTRACT

Dental caries is the most common oral disease worldwide. Caries is primarily caused by bacteria that colonize the mouth and breaks down sugars to produce an acid that de-mineralizes the surface of teeth. The resulting hole is called a carious lesion, and is the symptom used to diagnose this disease. Untreated caries provides an opportunity for other bacteria to infect the inner structure of the tooth, and potentially, get into the bloodstream. Caries is a multi-factorial disease that is affected by many host and environmental factors such as: diet, access to dental care, saliva composition, immune response, air quality, availability of fluoride, and genes.

Caries can be an expensive disease to treat. Since many people in the United States of America do not have dental insurance and public resources in this area are scarce, most of this cost comes directly from the consumer. The effects of untreated caries, particularly in children, can be severe. Tooth pain can lead to reduced food intake and loss of sleep. This can lead to poor nutrition and poor performance in school, stunting the child's physical and mental development. Teeth extracted due to caries can impair a child's ability to speak, as well as impact their sense of self-worth.

A focus on preventing caries will not only prevent these extreme outcomes, it will also help prevent more serious chronic conditions. Poor diet and malnutrition contribute to severe caries as well as heart disease, obesity, and diabetes. Preventing caries is of public health importance because it can have severe short and long term outcomes, it is a financial burden on healthcare consumers, and it is easily preventable. Genetic testing can play a role in preventing caries. If people know they have a higher susceptibility to caries they can try to mitigate their increased susceptibility by changing their diet or brushing more. If they know that they do not have a higher susceptibility, they may focus on other areas of their health.

TABLE OF CONTENTS

1.0 Introduction.....	1
1.1 Host-Related Factors.....	2
1.1.1 Dental Enamel.....	2
1.1.2 Tooth Morphology.....	2
1.1.3 Saliva.....	3
1.1.4 Immunology.....	3
1.1.5 Hygiene.....	4
1.1.6 Gender.....	4
1.2 Diet.....	5
1.3 Bacteria.....	5
1.4 Environment.....	6
1.4.1 Water Fluoridation.....	6
1.4.2 Air Quality and Asthma.....	7
1.4.3 Access to Care.....	8
1.5 Public Health Relevance.....	8
2.0 Genetics of Caries Research.....	9
2.1 Enamel Formation Genes.....	9
2.1.1 Case Control Studies.....	9
2.1.2 Tooth Cohort.....	10
2.2 Fine Mapping of Associated Regions.....	14
2.2.1 GWS.....	15
2.2.2 Fine Mapping.....	15
2.3 Detecting <i>S. mutans</i> in saliva.....	17
3.0 Application.....	21
Bibliography.....	23

LIST OF TABLES

Table 1: Allele p-values for SNPs in Five Study Population.....	12
Table 2: Significant Enamel Hardness Results, by Tooth Surface.....	13
Table 3: Summary of 5q13.3 Genotyping.....	16
Table 4: p-value for 14q11.2 Genotyping.....	17
Table 5: Results from <i>S. mutans</i> Real Time Detection Assay.....	19

LIST OF FIGURES

Figure 1: Surfaces of human teeth.....14

1.0 INTRODUCTION

Dental caries, commonly referred to as “dental cavities” in the US, is one of the most common sequela in the world, affecting 60% to 90% of children worldwide (1). Caries occurs when bacteria endogenous to the mouth break down sugars in the food we eat and produce an acid that de-mineralizes the surface of teeth, the enamel, producing small holes. If caries is not treated, bacteria can invade the inner structure of the tooth, the dentin, and cause an infection that can spread throughout the skull. In rare cases, these kinds of infections can cause death (2). Caries is a multi-factorial disease that is affected by many host and environmental factors. For instance, the morphology of the tooth will influence how easily it is colonized by bacteria. The amount of bacteria in the mouth is a result of diet, immune response, and saliva composition. Importantly, genes play a role in all of this (3). Non genetic factors, such as oral hygiene and access to dental care, will also be addressed in this essay.

The role of genetics in development of caries is well established. Over thirty years of studying monozygotic and dizygotic twins reared apart and together has shown that at least 40% of the risk for caries is genetic (4). The specific genes involved remain unclear. In the past 10 years more research has been done, by the Veira lab and others, into the contribution of genes involved in enamel formation to caries. Genes involved in tooth morphology and immune response are also promising areas of research (3).

Within this essay I will describe the various factors that influence an individual’s response to caries. Typically, to simplify these kinds of discussions, caries is examined as an intersection of 3 factors: host, diet, and bacteria. These factors exist within the context of other factors beyond the direct control of the individual, like water quality and access to dental care, which for the sake of this essay will represent the “environment.” I will also describe several projects I have participated in as manager of the Vieira Lab in the School of Dental Medicine at the University of Pittsburgh. Based on the data gathered from those projects and the available information on caries progression, I propose a public health intervention to reduce the prevalence of dental caries.

1.1 HOST-RELATED FACTORS

1.1.1 Dental Enamel

Several decades ago, individuals who never seemed to get cavities were said to have “strong teeth”, whereas individuals who were often afflicted with caries were said to have “soft teeth”. After the proliferation of fluoride toothpaste, this concept was abandoned in favor of judgments about hygiene. More recently, however, researchers have proposed that dental enamel strength may be one of the potential explanations for differences in caries susceptibility among individuals. One example is the genetic disorder Amelogenesis Imperfecta, a condition associated with pitting and thinning of the enamel, and subsequently, with a higher caries experience. This condition is caused by defects in the *amelogenin* and *enamelin* genes. Much of my early work in the lab of Dr. Vieira in the School of Dental Medicine at the University of Pittsburgh has focused on genes involved in enamel formation: *amelogenin*, *ameloblastin*, *enamelin*, *tuftelin 1*, and *tuftelin interacting protein 11*. All of these genes play different roles in the development and organization of the cells that become teeth.

Amelogenin (AMELX) is involved in the formation of the enamel surface and makes up 90% of the enamel matrix in humans. *Enamelin (ENAM)* is the next most common protein. Mutations in either of these genes can lead to enamel defects, called hypoplasias, that can make the enamel surface easier for bacteria to colonize (5). *Ameloblastin (AMBN)* is also present in the enamel matrix, but its role is more about stabilizing the crystalline structure of the enamel matrix and repairing early damage (6). *Tuftelin 1 (TUFT1)* is found in developing and mature enamel tissue, specifically at the junction between the enamel and the dentin of the tooth. It seems to be expressed very early in stages of mineralization, and may be involved in the differentiation of the two areas of the tooth (7). It also may play a role in nuclear cell signaling, as does *tuftelin interacting protein 11 (TFIP11)* (8).

1.1.2 Tooth Morphology

Investigating genes involved in enamel formation is a relatively recent occurrence (e.g., Deeley et al., 2008). Dental genetics has traditionally focused on genes affecting tooth morphology and position in the mouth (3). There are 4 main signaling pathways that affect the position teeth and general tooth

development: bone morphogenetic protein (BMP), fibroblast growth factor (FGF), sonic hedge hog (SHH), and wingless-type MMTV integration site (WNT). Products of the genes in this pathway have an impact on the enamel formation genes already mentioned. Alterations to the genes in these pathways are known to have a variety of effects, such as complete tooth loss, or the development of extra teeth (9). These developmental genes control when teeth emerge from the gums into the oral cavity and have an impact on genetic susceptibility to caries because teeth that erupt first have more exposure to factors in the oral environment that cause caries (10).

1.1.3 Saliva

One of the factors in the oral environment, saliva, does many things to help control caries. The amount and nature of saliva in the mouth is determined on a genetic level by a group of genes called aquaporins (11). Saliva helps create a neutral environment with a cycling carbonic acid/bicarbonate system. It allows for ion exchange during re-mineralization by providing phosphate and calcium to the teeth in a neutral pH for optimal reabsorption, a process helped by fluoride (12). It also helps to clean the oral cavity by diluting and washing away any acid produced by the fermentation of sugars by cariogenic bacteria. Saliva also raises the overall pH to a point where these bacteria cannot survive. The physical characteristics of saliva are also important. Having a low saliva flow rate or thick saliva means that that it does not cover all the teeth, it does not dilute anything, and in general, it is less effective in preventing caries.

1.1.4 Immunology

In general, individuals with immune deficiencies have a higher incidence of caries (3). Many studies have been done looking at the relationship between HLA types and caries. For example, there is a clear association between HLA-DR and enamel defects (13) and *Streptococcus mutans* antigens bind more strongly to certain HLA-DR types than others (14). Both of these observations are consistent with the possibility that HLA type influences caries susceptibility. However, there is no direct association between caries experience and HLA type (3). Another way the body responds to *S. mutans* infections on teeth is through the release of immunoglobulin A (IgA) (15). Higher levels of IgA have been noted in patients with low caries experience. In addition to creating antibodies, the body also produces anti-

microbial, like alpha and beta defensin (16) and lactotransferrin. Lactotransferrin in particular has been shown to destroy *S. mutans* in vitro (17). The amount of microbial or antibodies produced may be dependent on genetic expression, though more research in this area is needed.

1.1.5 Hygiene

Basic dental hygiene is considered by dentists to be: brushing ones teeth twice a day with fluoride toothpaste with an intact brush and flossing at least once a day with dental floss (18, 19). Though this may seem like common knowledge, a 2008 survey by the American Dental Association (ADA) showed that “1 in 5 Americans admits to not brushing twice a day” and 43% of people surveyed did not know when to replace their toothbrushes (20). Despite the proven benefits of fluoride some people choose to use more environmentally friendly alternatives, like sodium bicarbonate (baking soda) based mixes (21). Fluoride is effective because it inhibits de-mineralization while promoting re-mineralization, allowing for a more efficient response to caries inducing challenges (22). Sodium bicarbonate physically removes plaque and raises the pH of the oral environment, discouraging the growth of acid producing bacteria and facilitating re-mineralization (23). While oral rinses with a sodium bicarbonate solution has been recommended, the ADA does not officially advocate using just sodium bicarbonate to brush one’s teeth.

1.1.6 Gender

Gender also appears to have an impact on caries susceptibility because women, on average, have more caries than men (24). There are many possible explanations for this difference. First of all, the X chromosome carries the *amelogenin* gene that is critical in the development of enamel. There is a version of *amelogenin* on the Y chromosome, but it does not function the same. For example, mutations in X-linked *amelogenin* have a more severe phenotype than mutations in the Y-linked version. Also, women have lower rates of salivary flow, lessening the mechanical and chemical benefits of saliva on the teeth. The saliva in women’s mouths also has less immunoglobulin A (IgA), which has been shown to be protective against caries (25). Lastly, permanent teeth erupt earlier in women, leaving their teeth exposed to cariogenic agents in the oral cavity longer than those of men (10).

Hormones are also thought to play a role in the differential caries susceptibility between genders because women typically experience more caries during pregnancy (24). Animal studies have shown that an increase in estrogen in rats influences the thyroid gland and inhibits salivary flow, leading to more caries, whereas an increase in androgens has no effect (24). People with conditions that cause reduced salivary flow, such as hyperthyroidism, have been observed to have an increased amount of caries (24).

1.2 DIET

Ingestion of sugary foods contributes to caries development because monosaccharides, disaccharides, and polysaccharides are broken down into simple sugars that feed the bacteria that contribute to caries. Complex carbohydrates, such as: fiber, polyol-monosaccharides, polyol-disaccharides, and polyol-polysaccharides are not broken down as easily and do not influence caries progression (26). However, a diet high in carbohydrates may contribute to obesity, which is then associated with caries and tooth loss (27). This relationship between caries and obesity may simply be a correlation, in other words, a poor diet may lead to obesity as well as caries. Alternatively, there may be a causal relationship; that is, obesity may depress the immune system and thus increase the risk of developing more caries (28).

1.3 BACTERIA

Within the mouth many species of bacteria contribute to the development of caries. After brushing, various *Streptococci* species (collectively called mutans streptococci) begin to replicate and colonize teeth (29). They form the foundation for the plaque that grows on the enamel surface. As more food is ingested, acid-producing bacteria, such as *Streptococcus mutans* and *Streptococcus sobrinus*, begin to thrive and colonize the plaque. The longer the bacteria remain on the plaque, the more they grow and the more lactic acid they produce via the breakdown of sugars (29). This acid then attacks the

enamel, making a small hole that allows the acid to reach the inner dentin. At this point, a second group of bacteria, the *Lactobacillus* family, will divide and digest the dentin in the inner tooth. If the cavity is not repaired, the infection can spread through the roots and into the blood stream (29).

Most discussions of bacterial involvement with caries focus on *S. mutans*. However, *S. mutans* is not the only bacteria responsible for the breakdown of enamel (29). While it is more acid tolerant than its neighbors, other species of mutans streptococci have been observed to de-mineralize enamel, and the presence of *S. mutans* is not always directly connected to caries. How these different species interact is more important than how they function separately. For example, when there is one deep carious infection caused by *Lactobacillus* species, new lesions do not often form. This is because some *Lactobacillus* species, *L. rhamnosus* and *L. reuteri*, inhibit the growth of *S. mutans* (29).

People are not born with *S. mutans*; babies usually acquire these bacteria within the first or second year of life (30). In the majority of cases, babies acquire the bacteria via vertical transmission from their mother. Sometimes it is horizontal transmission from siblings. This process has an impact on overall oral health, because children who acquire the bacteria earlier have more caries than children who acquire it later (30). Some of the behaviors that spread the bacteria are innocuous habits of motherhood: blowing over hot food, testing food before giving it to a child, and kissing. Interestingly, vertical transmission is higher from mothers to daughters than from mothers to sons (30).

1.4 ENVIRONMENT

1.4.1 Water fluoridation

Before the advent of fluoridated toothpaste, water fluoridation greatly reduced the number of caries in the populations of western countries. By some reports, it continues to reduce the gap between health disparities within different socio-economic groups (31). Most of the fluoride we ingest is absorbed into our bodies and approximately one-half of that is incorporated into bones. As permanent teeth are developing, fluoride is incorporated into the enamel matrix, hardening the enamel that later develops (32). Tooth enamel may be malformed from too much fluoride being absorbed into developing teeth, causing a condition called fluorosis. It is rare, being primarily caused by poor nutrition or children habitually

swallowing toothpaste (31). The effects are mostly aesthetic, although severe forms result in pitting of the enamel with dark brown staining (32). While fluoride plays an important role in the developing tooth, its benefit is lost if the fully formed tooth is not exposed to fluoride continually throughout the individual's life (32). Fluoride also interferes with the ability of mutans streptococci to produce acid.

1.4.2 Air quality and Asthma

Although reports may vary, the consensus is that children with asthma have more caries experience than children who do not have asthma (33). Our lab has investigated a possible genetic link between the two conditions and found a very strong association with the *ameloblastin* gene ($p=2.525e-007$). This effect is likely due to the medications asthmatics take. These medications typically cause xerostomia, or dry mouth, which removes the protective effect of saliva. In addition, the constant drying out of the front teeth and gums observed with mouth breathing has been associated with increased incidence of gingivitis. Lastly, asthma medication is often acidic, and when taken in the form of an inhaler, can cause some dental erosion (33).

Smoking leads to poor oral health and, in young children, second hand smoke is significantly associated with an increase in early childhood caries experience (34), although the exact mechanism is unknown. Tobacco smoke is an immunosuppressant, which may make mutans streptococci infections worse. It also contains free radicals that draw vitamin C out of a person's system, promoting the growth of cariogenic bacteria. Children of smokers have higher rates of asthma and respiratory infection, that subsequently may lead to children breathing through their mouths, a possible risk factor for dental caries, as noted previously (34).

1.4.3 Access to care

Dental care varies greatly from person to person and place to place. In 2009, only 38.6% of the general U.S. population had seen a general practice dentist during the course of the year (35). This low rate is primarily due to two factors: a societal belief that general dental checkups are not important and a lack of effective dental insurance. More than one-third of Americans have no dental insurance, private or public, and many private policies are ineffective due to high co-pays (36). Most state Medicaid programs do not offer basic dental care to adults, and only 38% of CHIP (children's health insurance program)-

covered children see a dentist annually. Among the many barriers to obtaining health care with public assistance, the largest is finding dentists who will accept Medicaid/CHIP (37). In order to save money, many states cut back the reimbursements for dental care, to the point where the reimbursements do not cover the basic costs of performing the dental procedure (35). For this reason, many dentists are unwilling to accept patients covered by Medicaid or CHIP.

1.5 PUBLIC HEALTH RELEVANCE

With dental care, the focus is more on treating the symptoms of the illness instead of preventing it. This ends up being a costly decision, as 5 to 10% of public health funding in industrialized nations is spent on dental care. This makes oral diseases the 4th most expensive illness to treat in these countries (38). In addition to cost saving, preventative care would have an impact on individual health beyond improved oral health. Many of the risk factors for dental caries: poor diet, malnutrition, tobacco and alcohol use, are the same for other chronic disease: cardiovascular disease, diabetes, cancer, chronic obstructive pulmonary disease. Early intervention against these risk factors could help prevent the severity of these diseases later in life (38).

Caries and tooth loss also have an impact on a person's quality of life. In addition to contributing to low self-esteem and depression, severe caries can lead to persistent pain, trouble chewing, and problems with speech (38). A recent study in the United Kingdom found that 96% of 5 years-old had untreated caries. This was related to failure to thrive symptoms that were reversed after the caries was treated (39). Untreated caries can also contribute to loss of sleep due to pain, reduced food intake, more missed school, and a higher risk of hospitalization (39). These effects are seen more in poor and disadvantaged groups worldwide than in their more affluent counterparts (38). Women in developing countries, where they have less access to health care, are more affected by oral health problems, as are children, who are more susceptible to caries (38).

2.0 GENETICS OF CARIES RESEARCH

2.1 ENAMEL FORMATION GENES

As stated earlier, the focus of the Viera lab is to investigate the role of enamel formation genes in caries. A standard measure of caries experience is to count the number of decayed, missing due to caries, or filled due to caries, teeth that a person has. The sum of which is called DMFT (decayed, missing, filled, teeth). DMFT (or dmft when referring to children's teeth) will be used in this essay as a surrogate for caries experience.

2.1.1 Case Control Studies

To look at the impact of these enamel formation genes on caries we designed a case control study using available populations. The reasoning was that because DMFT varies widely among individuals (for the many reasons noted previously), the best chance of detecting the genetic impact was to look at people with extreme DMFTs. That is, the study comprised people in the same location, with the same access to fluoridated water and healthcare, with a similar diet, except one group has not had any caries while the other has had a lot. Our hypothesis was that there would be significant allelic differences in enamel formation genes in the low DMFT group when compared to the high DMFT group.

The first population we studied was a group of 110 adult Guatemalans from families with a history of cleft lip and palate (40). Samples from these individuals had been collected for a larger project in an affiliated lab at the dental school. All individuals in this group lived in the same village, were of the same ethnicity, and had a similar socioeconomic level, thus mitigating the effects of several non-genetic factors. The individuals were categorized by their DMFT scores into two groups: high or "cases" (DMFT ≥ 3) and low or "controls" (DMFT ≤ 2). Initial results showed no associations between the aforementioned genes and DMFT. However, when the definition of cases was restricted to those with very high caries experience, DMFT >20 , we saw an association (p-value= 0.002) with the rarer variant of X-linked amelogenin, the single nucleotide polymorphisms (SNP) hCV2190967.

Our next study was performed on a group of 173 children from Istanbul (41). These samples were collected by a visiting post-doctoral student for the purpose of testing enamel formation genes in a

child population. In this study, cases were defined as $dmft \geq 4$ and controls were children with no caries. Again, initial results showed no difference, but when cases were confined to children with high caries, $dmft > 8$, a significant ($p=0.01$) overrepresentation of the C allele of SNP rs17878486 in the amelogenin gene was seen. Overrepresentation of the T allele in SNP rs34538475 in the *ameloblastin* gene had a borderline association with cases with a $dmft > 10$ ($p=0.05$), and the CT genotype of SNP rs3790506 in *tuftelin* was overrepresented in cases with a $dmft > 5$ ($p=0.05$). These results are consistent with our previous findings, and also suggest that the cutoff for caries affection status should be $dmft \geq 5$

These observations raise a concern about this method of evaluating results. The $dmft/DMFT$ score of 3 or 4 that was initially considered “caries affected” in both these groups was chosen based on general assumptions about caries progression. A DMFT score = 3 or 4 is not uncommon, but a higher score would indicate a problem with the individual or their dental care habits. The fact that differences were not observed until higher $dmft/DMFT$ scores were considered could mean that this cut off is too low. There is also the possibility that no one cut off will be valid, as these samples come from different cultures with different access to dental care. In the Turkish population, a case definition with $dmft \geq 4$ results in 86 cases and 90 controls, whereas when $dmft \geq 8$ there were 49 cases and 127 controls. In the Guatemalan population, a case $DMFT \geq 3$ results in 66 cases and 44 controls, whereas a case $DMFT \geq 20$ results in 7 cases and 103 controls.

2.1.2 Tooth Cohort

Our most recent study uses a different approach. In addition to DNA from our standard population sets, DNA and extracted (due to caries) tooth samples were collected from patients at the School of Dental Medicine at the University of Pittsburgh. These patients are typically older and from low socioeconomic communities. The carious part of the teeth were cut off, the remaining enamel mounted in a stand and subjected to artificial de- and re-mineralization. The hardness of the enamel was measured at various points and this data was correlated to the results of DNA screening for variations in enamel formation genes (42).

The DNA used in this screening came from a variety of countries. The reason for this is twofold. First, the Vieira lab hosts many visiting graduate students from other countries who bring their own samples. In this way, we have samples from Brazil, Turkey, the Philippines, and many other locations. The second reason is because allele frequency varies between different ethnic groups. When analyzing genotyping results, it helps to be working in a homogenous population. Countries like Brazil and the

United States have long histories of ethnic mixture, making it difficult to determine if allelic variations are common or not. Populations with little mixture, like Guatemala and Turkey, have more stable frequencies.

The main group in this screening is a set of samples from cleft patients and their families in the Philippines. These samples came from rural, isolated areas without access to stable dental care or fluoridated water. Next was the Turkish children samples already described. There were two sample sets from Brazil, one from a pediatric dental clinic in Rio de Janeiro, and the other from hospitals in Curitiba, a capital city in a southern state of Brazil. While both these sets are from large cities with free access to dental care and fluoridated water, Curitiba is in a more rural area, and the sample set contained adults as well as children. The Rio de Janeiro set was exclusively children. Finally, there was a set of samples from the Patagonia region of Argentina composed of cleft patients and their families. This is also a rural area with access to fluoridated water and public services.

In total, 1,831 DNA samples from these different populations were tested and 48 teeth (from 28 individuals) were examined. Again, a SNP in X-linked *amelogenin*, rs946252 was associated with high caries experience (p=0.01). SNP rs4694075 in *ameloblastin* was also associated with high caries experience (p=0.007). However, at least one SNP in all of the enamel formation genes studied, except for *tuftelin interacting protein 11*, was associated with caries experience in at least one study population.

Table 1: Allele p-values for SNPs in Five Study Population

SNP	Gene	Filipino	Turkish	Brazilian (Rio de Janeiro)	Brazilian (Curitiba)	Argentinean
rs946252	<i>AMELX</i>	0.01	0.04	0.31	0.22	0.2
rs4970957	<i>TUFT1</i>	0.53	0.5	0.04	0.8	0.03
rs4694075	<i>AMBN</i>	0.007	0.29	0.94	0.22	0.29
rs12640848	<i>ENAM</i>	0.52	0.73	0.29	0.04	0.76
rs5997096	<i>TFIP11</i>	0.76	0.61	0.24	0.61	0.78

The enamel hardness association results were less specific, showing an association in every gene studied in nearly every SNP used, as shown in the following table.

Table 2: Significant Enamel Hardness Results, by Tooth Surface

SNP	Gene	Low Baseline	Low After Caries	High After Fluoride	Ratio of Change after pH Cycling
rs17878486	<i>AMELX</i>	0.03 B			0.03 B+L
rs946252					0.03 M 0.03 B+L
rs3790506	<i>TUFT1</i>	0.03 M 0.02 B+L	0.02 B+L		
rs2337360			0.006 D	0.03 D	
rs4970957					0.02 O
rs34538475	<i>AMBN</i>	0.04 D			
rs4694075					
rs3796704	<i>ENAM</i>				
rs12640848			0.02 D		0.01 O
rs134136	<i>TFIP11</i>		0.006 B+L 0.009 O		0.04 B+L 0.009 O
rs5997096					

M=mesial, D=distal, B+L=buccal and lingual, O=occlusal see Image below for visual explanation

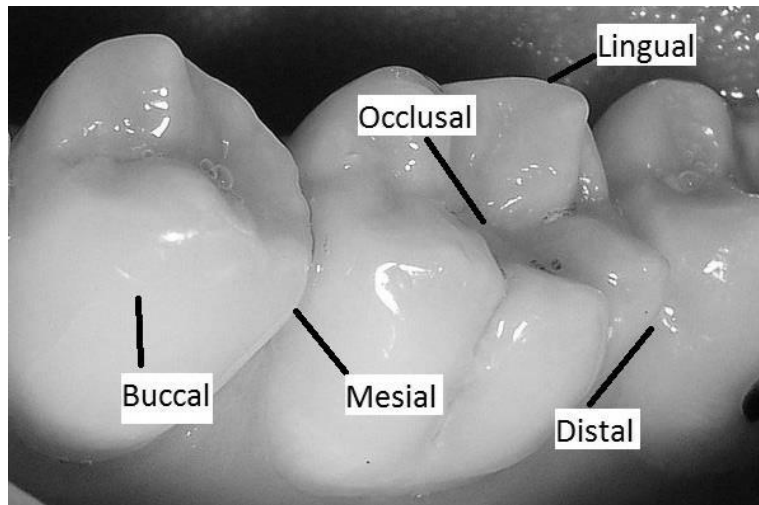


Figure 1: Surfaces of human teeth (43)

The *amelogenin* results are expected because it plays such a large role in enamel formation, but the *tuftelin* results are interesting. Knowledge of the role *tuftelin* may play in the formation of the tooth is limited and the results of our study may offer clues into its exact function. However, the teeth that were assessed had been removed due to caries. Thus, there may have been underlying issues with the enamel on these teeth. In addition to this, each population had its own limitations. The two populations from Brazil had lower DMFTs overall than the other populations. The Turkish population and one of the Brazilian populations was made up entirely of children, and we know that primary teeth respond to caries differently than permanent teeth (44). The Argentinean and Filipino populations were from families affected by cleft lip and/or palate, though the samples for this study were unaffected individuals. It is unclear if having a family history of cleft lip and palate puts one at a higher risk of caries than the general population.

2.2 FINE MAPPING OF ASSOCIATED REGIONS

While the studies focusing on enamel formation genes had promising results, they did not offer definitive results regarding caries experience. Even when controlling (as much as possible) for outside factors, no genes stood out as consistently being associated with high or low caries experience. For this reason, other areas of the genome were investigated for possible genetic links to caries experience.

2.2.1 GWS

Our lab performed one of the first genome wide scans for genes related to caries (45). A panel of 392 fluorescent genotype markers was used to assay 46 families consisting of 458 total individuals. After performing linkage analyses to detect co-segregation between the DNA markers and caries experience (as measured by DMFT), significant evidence for linkage was found in five chromosomal regions: three for low caries susceptibility: 5q13.3 (dominant midpoint LOD score=2.30), 14q11.2 (recessive single-point LOD score=2.29), and Xq27.1 (non-parametric LOD p-value=0.00005); and two for high caries susceptibility: 13q31.1 (recessive single point LOD score=2.33 and recessive multipoint LOD score=2.20) and 14q24.3 (recessive single-point LOD score=2.06). There are no enamel formation genes in any of these locations. In fact, the genes and gene products found in the above mentioned regions are not known to be related to caries formation or tooth development (45). This result is not unexpected because many factors influence the onset and progression of caries. How these factors interact is unknown, but these results can be used for generating additional hypotheses.

2.2.2 Fine Mapping

Currently, our lab is fine-mapping these 5 regions by first genotyping a population with unlinked SNPs from each region, then determining which SNPs are associated with high or low caries experience, and finally investigating the genes that contain those associated SNPs (45). The genotyping of 5q13.3 revealed 4 markers in 4 different genes that were significantly associated with low caries experience.

Table 3: Summary of 5q13.3 Genotyping

Name	Abbreviation	Marker	p value
prostate androgen-regulated transcript 1	<i>PART1</i>	rs27565	0.00021
SWIM-type containing zinc finger 6	<i>ZSWIM6</i>	rs4700418	0.0089
cyclin B1	<i>CCNB1</i>	rs875459	0.0046
basic transcription factor 3	<i>BTF3</i>	rs6862039	0.0084

In this analysis, the definition of caries experience varied with age: low was defined as DMFT ≤ 2 in children, ≤ 5 in teenagers, and ≤ 8 in adults.

None of these genes have a known function associated with caries. To further assess whether any of these genes may be involved in caries, RNA extracted from whole saliva samples was tested for the mRNA of these 4 genes. RNA expression was only observed for *PART1* and *BTF3*, and only expression of *BTF3* was correlated with low caries experience. This gene is involved in transcription regulation and cell cycle regulation (46), but how these processes may influence caries is unclear (47).

Despite the evidence for linkage in the GWS, the 13q31.1 chromosomal region had no associated SNPs. In the 14q24.3 region, the gene *ESRRB* showed some promising results. *ESRRB* codes for a protein that is similar to an estrogen receptor, but does not actually interact with estrogen; its complete function is unknown. In mice, it plays an important role in the proliferation of stem cells (48). In humans, however, it has only been associated with non-syndromic hearing loss (49).

Significant results were found in the next region, 14q11.2. Four SNPs were associated with low caries experience in two of the four populations studied (50).

Table 4: p-value for 14q11.2 Genotyping

	Filipino	Turkish	Brazilian (Rio de Janeiro)	Guatemalan
rs1997532	0.04	0.0005	not significant	0.03*
rs1997533	0.02	0.01	not significant	not significant
rs8011979	0.01	0.001	not significant	not significant
rs7150049	0.02	0.001	0.05	not significant

* this result was significant only after adjusting for sex

As in the last study, the definition of low caries experience varied with age: dmft ≤ 2 in children, ≤ 5 in teenagers, and ≤ 8 in adults. Unfortunately, all of these SNPs are in between *T-cell receptor alpha variable (TRAV)* genes and may not actually code for anything. In general, TRAV genes help determine the variability of the alpha region of T-cells, that is, they influence how well T-cells respond to infection (50). The associated SNPs are closest to the gene *TRAV4*, and RNA expression of this gene was found in the saliva of children and teenagers with low caries experience. The possible nature of the involvement of *TRAV4* in caries can only be hypothesized. The theory in our lab is that *TRAV4* is involved in a higher immune response, attacking *S. mutans* infections early so that cavities progress more slowly or not at all.

While none of these regions revealed any obvious genes that influence caries development, it did provide further areas for investigation. It also highlighted some of the difficulties in dealing with a multifactorial disease. So many factors interact to create this disease that many genes with a variety of functions could be involved in its acquisition and progression. Hypotheses generated from these results need to be tested and replicated in additional studies.

2.3 DETECTING *S. MUTANS* IN SALIVA

Due to the strong influence of *S. mutans* on caries, our lab was interested in developing an assay that would detect bacterial DNA in samples extracted from buffered saliva (51). Many such assays exist for whole saliva, or for DNA extracted from whole saliva. The assay our lab developed was based on one

previously performed with DNA from whole saliva (52). The DNA our lab uses comes, primarily, from saliva collected in kits that buffers saliva upon collection. The manufacturers of this kit claim that the buffer not only neutralizes the DNAses in the saliva, but that it also suppresses and removes the majority of bacteria from the saliva. Our assumption was that the buffer included in the saliva samples we worked with would still, despite manufacturer's claims, contain a measurable amount of bacterial DNA. Thus, we created primers specific to the glucosyltransferase gene of *S. mutans* and ran an absolute quantification real time PCR reaction with our samples.

This reaction works in the same manner as a standard PCR, primers for specific regions are created, they match up to those regions, replicate them, then those copies get replicated with the copy in the genome, causing an exponential increase in the amount of the genome region that the primer is designed to find. If there is a lot of *S. mutans* in the sample, and as a result a lot of glucosyltransferase for the primers to recognize, the number of replications per cycle of PCR will sharply increase early on. If there is a small amount of *S. mutans* DNA in the sample, it will take many cycles before a significant amount of replication is seen. Unfortunately, while this reaction can tell us how many reactions are happening per cycle, we cannot accurately measure the specific amount of *S. mutans* DNA present in the samples. This reaction can tell us, in general, if someone has a lot of *S. mutans* or not. Within this constraint, as the following table shows, significant results were only found in populations containing mostly children (51).

Table 5: Results from S. mutans Real Time Detection Assay

Population	N	Average age	DMFT/dmft average	Spearman's rho	T statistic	Degrees of freedom	Two- tailed p- value
Argentina	98	31.5	7.44	-0.0831	-0.82	96	0.41
Guatemala	109	28	6.87	0.07244	0.7513	107	0.45
Turkey	175	5.4	3.68	0.3328	3.23	84	0.002
Dental Registry and DNA Repository (DRDR)	666	42.9	15.98	0.02069	0.53	655	0.6
Cariology Course	158	43.3	3.77	0.05079	-0.6352	156	0.53
Center for Oral Health Disparities in Appalachia (COHRA)	44	3.3	1.64	0.4343	3.161	43	0.003

This result may seem obvious, because children are more prone to caries and therefore to having a detectable amount of *S. mutans*. It is also possible that there are carious lesions that can't be seen by the naked eye, which would elevate the level of *S. mutans* present. Another limitation is that this is just a snapshot of one moment in the *S. mutans* profile of the volunteer. It tells us whether they had high or low caries on the day they donated. We assume, for the purposes of research, that it represents a general picture of the oral biome from which to extrapolate.

We can use this assay for other studies to categorize subjects into different groups. For example, if someone has low caries and high *S. mutans*, we can design a study to look at other factors in their body/lifestyle that will impact their caries outcome. Alternatively, using data on subjects with high caries but no *S. mutans*, we can investigate what other bacteria in their mouth are creating the acid. We can also assess their diet and investigate the level of proteins versus carbohydrates.

3.0 APPLICATION

As described previously, dental caries among the pediatric population is a serious public health problem. Given my background in the genetics of dental caries, I propose a pilot public health intervention to assess whether parent and child's knowledge of the child's possible increased susceptibility to dental caries would affect the oral health habits of the child. As part of this proposal, a genetic panel of SNPs associated with high or low caries risk will be developed. This panel would then be tested with children from a pediatric clinic whose parents would be notified of the results of the test. The parents and child will then be questioned after hearing the results if they plan on making any lifestyle changes. They will be asked the same questions at their next two regular visits (6 months, 1 year later), to see if they actually implemented any of these changes.

The primary resource required for this project is a pediatric clinic with dentists and patients willing to participate. I propose the pediatric clinic at the University of Pittsburgh School of Dental Medicine because it has been the site of other research projects in the past, so the staff is familiar with dealing with research protocols. The people who attend to this clinic are typically from a lower social economic status and from neighborhoods near the University. The children volunteering for this study should be patients between the ages of 7 and 12. In this age range, children are beginning to get their permanent dentition (adult teeth). As new teeth come in, parents will be more observant of their children's oral health habits.

Each volunteering patient will be given a standard dental exam and cleaning. The dentist will then explain to the patient and their parents what constitutes good oral health, and what they need to do to improve their oral health. Half of these volunteer will then be asked to provide a saliva sample, from which DNA will be extracted. All parents and children will need to provide informed consent to allow specific items from the child's medical record to be accessed and for having their DNA used. The child's dmft/DMFT from their medical records will be used in this study as a measure of caries experience. Other medical information, such as current medications and chronic medical conditions, will also be noted. Parents will be asked what they expect the outcome of the testing to be and if they plan to make any changes in their child's current oral health care regime.

The panel will be designed to evaluate many of the above mentioned genes, as well as any gathered from a more exhaustive literature search. Specific SNPs in each of these genes will be on the panel. A laboratory would be required to create the physical panel, extract the DNA, and run the assay.

SNP results will be compared to DMFT scores to see if there are any statistically significant correlations. Within a month of running the assay, the children and their parents will be notified of the results. They will be asked again if they plan on making changes in their lifestyle. At the next routine visit with the dentist for all the children in this study, 6 months after the recruiting visit, the child and the parents will be asked if they made any of the changes in dental health care that they previously mentioned. The child's dmft/DMFT will be noted to see if there has been any improvement or not. This process will be repeated at the next 6 month visit (one year after the genetic screening was implemented) to see if there is any difference between the dmft/DMFTs of the children who got the genetic screening and those who didn't.

There are two goals with this project. The first is to assess if it is possible to design a predictive genetic panel that could help customize oral health care. This panel could be used by larger entities like Medicaid or dental insurance providers to help determine how better to spend resources on patients. For example, whether preventive care or restorative care would be better for a specific patient.

The second goal is to assess whether knowledge of genetic testing results impacts individual choices for health care. All the participants in this study will receive a comprehensive education about the causes of dental caries, what each patient can do to prevent caries, and the evidence for a genetic component to the disease. Those who receive genetic profile results may or may not act any differently than those who did not. Less susceptible people may reduce brushing their teeth and get more cavities; more susceptible may be more diligent about oral care and improve their oral health. An individual's action depends on how he/she internalizes the risk. Because the underlying message, i.e., that everyone still needs to brush their teeth at least twice a day, is the same for both groups, the parents of the children who get the test result may not react at all to the results.

In the short term, the patients involved in this study along with their parents will have increased general knowledge about oral health. Those who received the genetic screening will have knowledge about their susceptibility to caries. In the medium term, patients and their parents will be reminded of healthy lifestyle choices they could make to improve their child's oral health. In the long term, this study could help in addressing the larger question of whether genetic testing for more common diseases (such as dental caries) is useful, that is, do individuals change their behaviors to mitigate risk. The study may also indicate additional procedures that would increase its effectiveness.

BIBLIOGRAPHY

1. World Health Organization. Oral health, Fact sheet N°318. April 2012.
<<http://www.who.int/mediacentre/factsheets/fs318/en/index.html>> Accessed 19 June 2013.
2. Owing L. 2007 Mar. 5. ABC News.
<<http://abcnews.go.com/Health/Dental/story?id=2925584&page=1>> Accessed 2013 Mar. 9.
3. Shuler CF. Inherited risks for susceptibility to dental caries. *J Dent Educ.* 2001;65(10):1038-45.
4. Conry JP, Messer LB, Boraas JC, Aeppli DP, Bouchard TJ Jr: Dental caries and treatment characteristics in human twins reared apart. *Arch Oral Biol* 1993; 38: 937–943.
5. Wright JT. The molecular etiologies and associated phenotypes of amelogenesis imperfecta. *Am J Med Genet A* 2006;140:2547–2555.
6. Tamburstuen MV, Reseland JE, Spahr A, Brookes SJ, Kvalheim G, Slaby I, Snead ML, Lyngstadaas SP. Ameloblastin expression and putative autoregulation in mesenchymal cells suggest a role in early bone formation and repair. *Bone* 2011; 48:406-413.
7. Zhengkuan M. The human tuftelin gene: cloning and characterization. *Gene* 279 (2001) 181–196
8. Tannukit S. Identification of a novel nuclear localization signal and speckle-targeting sequence of tuftelin-interacting protein 11, a splicing factor involved in spliceosome disassembly. *Biochem Biophys Res Commun.* 2009 Dec 18;390(3):1044-50.
9. Bei M. Molecular genetics of tooth development. *Genetics and Development.* 2009; 19:504-510.
10. Ferraro M, Vieira AR. Explaining gender differences in caries: a multifactorial approach to a multifactorial disease. *Int J Dent.* 2010;649643.
11. Wang X, et.al. Genetic and Environmental Factors Associated with Dental Caries in Children: The Iowa Fluoride Study. *Caries Res.* 2012;46:177–184
12. Joana Cunha-Cruz, JoAnna Scott, Marilyn Rothen, Lloyd Mancl, Timothy Lawhorn, Kenneth Brossel and Joel Berg. Salivary characteristics and dental caries: Evidence from general dental practices. *JADA* 2013;144(5):e31-e40
13. Aguirre JM, Rodriguez R, Oribe D, Vitoria JC. Dental enamel defects in celiac patients. *Oral Surg* 1997;84:646-50.
14. Senpuku H, Yanagi K, Nisizawa T. Identification of *Streptococcus mutans* PAC peptide motif binding with human MHC class II molecules (DRB1-0802, 1101, 1401 and 1405). *Immunology* 1998;95:322-30.
15. Sanui T, Gregory RL. Analysis of *Streptococcus mutans* biofilm proteins recognized by salivary immunoglobulin A. *Oral Microbiol Immunol.* 2009 Oct;24(5):361-8.
16. Gomes Pde S, and Fernandes MH. Defensins in the oral cavity: distribution and biological role. *J Oral Pathol Med.* 2010 Jan;39(1):1-9.

17. Fine DH, Toruner GA, Velliyagounder K, Sampathkumar V, Godbole D, Furgang D. A lactotransferrin single nucleotide polymorphism demonstrates biological activity that can reduce susceptibility to caries. *Infect Immun*. 2013 May; 81(5):1596-605.
18. Mayo Clinic Staff. 2013 May 14. Oral Health: Brush Up on Dental Care Basics. <<http://www.mayoclinic.com/health/dental/DE00003>> Accessed 2013 June 11.
19. Graves RC, Disney JA, Stamm JW. Comparative effectiveness of flossing and brushing in reducing interproximal bleeding. *J Periodontol*. 1989 May;60(5):243-7.
20. The Public Speaks Up on Oral Health Care: An ADA and Crest/Oral B Survey. October 2008. <<http://www.crest.com/ada-webcast/surveyfindings.pdf>> Accessed 2013 June 11.
21. Beavan C. 2007 Mar. 20. No Impact Man. <http://noimpactman.typepad.com/blog/2007/03/i_hereby_sacrif.html> Accessed 2013 Mar. 10.
22. Ten Cate JM. Contemporary perspective on the use of fluoride products in caries prevention. *Br Dent J*. 2013 Feb 22;214(4):161-7.
23. Walsh LJ. Preventive dentistry for the general dental practitioner. *Aust Dent J*. 2000 Jun;45(2):76-82.
24. Lukacs JR, Largaespada LL. Explaining sex differences in dental caries prevalence: saliva, hormones, and "life-history" etiologies. *Am J Hum Biol*. Jul-Aug;18(4):540-55. 2006
25. Lehner T, Clarry ED, Cardwell JE. Immunoglobulins in saliva and serum in dental caries. *Lancet*. Jun 17;1(7503):1294-6. 1967
26. Touger-Decker R, van Loveren C. Sugars and dental caries. *Am J Clin Nutr*. 2003 Oct;78(4):881S-892S.
27. Yuan JC, et.al. Dentistry and obesity: a review and current status in U.S. predoctoral dental education. *J Dent Edu*. 2012 Sep;76(9):1129-36.
28. Marshall TA and Eichenberger-Gilmore JM. Dental caries and childhood obesity: roles of diet and socioeconomic status. *Community Dent Oral Epidemiol*. 35: 449-458 2007
29. Marcotte H and Lavoie MC. Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiol Mol Rev*. 1998 Mar; 62(1):71-109.
30. Lapidattanakul J, Nakano K, Nomura R, Hamada S, Nakagawa I, and Ooshima T. Demonstration of mother-to-child transmission of *Streptococcus mutans* using multilocus sequence typing. *Caries Res*. 2008;42(6):466-74.
31. McGrady MG, Ellwood RP, Maguire A, Goodwin M, Boothman N, Pretty IA. The association between social deprivation and the prevalence and severity of dental caries and fluorosis in populations with and without water fluoridation. *BMC Public Health* 2012, 12:1122
32. Palmer CA, Gilbert JA; Academy of Nutrition and Dietetics. Position of the Academy of Nutrition and Dietetics: the impact of fluoride on health. *J Acad Nutr Diet*. 2012 Sep;112(9):1443-53.
33. Widmer RP. Oral health of children with respiratory diseases. *Paediatr Respir Rev*. 2010 Dec;11(4):226-32.

34. Leroy R, Hoppenbrouwers LR, Hoppenbrouwers K, Jara A, Declerck D. Parental smoking behavior and caries experience in preschool children. *Community Dent Oral Epidemiol* 2008; 36: 249–257
35. American Dental Association. 2012 April 26. Breaking Down Barriers to Oral health for All Americans: The Role of Finance. <http://www.ada.org/sections/advocacy/pdfs/7170_Breaking_Down_Barriers_Role_of_Finance-FINAL4-
36. Sanders, B. 2012 Feb. 29. U.S. Senate Committee on Health, Education, Labor & Pensions, Subcommittee on Primary Health and Aging. Dental crisis in america: The Need to Expand Access. <<http://www.sanders.senate.gov/imo/media/doc/dentalcrisis.report.pdf>>. Accessed 2013 Mar. 10.
37. Center for Medicare and Medicaid Services. Dental Care. <<http://www.medicare.gov/Medicare-CHIP-Program-Information/By-Topics/Benefits/Dental-Care.html>>. Accessed 2013 Mar. 9.
38. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. *Bull World Health Organ*. 2005 Sep;83(9):661-9.
39. Sheiham A. Dental caries affects body weight, growth and quality of life in pre-school children. *Br Dent J*. 2006 Nov 25;201(10):625-6.
40. Deeley K, Letra A, Rose EK, Brandon CA, Resick JM, Marazita ML, Vieira AR. Possible association of amelogenin to high caries experience in a Guatemalan-Mayan population. *Caries Res*. 2008;42(1):8-13.
41. Patir A, Seymen F, Yildirim M, Deeley K, Cooper ME, Marazita ML, Vieira AR. Enamel formation genes are associated with high caries experience in Turkish children. *Caries Res*. 2008;42(5):394-400
42. Shimizu T, Ho B, Deeley K, Briseño-Ruiz J, Faraco IM Jr, Schupack BI, Brancher JA, Pecharki GD, Küchler EC, Tannure PN, Lips A, Vieira TC, Patir A, Yildirim M, Poletta FA, Mereb JC, Resick JM, Brandon CA, Orioli IM, Castilla EE, Marazita ML, Seymen F, Costa MC, Granjeiro JM, Trevilatto PC, Vieira AR. Enamel formation genes influence enamel microhardness before and after cariogenic challenge. *PLoS One*. 2012;7(9). Epub 2012 Sep 24.
43. dozenist. "MandibularLeftFirstMolar08-15-06." Photograph. *Wikimedia Commons*. 15 August 2006. <<http://commons.wikimedia.org/wiki/File:MandibularLeftFirstMolar08-15-06.jpg>>
44. Hayashi-Sakai S, Sakai J, Sakamoto M, Endo H. Determination of fracture toughness of human permanent and primary enamel using an indentation microfracture method. *J Mater Sci Mater Med*. 2012 Sep;23(9):2047-54.
45. Vieira AR, Marazita ML, Goldstein-McHenry T. Genome-wide scan finds suggestive caries loci. *J Dent Res*. 2008 May;87(5):435-9.
46. Green CD, Thompson PD, Johnston PG, El-Tanani MK. Interaction between transcription factor, basal transcription factor 3, and the NH2-terminal domain of human estrogen receptor alpha. *Mol Cancer Res*. 2007 Nov;5(11):1191-200.
47. Shimizu T, Deeley K, Briseño-Ruiz J, Faraco Jr IM, Poletta FA, Brancher JA, Pecharki GD, Küchler EC, Tannure PN, Lips A, Vieira TC, Patir A, Yildirim M, Mereb JC, Resick JM, Brandon CA, Cooper ME, Seymen F, Costa MC, Granjeiro JM, Trevilatto PC, Orioli IM, Castilla EE, Marazita ML, Vieira AR. Fine-Mapping of 5q12.1-13.3 Unveils New Genetic Contributors to Caries. *Caries Res*. 2013 Jan 30;47(4):273-283.

48. Martello G, Sugimoto T, Diamanti E, Joshi A, Hannah R, Ohtsuka S, Göttgens B, Niwa H, Smith A. Esrrb is a pivotal target of the Gsk3/Tcf3 axis regulating embryonic stem cell self-renewal. *Cell Stem Cell*. 2012 Oct 5;11(4):491-504.
49. Ben Saïd M, Ayedi L, Mnejja M, Hakim B, Khalfallah A, Charfeddine I, Khifagi C, Turki K, Ayadi H, Benzina Z, Ghorbel A, Castillo ID, Masmoudi S, Aifa MH. A novel missense mutation in the ESRRB gene causes DFNB35 hearing loss in a Tunisian family. *Eur J Med Genet*. 2011 Nov-Dec;54(6):e535-41.
50. Briseño-Ruiz J, Shimizu T, Deeley K, Dizak PM, Ruff TD, Faraco IM Jr, Poletta FA, Brancher JA, Pecharki GD, Kuchler EC, Tannure PN, Lips A, Vieira TC, Patir A, Koruyucu M, Mereb JC, Resick JM, Brandon CA, Letra A, Silva RM, Cooper ME, Seymen F, Costa MC, Granjeiro JM, Trevilatto PC, Orioli IM, Castilla EE, Marazita ML, Vieira AR. Role of TRAV locus in low caries experience. *Hum Genet*. 2013 May 9.
51. Vieira AR, et.al.. Detection of *Streptococcus mutans* Genomic DNA in Human DNA Samples Extracted from Saliva and Blood. *ISRN Dent*. 2011;2011:543561.
52. A. Yano, N. Kaneko, H. Ida, T. Yamaguchi, and N. Hanada. Real-time PCR for quantification of *Streptococcus mutans*. *FEMS Microbiology Letters*, vol. 217, no. 1, pp. 23–30, 2002