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Caries: Review of Human Genetics Research

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Abstract

The NIH Consensus Development Program released a statement in 2001 (NIH Consensus Statement, 2001) and listed six major clinical caries research directions. One of these directions was the need for genetic studies to identify genes and genetic markers of diagnostic, prognostic, and therapeutic value. This last decade has seen a steep increase in studies investigating the presence of genetic factors influencing individual susceptibility to caries. This review revisits recent caries human genetic studies and provides a perspective for future studies in order to fulfill their promise of revolutionizing our understanding of and the standard of care for the most prevalent bacteria-mediated non-contagious disease in the world.

Keywords

Review; Genomics; linkage analysis; association analysis; candidate genes; heritability

Introduction

Despite more than 100 years of accumulated knowledge on the pathogenesis of this disease, caries is still a major oral health problem in most industrialized countries, affecting 60-90% of schoolchildren and the vast majority of adults. According to the World Health Organisation, caries is the most prevalent oral disease in several Asian and Latin American countries, while it appears to be less common and less severe in most African countries [World Health Organisation, 2003]. In light of changing living conditions, however, it is expected that the incidence of caries will increase in many developing countries in Africa, due particularly to a growing consumption of sugars and inadequate exposure to fluorides.

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The interest in understanding the mechanisms underlying individual susceptibility to caries coincides with the development of feasible approaches to understand genetic susceptibility to complex human disease, thanks in large part to tools developed by the Human Genome Project (www.genome.gov). The combination of the high prevalence of caries among certain groups and the evidence that fluoride exposures do not protect all individuals [Slade et al., 2013] has specifically motivated research towards identifying genetic contributors to caries. A combination of candidate gene and genome-wide studies has arisen in the literature, with some notable successes, but also notably exemplifying the difficulties of developing a study with robust phenotype definitions and sample sizes that allow enough statistical power to detect genetic effects.

Further, note that the human genome is not the only genome involved in caries, i.e., the oral microbiome clearly plays a role in pathogenesis and a number of groups are simultaneously working on the metagenomic contributions to caries. The oral cavity contains more than 700 species of bacteria, which grow primarily in multi-species biofilms (for example, dental plaque) that play vital roles in homeostasis of the mouth and in diseases of the oral cavity [Zarco et al., 2011]. Although caries is strongly associated with differences in diet and environmental factors, a study of the taxonomy of the salivary microbiome of 120 individuals selected from 12 different locations worldwide (10 per location) found little geographic structure; only 13.5% of the variance in genera composition observed between populations was explained by differences among individuals [Nasidze et al. 2009]. This suggests that microbial taxonomy alone – even after adjusting for environmental exposures – is insufficient to explain individual, familial, and regional variations in oral microbiota and particularly caries susceptibility.

In this paper, we focus on recent human genetic studies, provide an overview of where the field stands, and summarize the promise of these studies in devising new strategies to prevent and manage caries.

Pathogenesis

The term caries is thought to derive from words meaning: rottenness, decay, injure, break, death, destruction, withered, or faded. It alludes to a disease process, but does not describe it. According to Fejerskov, caries describes the signs and symptoms of a disease. In other words, it is the result of a localized chemical dissolution of the tooth surface caused by metabolic events that take place in the biofilm (dental plaque) that covers the affected area. Carious lesions result from a shift in the ecology and metabolic activity of the biofilm, whereby an imbalance in the equilibrium between tooth mineral and biofilm fluid has developed. Although the biofilm is a prerequisite for carious lesions to occur, its presence on solid surfaces does not necessarily result in the development of clinically visible carious lesions. Some chemical modifications are so subtle that they can only be recorded microscopically. When cumulative numerous pH fluctuations result in a net loss of calcium and phosphate of an extent that makes the enamel porous and visible, it is considered a “white spot” lesion [Fejerskov et al., 2008].

Featherstone [2006] explains the caries process as a balance between pathological factors and protective factors. Pathological factors that include cariogenic bacteria, frequent ingestion of fermentable carbohydrates and salivary dysfunction drive the caries process toward demineralization. Protective factors include salivary components, fluoride and remineralization, and targeted biofilm control drive the caries process toward remineralization [Featherstone, 2004]. If pathological factors outweigh the protective factors, caries progresses as a consequence [Featherstone, 2006]. Behaviors related to oral hygiene practices, dietary choices, and decisions involving seeking professional oral health care also contribute to caries and it is a major challenge defining these factors and include them along analyses of biological underpinnings.

The American Academy of Pediatric Dentistry currently recommends that caries risk assessment based on a child's age, biological factors, protective factors, and clinical findings should be a routine component of new and periodic examinations by oral health and medical providers [American Academy of Pediatric Dentistry, 2012].

There is no single test that takes into consideration all these factors and accurately predicts an individual's susceptibility to caries. The caries risk may be determined by analyzing and integrating several factors such as caries experience (initial caries lesions and established caries defects, secondary caries and present caries activity), fluoride use, extent of plaque present, diet, bacterial and salivary activity, and social and behavioral factors [Reich et al, 1999]. Although the best tool to predict future caries is past caries experience, this risk factor is not applicable for young children due to the need to determine caries risk before the disease is present [American Academy of Pediatric Dentistry, 2012].

Host susceptibility is underlined by a potential genetic contribution for caries risk. The understanding of genetic contributions to caries can be highly valuable for dental practitioners. In the future, clinicians might be able to explain to patients that some forms of caries are more strongly associated with inherited risk. This could offer an explanation for both the patient and dental practitioner why persons with similar behavioral risks (*i.e.*, tooth brushing frequency or dietary habits) have different caries risk and/or caries activity [Bretz et al., 2003].

Early Evidence for a Genetic Component of Caries

Although a genetic contribution to caries in humans has been controversial, even the earliest studies of family patterns in twins [Boraas et al., 1988; Conry et al., 1993], families [Klein and Palmer, 1940], and animal breeding [Hunt et al., 1944] were consistent with a genetic component. The most compelling early evidence for a genetic component to caries comes from studies of twins reared apart. In two related studies, investigators found significant resemblance for percentage of teeth and surfaces restored or carious within monozygotic but not dizygotic twin pairs reared apart and estimated the genetic contribution to caries as 40% [Boraas et al., 1988; Conry et al., 1993]. More recent studies of twins reared together [Bretz et al., 2003 and 2005] or of families [Wang et al., 2010] estimated the heritability for caries measured by the DMFT/S as ranging from 45-64%, with primary dentition caries in general showing higher heritability than permanent dentition caries [Wang et al., 2010]. Heritability

studies alone are not sufficient to demonstrate a genetic component in caries because shared behavior and other environmental factors can contribute to covariance between relatives and mimic genetic correlation. Notably however, genetic studies in animal models (see e.g. Nariyama et al. [2004]) and recent human molecular genetic studies bear out the heritability results. A full summary of the long history of animal model studies in caries is beyond the scope of this review, but note that many of the candidate gene studies done in humans (see Table 1 for a summary) were motivated by the findings in animal models. This review focuses in large part on the recent strides in caries molecular genetic studies in human populations.

Caries Phenotypes

Shared behavior, practice, and habits within families can be expected to contribute to covariance between relatives and to mimic genetic correlation if not controlled by the experimental design [Potter, 1990]. This is particularly true for caries, which can be profoundly affected by dietary sugar intake and/or oral hygiene practices within families. This effect can explain results that suggest a major gene effect [Werneck et al., 2011], despite the fact that one would not expect that to be the case for caries.

Successful genetic studies require careful measurement of the phenotype of interest (i.e. the disorder or physical characteristic under study), ideally in a biologically meaningful way. Genetic studies of caries are thus inherently difficult because the usual disease measures originally developed for clinical and epidemiological studies more directly assess the consequence of the disease rather than its pathogenesis. The DMFT/DMFS score (i.e. a count of the number of **D**ecayed/**M**issing-due-to-caries/**F**illed **T**eeth or **S**urfaces) is the most widely used index and gives a good estimate of the prevalence of the disease, as well as an estimate of the severity of the affection based on the number of teeth (or surfaces) affected by caries. The DMFT distribution in 12-year-olds across many countries shows that there is a skewed distribution of caries prevalence [Nishi et al., 2002]. While a proportion of 12-year-olds are caries-free, a considerable number still have DMFT values higher than the goal of 3 set by the Health Assembly of the World Health Organisation (Bratthall [2000]; see also Figure 1). Caries prevalence assessed in the third of the population with higher DMFT scores (SiC or Significant Caries Index) brings to attention the individuals that are experiencing most severe disease. It has been proposed that the SiC index for countries should be less than 3 DMFT in the 12-year-olds by the year 2015 [Bratthall, 2000].

Over the life course, populations tend to have increased DMFT/DMFS scores (Figure 2), in part due to untreated caries disease, in part due to restorative procedures and other reasons. Therefore, genetic studies defining the phenotype based on caries experience scores will have to deal with the limitation that these scores provide an estimate of the prevalence of the disease but not necessarily directly determine the individual factors involved in its pathogenesis. DMFT scores have limited ability to provide insight into the severity or pathogenesis of the caries experience in an individual. With the widespread utilization of fluorides, carious lesions have become more difficult to detect and many are incipient. Epidemiological assessments usually have study participants at only one time and judgment of lesion status needs to be made at the visit. Dentists in their practices have the ability to

follow-up cases and make decisions based on how an apparent lesion progresses or stay arrested and treatment decisions can be planned accordingly. To sophisticate the power of discrimination of caries experience evaluations at the population level, another index was proposed, the International Caries Detection and Assessment System (ICDAS; <https://www.icdas.org/>; Pitts [2004]). This index provides a more sophisticated assessment of the caries experience but at the same time brings to attention the difficulties of discerning between specific codes of early alteration of the enamel (codes 0 to 3) and the feasibility of applying this approach to large settings.

Both DMFT/DMFS and ICDAS are used to obtain information on primary dentition, which is ideally examined after complete, after 4 and before 6 years of age. Caries experience data has been generated for individuals older than 12 years of age as well, but in these cases, total caries experience tends to increase due to other factors that cannot be easily discerned from caries: sound surfaces may have been prepared to receive tooth restorations or are bases for fixed prosthodontics, teeth can be lost due to trauma, periodontal disease, orthodontic indications, and depending on the study, these factors cannot be easily detected during the clinical assessment.

For human genetic study purposes, research groups have begun to use tooth and/or surface specific scores, for example dividing the surfaces into pit-and-fissure versus smooth surfaces [Zeng et al., 2013]. An intriguing recent study used cluster analysis to divide the tooth surfaces into groups with similar caries experience [Shaffer et al., 2013b], resulting in five primary clusters summarized in footnote “e” of Table 2. Of note some clusters of surfaces did not show significant heritability (e.g maxillary incisors, see Table 2), indicating that perhaps some clusters are not under genetic control while other clusters do have genetic contributions to risk of caries.

Human Genetic Studies of Caries

There are two major ways to study the genetics of a complex trait such as caries, and both have been applied to caries: candidate gene studies (summarized below and in Table 1) and genome-wide studies (summarized below and in Table 2). Candidate gene studies test hypotheses regarding association between specific genes or gene variants and caries experience. Genome-wide studies test either linkage or association between anonymous DNA variants with known locations throughout the genome, and thus represent a hypothesis-generating procedure. DNA variants with genome-wide significant statistical signals imply that the anonymous variants are near caries etiologic variants; such positive signals then require follow-up studies for identification of the etiologic variants. Because genome-wide studies are hypothesis-generating, one typically targets for follow-up both strictly genome-wide significant results and also suggestive results, i.e. those results that are near strict significance.

Candidate Gene Studies

Most of the genetic studies of caries to date approach the problem of detecting a genetic factor contributing to caries by testing genetic variation in specific genes, based on their assumed or known function which is thought to be relevant to the disease, using the standard

statistical approach of testing for association between specific variants or alleles at a genetic locus and caries. These genes can be grouped in certain categories, based on the factor influencing caries. The major candidate gene categories studied to date include enamel formation genes, immune response genes, genes related to saliva, genes related to taste, and others (see Table 1). Associations have not always been replicated in other independent studies and these conflicting results are probably due to population heterogeneity and issues with statistical power. Due to such study design issues, the associations described in Table 1 cannot yet be fully refuted by the negative reports [Brancher et al., 2011; Yarat et al., 2011; Buczkowska-Radli ska et al., 2012; Olszowski et al., 2012; Gasse et al., 2013; Yang et al., 2013] that followed the original studies.

The most studied group of candidate genes includes the enamel formation genes. The aggregate association data [Slayton et al., 2005; Deeley et al., 2008; Patir et al., 2008; Shimizu et al., 2012; Ergöz et al., 2013; Jeremias et al., 2013] have only one mostly consistent result, the lack of association between *TFIP11* and caries (Jeremias et al. [2013] is the exception). However, association with this gene was found when the phenotype tested was microhardness of enamel after the creation of an artificial caries lesion [Shimizu et al., 2012]. These data suggest that the bulk of candidate gene studies can suffer from the definition of the phenotype as discussed earlier. Most studies compare individuals who are caries-free to individuals with at least one affected tooth. The obvious question is “Are individuals with DMFT=1 the same as individuals with DMFT=10?” The studies that take into consideration age when evaluating the DMFT likely provide a better estimate of genetic associations.

Another interesting phenotype is a surrogate of the carious lesion severity. Some individuals can have a more dramatic progression of the lesion into dentin than others, and these lesions can involve the pulp to a point that these cases can be more susceptible to developing periapical lesions detected radiographically. *MMP2* expression is higher in dentin affected by caries [Toledano et al., 2010]. When the presence of periapical lesions associated with deep carious lesions in dentin was used as a phenotype (in comparison to absence of periapical lesions despite the presence of deep carious lesions in dentin), associations were found with *MMP2* and *MMP3* [Menezes-Silva et al., 2012], demonstrating the promise of exploring phenotypes related to the severity of the carious lesions.

Genome-Wide Linkage Studies

With early molecular genetic tools such as restriction fragment polymorphisms (RFLPs) and single nucleotide polymorphisms (SNPs), genome-wide studies began to identify regions in the genome likely to harbor caries risk genes. The first genome-wide attempt to identify genetic contributors to caries used the linkage approach [Vieira et al., 2008] (see also Table 2). Linkage studies utilize the recombination that occurs between genetic loci that are near each other on the same chromosome during crossing over of homologous chromosomes during meiosis I. The recombination frequency is a function of distance between loci, and the larger the estimated recombination frequency the lower the likelihood that the loci are linked (*i.e.*, close to each other). If a genetic marker is physically close or in the vicinity of the genetic variant causing the disease, then there will be statistically significant evidence of

linkage, as estimated by a statistic termed the LOD score which has a value of 3.4 for genome-wide statistically significant linkage or between 2 and 3.4 for suggestive evidence of linkage.

The genome-wide linkage scan performed for caries controlled for several key environmental factors that influence caries. The 46 families studied shared similar cultural habits, were all from a specific geographic area in the Philippines, and had very limited access to dental care. The analysis was done twice, once utilizing a definition for high caries experience and the second for low caries experience. The definitions of caries experience relied on DMFT scores but took into consideration the age of the subjects to overcome the limitations of DMFT scores (see footnote d in Table 2).

The results suggested five loci linked to caries experience (LOD scores above 2.0 or non-parametric p-values below 0.0009), three for low caries experience (5q13.3, 14q11.2, and Xq27.1) and two for high caries experience (13q31.1 and 14q24.3). Follow-up fine mapping of these regions using association tests was completed for 5q13.3 [Shimizu et al., 2013], 14q11.2 [Briseño-Ruiz et al., 2013], and 13q31.1 [Küchler et al., 2013] in 72 Filipino families and additional population data sets from multiple sources. In chromosome 5, *BTF3* was a gene in the region flanking association signals that showed gene expression levels in whole saliva associated with caries experience. Similarly, in chromosome 14, genetic markers flanking *TRAV4* were associated with low caries experience and *TRAV4* expression in whole saliva of individuals with low caries experience was higher in children and teenagers in comparison to adults. An intergenic single nucleotide polymorphism (SNP) at 13q31.1 was associated with high caries experience and was predicted to disrupt the binding sites of two different transcription factors. Fine mapping of the two remaining regions is ongoing.

Genome-Wide Association Studies

Genome-wide association studies generally utilize large panels of SNPs, with typically 600,000 SNPs actually genotyped and millions more SNPs imputed from the genotyped data, all of which are used for analysis. A typical threshold for genome-wide statistical significance for such studies is a p-value 10^{-7} for a 600,000 SNP panel, and between 10^{-5} and 10^{-5} is considered suggestive. Caries genome-wide association studies are summarized in Table 2.

The first genome-wide association studies for caries, one for the primary dentition [Shaffer et al., 2012] and one for the permanent dentition [Wang et al., 2012a], suggested different loci than the ones reported in the earlier genome-wide linkage study, not surprising given the relative strengths of the two approaches (see below). The analysis of the primary dentition [Shaffer et al., 2012] did not unveil any formal statistically significant association if multiple testing corrections are implemented but had three loci with suggestive results: 1q42-q43, 11p13, and 17q23.1. This study was done in 1,305 US children three to twelve years of age (see Table 2). The analysis was repeated taking into consideration home fluoride exposure data, which were available for 720 children; suggestive results were found for 22q12.1 when fluoride exposure was sub-optimal, and 1p34 and 6q16.1 when fluoride exposure was

optimal. However, when these initial results were tested in an independent sample of 1,695 Danish children, ages two to seven, no associations were replicated with statistical significance [Shaffer et al., 2012].

The genome scan of caries in the permanent dentition [Wang et al., 2012a] included 7,443 subjects from five studies from the US that were analyzed separately because caries assessments were done differently, DNA sources were different, and genotyping was done at different times in distinct platforms. Individuals having at least one affected tooth in any of the studies were considered affected. Similar to the caries in the primary dentition scan, the results for the permanent dentition yielded a few loci with nominal genome-wide significance (*i.e.*, p -value 10^{-7}) after adjustment of multiple testing. Given the multiple populations included in this study, these results were appropriately considered suggestive, as were several other results with p -values between 10^{-5} and 10^{-7} (see Table 2).

A portion of the data included in the permanent dentition analyses [Wang et al., 2012a] was reanalyzed twice under surface-specific phenotypic definitions that might be more biologically meaningful than the overall DMFS. The first re-analysis defined affection status by hierarchical cluster analysis of tooth-surface level data [Shaffer et al., 2013a and b). These analyses yielded five distinct phenotype definitions (DMFS1-5, see footnote e in Table 2) and resulted in genome-wide statistically significant results for two loci: one for caries in anterior mandibular teeth (DMFS2) and a marker close to *LYZL2* (10p11.23; p -value = $9E^{-9}$), and one for caries in canines and pre-molars (DMFS5) and a marker close to *AJAPI* (1p36.32; p -value = $2E^{-8}$). Additional suggestive loci are summarized in Table 2.

A second reanalysis of the permanent dentition data included two affection definitions: the presence of at least one molar occlusal surface affected (pit and fissure) or the presence of at least one smooth surface affected [Zeng et al, 2013]. These analyses yielded a few genome-wide significant or suggestive associations for each phenotype definition. For occlusal caries, markers flanking *BCOR* (Xp11.4; p -value = $1.8E^{-7}$) and *INHBA* (7p15-p13; p -value = $6.5E^{-6}$) showed a trend for association. For smooth surface caries, markers flanking *BCORL1* (Xq26.1; p -value = $1.0E^{-5}$) and *CXCR1* and *CXCR2* (2q35; p -value = $1.9E^{-6}$) had suggestive results. Similarly, reanalysis of the primary dentition data in Shaffer et al. [2012a] included two affection definitions: the presence of at least one molar occlusal surface affected (pit and fissure) or the presence of at least one smooth surface affected [Zeng et al, 2013 in press]. Full results are summarized in Table 2. Genome-wide significant association was observed with *KPNA4* (3q26.1) and pit and fissure caries, and suggested associations with three genes that were observed in previous studies: *MPPED2* (p -value= $6.9E^{-6}$), *AJAPI* (p -value= $1.6E^{-6}$), and *RPS6KA2* (p -value= $7.3E^{-6}$).

In addition to the above association analyses of individual SNPs with caries, a recent analysis [Wang et al., 2013] applied a new statistical methodology to the primary dentition association results from Shaffer et al. [2012]. Instead of analyzing SNPs one by one, they used gene set-based analysis, which has recently emerged as a useful approach to examine the joint effects of multiple risk loci in complex human diseases or phenotypes such as caries. Wang et al. [2013] used four complementary gene set statistical analysis methods, and analyzed 1,331 gene sets under Gene Ontology (GO) terms [Ashburger et al., 2000].

Identified were 13 significantly associated GO terms/gene sets (see Table 2). Seventeen additional sets were further identified as marginally relevant. The identified gene sets encompass broad functions that potentially interact and contribute to the oral immune response related to caries development, which have not yet been reported in any standard single marker based analysis. These approaches are under development, but it appears that the gene set enrichment analysis approach can provide complementary insights into the molecular mechanisms and polygenic interactions in caries.

As summarized by Wang et al. [2013] five genes from either ligase activity (*WWP2* and *RNF217*), neuronal development (*ROBO2* and *SLIT2*), or cytokine/protein secretion (*INS*) gene sets listed in Table 2 might be potentially associated with dental traits. *WWP2* is a member of ligase activity pathways and functions as a ligase for and mediates degradation of PTEN, whose gene is expressed in mouse oral development. *RNF217* is located at 6q22.31, a genomic region reported to be associated with oral clefts. *ROBO2* is a receptor for *SLIT2* and possibly *SLIT1* genes, which appear to work cooperatively to establish anatomical midlines during neuronal development and establishment of olfactory organization. Gene *SLIT1* is also expressed in the primary and secondary enamel knots during molar tooth cusp formation. *INS* can impact caries through insulin sensitivity. Insulin receptor binding sites are present on rat incisors. None of these relationships are apparently relevant for caries development, but the gene sets and the subset of tooth-related genes raise interesting possible mechanisms for caries.

Summary of Genome-Wide Analyses

When we compare the results across all these genome-wide analyses there are three notable conclusions: (1) very little overlap exists across studies but the promising associations with 1p36.32 and 10p11.23 should be prioritized for future studies for the identification of genetic factors contributing to caries; (2) phenotype definitions for caries warrant further refinement because suggestive but not formally significant results were obtained for the traditional DMFS/T caries definitions, but genome-wide significant results *were* found for surface-specific caries scores; and, (3) utilizing approaches that look for joint effects of DNA variants across sets of related genes (such as the gene set enrichment approach of Wang et al. [2013]) appear to hold promise for identifying possibly functional relationships between caries associated genetic factors.

Interestingly, as mentioned above, the significant or suggestive signals to date from genome-wide association studies of caries are different from the significant signals found from genome-wide linkage analyses. This fact emphasizes that the two approaches have different strengths: association studies are more sensitive in detecting common variants of small effect size than are linkage studies, but linkage is more robust in detecting etiologic genes that exhibit allelic heterogeneity—if multiple different variants (especially rare variants) within a gene can lead to caries, linkage is much more likely to detect such genes [Risch and Merikangas, 1996]. Thus further follow-up studies are necessary of the results from both types of studies to confirm genetic loci for caries.

Final Remarks

Advances in molecular genetics, engineering, management, research and education offer many new ways to treat patients regarding caries management and prevention. Advancement is underway in caries risk assessment, carious lesion detection, genetic predisposition technologies, and restorative techniques [Berg, 2013]. The understanding of genetic contributions to caries can be highly valuable for dental practitioners as the starting point of host susceptibility. In the future, clinicians might be able to explain to patients that some forms of caries are more strongly associated with inherited risk, offering an explanation for both the patient and dental practitioner why similar behavioral risks (*i.e.*, tooth brushing frequency or dietary habits) have different caries risk [Bretz et al., 2003]. Individuals at higher genetic risk could then be monitored more closely and provided with more aggressive caries management and prevention programs [Bretz et al., 2003].

There has been much interest and progress recently in identifying the genetic contributions to caries. The disease is still highly prevalent despite more than 100 years of studies clearly defining its pathogenesis. New strategies that can protect individuals at higher risk even when exposed to fluorides through drinking water or other sources are needed. The recent studies have shown promise in identifying promising biomarkers that have the potential to help in determining and personalizing treatment for individuals at higher risk to develop the disease. Initial findings suggesting differential expression of genes and proteins in whole saliva (*BTF3* [Shimizu et al., 2013], *TRAV4* [Briseño- Ruiz et al., 2013], and total protein count [Roa et al., 2008]) bring the promise that personalized treatment for caries is on the horizon. Future studies should carefully consider phenotypic definitions and incorporate environmental exposures and demographic variables such as age and sex in the analysis. Models that take into consideration longitudinal evaluations of caries [Isaksson et al., 2013] can be the most promising. Taking advantage of clinical studies such as randomized trials [Bader et al., 2013] also can provide novel insight. In future work, an integration of other genetic and genomic information (such as metagenomics, gene expression, protein-protein interaction network, evidence from multiple species, and multi-dimensional functional module analysis) can also open new avenues to understand the etiology of caries.

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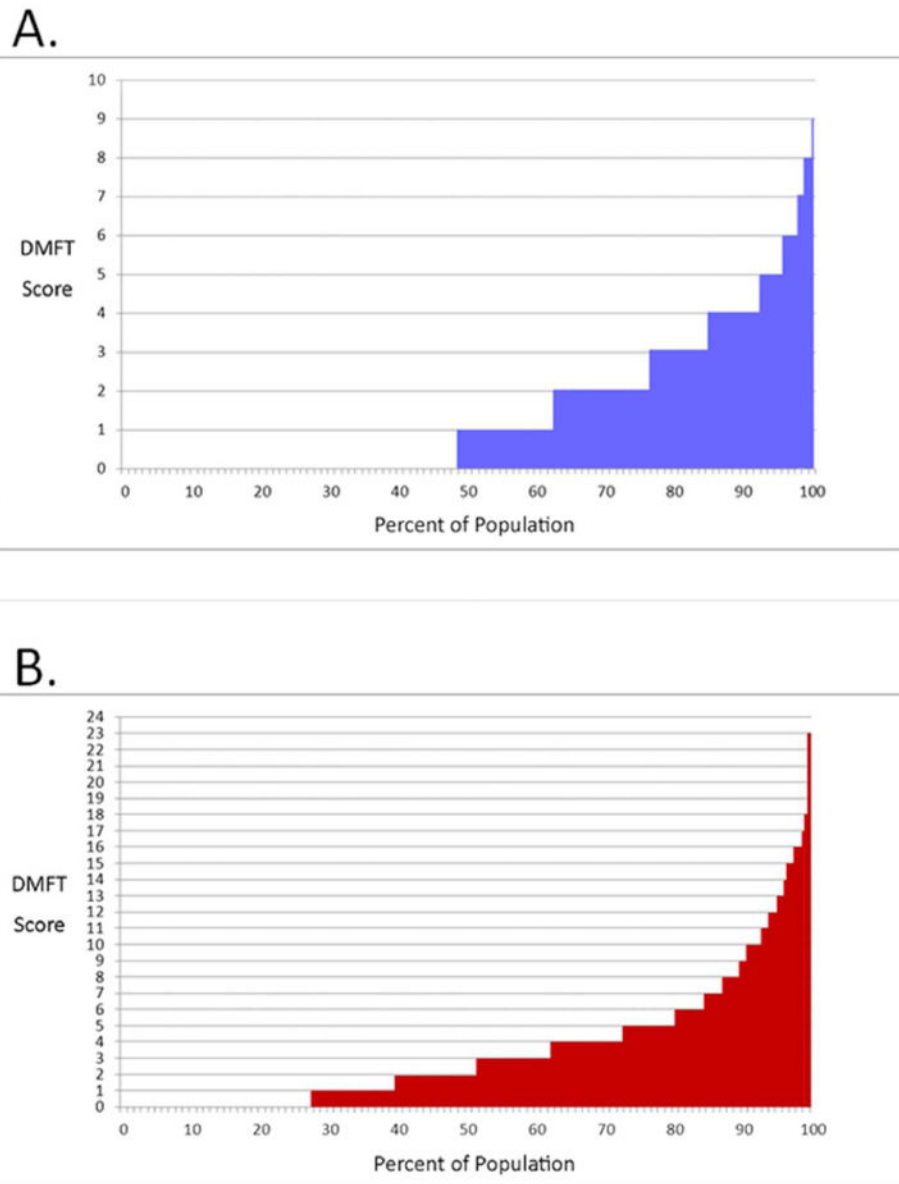


Figure 1. Graphs show caries data for two populations of 12 year-olds, expressed as frequency distribution. (A) Data (N=539) from Curitiba, Brazil (Principal Investigator Paula C. Trevilatto) included in Shimizu et al. (2012). (B) Data (N=368) from the Center for Oral Health Research in Appalachia, USA (Principal Investigator Mary L. Marazita) included in Wang et al. (2012). 48% of children from the Brazilian site (N=258) and 28% of the children from the US site (N=102) were caries-free. The mean DMFT was 1.45 (Brazilian site) and 3.45 (US site), respectively. The Significant Caries Index (SiC) calculated from the third of the population with the highest caries scores is 3.64 (Brazilian site) and 7.82 (US site), respectively. The third of the population with the highest caries experience likely include individuals that accumulate the greatest number of genetic factors increasing susceptibility to caries. Among the caries-free individuals are probably some individuals that

have greater number of genetic factors protecting against caries risk. Environmental factors such as dietary habits, fluoride exposure, and access to dental care can easily overcome genetic factors and also explain differences in caries experience in the two cohorts.

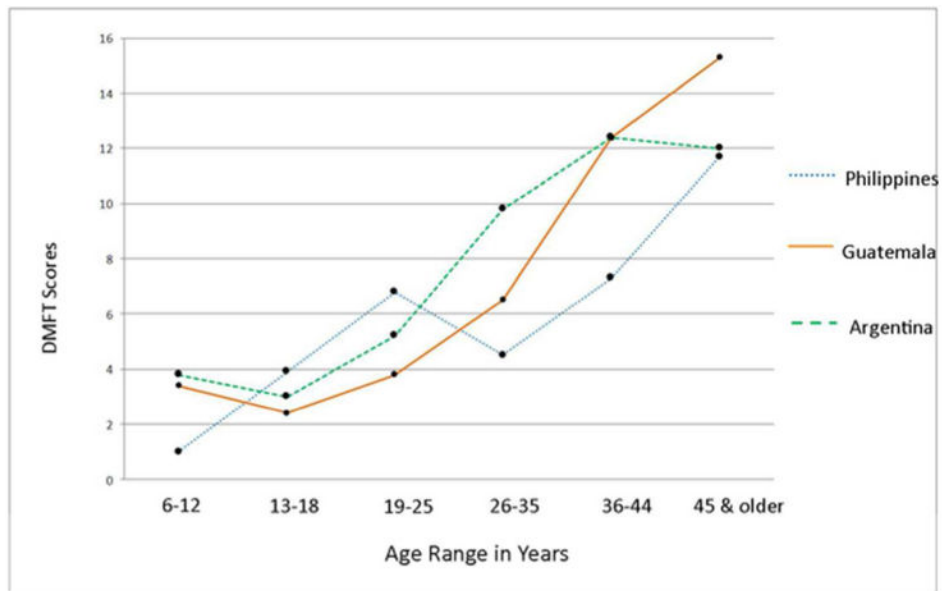


Figure 2. DMFT scores tend to grow as individuals grow older. The effect of age in any caries experience score will potentially add undetectable bias since it is not possible to account for all factors contributing to increasing levels of tooth loss and affection g and these likely impact genetic analyses (data from Jindal et al. [2011]).

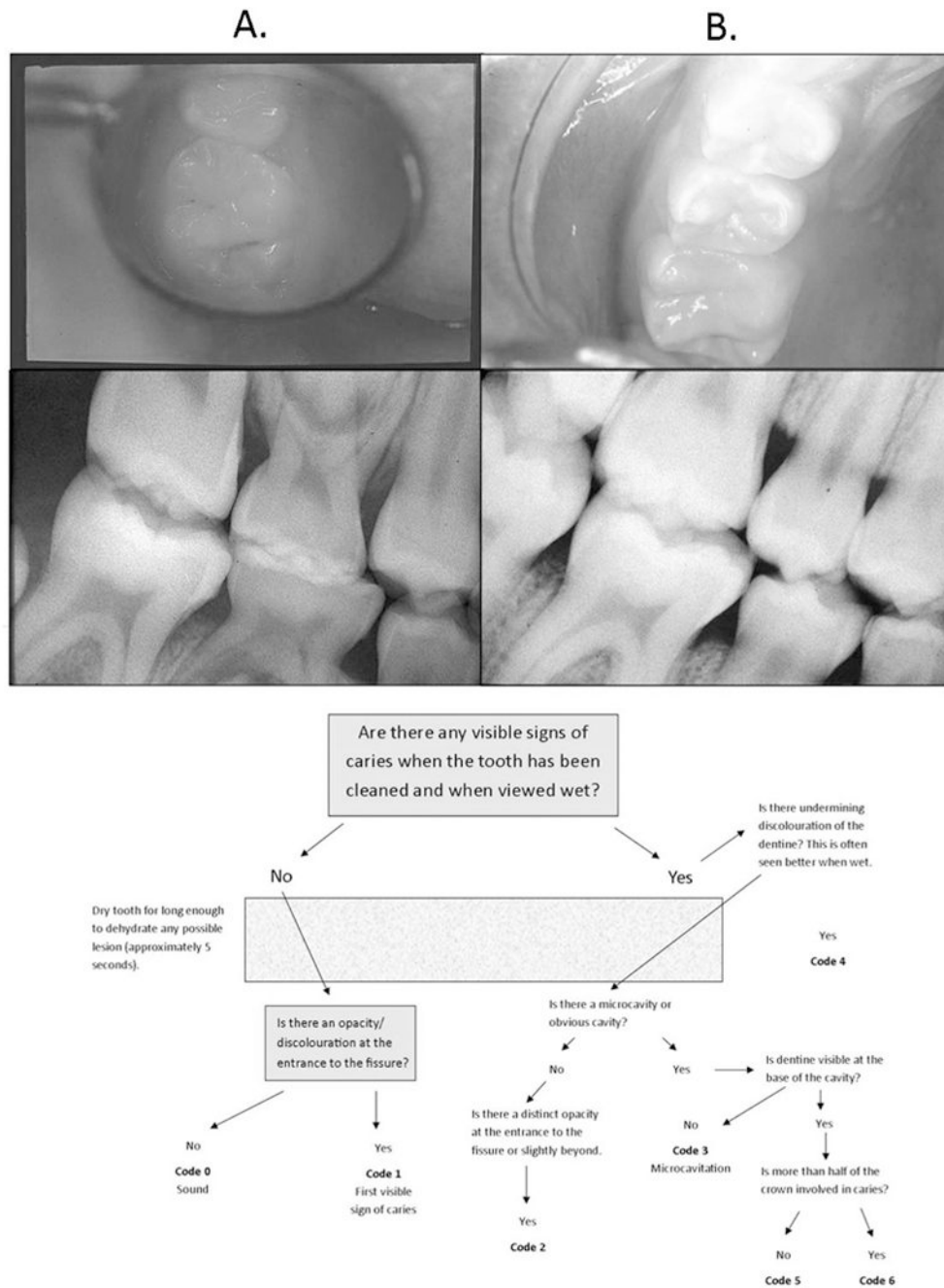


Figure 3. Questionable carious lesions can be managed with minimal intervention. A. 9-year old patient with a discoloration on an occlusal fissure of the right maxillary first molar. Radiographic exam showed no signs of dentine affection. The dental explorer was used gently and suggested the fissure was retentive. The mother of the child was instructed to carefully brush that tooth with an over the counter fluoridated toothpaste every day at least before the child going to bed. B. After 5 years, there are no signs of lesion progression, both clinically and radiographically, which suggests the decision of not performing restorative

treatment was for the benefit of the child. C. Diagram of the decision making tree of the ICDAS (The International Caries Detection and Assessment System. Modified from <https://www.icdas.org>). The surface of the tooth above would have been possibly coded as 3 in a larger scale study, even though it was sound.

Table 1

Summary of the candidate genes studied for caries in humans.

Gene	Function	Summary of Reported Results	References
Enamel Formation Genes			
Tuftelin (<i>TUFT1</i>)	Expressed in the developing and mineralized tooth and nonmineralizing soft tissues (Deutsch et al., 2002)	Associated with higher caries experience. This association can be dependent of the presence of <i>Streptococcus mutans</i>	Slayton et al. [2005]; Deeley et al. [2008]; Patir et al. [2008]; Shimizu et al. [2012]
		No evidence of association	Wang et al. [2012b]; Ergöz et al. [2013]; Jeremias et al. [2013]
Amelogenin (<i>AMELX</i>)	Involved in the mineralization during tooth enamel development	Associated with higher caries experience.	Deeley et al. [2008]; Patir et al. [2008]; Kang et al. [2011]; Shimizu et al. [2012]; Jeremias et al. [2013]
		No evidence of association	Slayton et al. [2005]; Olszowski et al. [2012]; Ergöz et al. [2013]; Gasse et al. [2013]
Enamelin (<i>ENAM</i>)	Involved in the mineralization and structural organization of the enamel	Associated with higher caries experience.	Patir et al. [2008]; Shimizu et al. (2012); Jeremias et al. [2013]
		No evidence of association	Slayton et al. [2005]; Deeley et al. [2008]; Olszowski et al. [2012]; Wang et al. [2012b]; Ergöz et al. [2013]
Tuftelin Interacting Protein 11 (<i>TFIP11</i>)	Thought to interact with tuftelin and can play a role in spliceosome disassembly in Cajal bodies (Stanek et al., 2008)	Associated with initiation of carious lesions and higher caries experience	Shimizu et al. [2012]; Jeremias et al. [2013]
		No evidence of association when DMFT scores are analyzed	Slayton et al. [2005]; Deeley et al. [2008]; Patir et al. [2008]; Shimizu et al. [2012]; Ergöz et al. [2013]
Ameloblastin (<i>AMBN</i>)	Involved in enamel matrix formation and mineralization	Associated with higher caries experience	Patir et al. [2008]; Shimizu et al. [2012]; Ergöz et al. [2013]
		No evidence of association	Slayton et al. [2005]; Deeley et al. [2008]; Jeremias et al. [2013]
Matrix Metalloproteinase 20 (<i>MMP20</i>)	Degrades amelogenin	Associated with higher caries experience in Whites with poor oral health habits	Tannure et al. [2012b]
		No evidence of association	Wang et al. [2012b]
Kallikrein-related peptidase 4 (<i>KLK4</i>)	Degrades enamel proteins	Associated with lower caries experience	Wang et al. [2012b]
Immune Response Genes			
CD14 molecule (<i>CD14</i>)	Mediates innate immune response to bacterial lipopolysaccharide	Absent in the saliva of individuals with active carious lesions	Bergandi et al. [2007]

Gene	Function	Summary of Reported Results	References
Human Leukocyte antigen; Major Histocompatibility Complex, class II, DR beta 1 (<i>HLA-DRB1</i>) and DQ beta 1 (<i>HLA-DQB1</i>)	Presents peptides derived from extracellular proteins	Frequency of allele 4 of DRB1 is increased in children with early childhood caries. Also allele 2 of DQB1 is increased in adolescents affected by caries. DRB1 allele 1 and DQB1 allele 3 frequencies are increased in the presence of <i>Streptococcus mutans</i>	Lehner et al. [1981]; Altun et al. [2008]; Bagherian et al. [2008]; Valarini et al. [2012]
		No evidence of association	de Vries et al. [1985]
Beta defensin 1 (<i>DEFB1</i>)	Antimicrobial peptide implicated in the resistance of epithelial surface to microbial colonization	Distinct <i>DEFB1</i> haplotypes are associated with low and high caries experience.	Ozturk et al. [2010]; Krasone et al. [2013]
Lactotransferrin (<i>LTF</i>)	Major iron-binding protein in milk and body secretions with antimicrobial activity	Associated with lower caries experience No mutations found in the promoter region	Azevedo et al. [2010]; Fine et al. [2013] Brancher et al. [2011]
Mucin 7 (<i>MUC7</i>)	Facilitates the clearance of bacteria in the oral cavity	Associated with higher caries experience with poor oral hygiene No evidence of association	Pol [2011] Buczowska-Radli ska et al. [2012]
Mannose-binding Lectin (protein C) 2, soluble (<i>MBL2</i>)	Recognizes mannose and N-acetylglucosamine on many microorganisms	Associated with higher caries experience No evidence of association	Olszowski et al. [2012] Yang et al. [2013]
Mannan-binding lectin serine peptidase 2 (<i>MASP2</i>)	Bactericidal factor that binds to the Ra and R2 polysaccharides expressed by certain enterobacteria	No evidence of association	Olszowski et al. [2012]
Saliva Genes			
Aquaporin 5 (<i>AQP5</i>)	Water channel protein that plays a role in the generation of saliva, tears, and pulmonary secretions	Associated with higher caries experience	Wang et al. [2012b]
Protein-rich Protein HaeIII subfamily 1 (<i>PRH1</i>)	Provide protective environment for the teeth	Associated with higher caries experience and colonization by <i>Streptococcus mutans</i>	Zakhary et al. [2007]
Other Genes			
Matrix Metalloproteinase 2 (<i>MMP2</i>)	Degrades type IV collagen	No evidence of association	Tannure et al. [2012a]
Matrix Metalloproteinase 9 (<i>MMP9</i>)	Degrades type IV and V collagens	No evidence of association	Tannure et al. [2012a]
Matrix Metalloproteinase 13 (<i>MMP13</i>)	Involved in endochondral ossification and bone remodeling. This genes is physically close to <i>MMP20</i> and maybe evolved at the same time before the divergence of ray-finned fish and lobe-finned fish (Kawasaki and Suzuki, 2011)	Associated with lower caries experience	Tannure et al. [2012a]
TIMP metalloproteinase inhibitor 2 (<i>TIMP2</i>)	Possibly critical to the maintenance of tissue homeostasis by suppressing the proliferation of quiescent tissues in response to angiogenic factors, and by inhibiting protease activity in tissues undergoing remodelling of the extracellular matrix	No evidence of association	Tannure et al. [2012a]

Gene	Function	Summary of Reported Results	References
Taste Receptor, type 2, member 38 (<i>TAS2R38</i>)	Controls the ability to taste glucosinolates	Associated with lower caries experience	Wendell et al. [2010]
Taste Receptor, type 1, member 2 (<i>TAS1R2</i>)	Sweet taste receptor	Associated with both lower and higher caries experience	Wendell et al. [2010]; Kulkarni et al. [2013]
Guanine nucleotide binding protein, alpha transducing 3 (<i>GNAT3</i>)	Believe to be involved in dietary preferences	No evidence of association	Wendell et al. [2010]
Solute carrier family 2 (facilitated glucose transporter), member 2 (<i>SLC2A2</i>)	Mediated facilitated bidirectional glucose transport	Associated with higher caries experience	Kulkarni et al. [2013]
Dentin sialophosphoprotein (<i>DSPP</i>)	Involved in the mineralization process of dentin	Associated with lower caries experience	Wang et al. [2012b]
Secreted phosphoprotein 1 (<i>SPP1</i>)	Involved in the attachment of osteoclasts to the mineralized bone matrix	No evidence of association	Wang et al. [2012b]
Carbonic anhydrase IV (<i>CA4</i>)	Involved in respiration, calcification, acid-base balance, bone resorption, and formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid	No evidence of association	Yarat et al. [2011]

Table 2

Summary of genome-wide studies of dental caries (DMFT/dft=decayed, missing, filled teeth; DMFS/dfs=decayed, missing filled surfaces).

Reference and Study Population	PHENOTYPE	RESULTS		Follow-up studies: locus (gene), reference
		Significant ^b Locus (gene)	Suggestive ^c Locus (gene)	
GENOME-WIDE LINKAGE STUDIES (GWL)^a				
Vieira et al. [2008] 46 Filipino extended kindreds, 642 individuals	DMFT, categorical ^d		Low caries experience: 5q13.3 14q11.2 Xq27.1	1) 5q13.3 (<i>BTF3</i>), Shimizu et al [2013] 2) 14q11.2 (<i>TRAV4</i>), Briseño-Ruiz et al [2013] 3) 13q31.1 (intergenic), Kùchler et al. [2013]
			High caries experience: 13q31.1 14q24.3	
GENOME-WIDE ASSOCIATION STUDIES (GWAS)^a				
Shaffer et al. [2012] 1,305 U.S. White children, aged 3-12	Primary dentition, categorical, dft 1		1q42-q43 (<i>ACTN2</i> , <i>MTR</i> , <i>EDARADD</i>) 11p13 (<i>MPPED2</i>) 17q23.1 (<i>LPO</i>)	1) No significant replication in 1,695 Danish children ages 2-7 years; Shaffer et al. [2012] 2) Significant replication, utilizing gene set enrichment; Wang et al [2013], see below in this table
	dft 1; population stratified by fluoride (sufficient or not) in household water		1p34 (<i>ZMPSTE24</i>) 6q16.1 (<i>EPHA7</i>) 22q12.1 (<i>TFPI1</i>)	
Wang et al [2012a] 7,443 U.S. White adults	Permanent dentition, categorical, DMFT 1	4q32 (<i>TLR2</i>) 5q11.1 (<i>ISL1</i>) 6q27 (<i>RP56KA2</i>) 7q21 (<i>FZD1</i>) 14q22 (<i>CDKN3</i> , <i>CNIH</i> , <i>GMFB</i> , <i>CGRRF1</i> , <i>BMP4</i>)	1q42.11-q42.3 (<i>RHOU</i>) 4q13.3 (<i>ADAMTS3</i>) 8p21.1 (<i>PTK2B</i>)	
Shaffer et al. [2013a] 920 US White adults (17-75; a subset of subjects from Wang et al. [2012a])	Five quantitative permanent dentition DMFS scores (DMFS1-5) derived from hierarchical cluster analysis ^e (Shaffer et al. [2013b])	DMFS2^e: 10p 11.23 (<i>LYZL2</i>) DMFS5^e: 1p36 (<i>AJAP1</i>) with	DMFS2^e: 4q31.22 (<i>EDNRA</i>) DMFS5^e: 1p36 (<i>SPSB1</i>) 2p24 (<i>TRIB2</i>) 4p15 (<i>PROM1</i>) 4q22 (<i>ABCG2</i> , <i>PKD2</i> , <i>SCPP</i>) 4q31.22 (<i>EDNRA</i>) 7q22 (<i>ATXN7</i>) 17q11 (<i>WSB1</i>)	

Reference and Study Population	PHENOTYPE	RESULTS		Follow-up studies: locus (gene), reference
		Significant ^b Locus (gene)	Suggestive ^c Locus (gene)	
Zeng et al. [2013] 1,017 US White adults (14-56; a subset of subjects from Wang et al. [2012a])	Two quantitative permanent dentition DMFS scores (pit/fissure, smooth) ^f	Smooth^f: 8q21.3 (no gene)	Pit/Fissure^f: 7p15-13 (<i>INHBA</i>) 8q21.3 (<i>no gene</i>) Xp11.4 (<i>BCOR</i>) Smooth^f: 2q35 (<i>CXCR1</i> , <i>CXCR2</i>) Xq26.1 (<i>BCORL1</i>)	
Zeng et al. [2013, in press] 1,006 White children (3-14), a subset of children included in Shaffer et al. [2012]	Two quantitative primary dentition dfs scores (pit/fissure, smooth) ^f	Pit/fissure^g: 3q26.1 (<i>KPNA4</i>)	Pit/fissure^g: 11p14.1 (<i>MPPED2</i>) Smooth^g: 1p36 (<i>AJAP1</i>) 6q27 (<i>RP56KA2</i>) 16p11.2 (<i>ITGAL</i>) 20q11.21 (<i>PLUNC</i>)	
GENOME-WIDE ASSOCIATION STUDIES (GWAS)^a WITH GENE SET ENRICHMENT				
Wang et al. [2013] 1,142 US White children (3-12), a subset of children included in Shaffer et al. [2012]	Categorical primary dentition dfs score, dfs 1 adjusted for age	GENE SETS^h: 1) Cytokine secretion (18, including <i>INS</i>) 2) Ligase activity forming carbon nitrogen bonds (68, including <i>WWP2</i> , <i>RNF217</i>) 3) Ubiquitin protein ligase activity (49) 4) Protein secretion (32) 5) Regulation of protein secretion (22) 6) Regulation of cytokine secretion (16) 7) Regulation of axonogenesis (10) 8) Regulation of neurogenesis (14, including <i>ROBO2</i> , <i>SLIT2</i>) 9) Central nervous system development (122) 10) Small conjugating protein ligase activity (51) 11) Glycoprotein catabolic process (12) 12) Axonogenesis (43) 13) Cell matrix junction (16; includes <i>ACTN2</i>)	Multiple suggestive gene sets, see Wang et al. [2013]	

^a genome-wide linkage=GWL, utilizes techniques of genetic linkage analysis and a panel of multi-allelic markers evenly spaced throughout the genome; genome-wide association=GWA, utilizes standard association analysis methods to detect relationship between a trait and anonymous bi-allelic SNP variants

^b "significant"= genome-wide significant, i.e. for GWL: LOD scores > 3.4; for GWA: p-value < 10⁻⁷, additional adjustments for multiple phenotypes are also done

^c "suggestive": for GWL: LOD scores between 2-3.4; for GWA p-value between 10⁻⁵ and 10⁻⁷

^d Categories (see Vieira et al. [2008]) in children (< 13): very low caries experience (DMFT=0-1), low (DMFT=2), moderate (DMFT=3-4), high (DMFT > 5); in teenagers (13-18): very low (DMFT=0-2), low (DMFT=3-5), moderate (DMFT=6-8), high (DMFT > 9); in adults: very low (DMFT=0-5); low (DMFT=5-8); moderate (DMFT=9-13), high (DMFT > 14)

^e Hierarchical clusters (see Shaffer et al. [2013b]): DMFS1 (DMFS scored from the occlusal surfaces of molars; heritability (h²)=27%, p=0.06), DMFS2 (mandibular incisors, canines and first premolars; h²=54%, p=0.002), DMFS3 (molars and maxillary premolars excluding occlusal molar surfaces; h²=43%, p=0.004), DMFS4 (maxillary incisors; h²=0, p=0.5) and DMFS5 (canines and premolars; h²=40%, p=0.008).

^fDMFS calculated on pit and fissure surfaces, and DMFS calculated on smooth surfaces, with each score adjusted for age, sex, presence of *Streptococcus mutans*, and home water fluoride level

^gdfs calculated on pit and fissure surfaces adjusted for age and age², dfs on smooth surfaces adjusted for age

^hGene sets are combination of genes that are functionally related (terms used here are from the Gene Ontology of Ashburner et al. [2000]); the number in parenthesis is the number of genes tested within the set [Wang et al. 2013]—selected gene names with known/suspected involvement in oral or dental phenotypes are also listed. Terms included in this table are those significant after adjustment for multiple testing (FDR < 0.05) under at least one of four statistical methods utilized for the gene-set analyses (GenGen, ALLIGATOR, SNP Ratio Test, Mixed Model, see Wang et al. [2013]). Bolded terms are those that were significant under more than one statistical method.