ORAL DISEASES

Oral Diseases (2010) 16, 613–623. doi:10.1111/j.1601-0825.2010.01675.x

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REVIEW ARTICLE

A critical review: an overview of genetic influence on dental caries

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Dental decay is a complex, chronic disease and one of the most common illnesses in dentistry today. Several dental decay risk factors have been identified during the last years; however, these variables alone may not entirely explain the disease development. Genetic research applied to dental decay began in the 1930s with experimental reports in animals and human observational research. Only recently, have some studies begun to search for genetic polymorphisms in humans and apply linkage analysis. However, due to the complex characteristics of the disease, the strong influence from several biological and environmental factors, and the small number of genetic studies related to dental caries, the genetic basis still requires further study. Therefore, the aim of this review is to provide a brief description of the current methodology for genetic analysis of complex traits, followed by a comprehensive evaluation of the literature related to genetic susceptibility/resistance to dental decay and a discussion of different aspects of the applied methodology. Advances towards the elucidation of the dental decay genetic basis may contribute to the understanding of the disease etiopathogenesis and to the identification of high risk groups, thus providing potential targets for effective screening, prevention and treatment.

Oral Diseases (2010) **16**, 613–623

Keywords: dental caries; genetics; genetic epidemiology; susceptibility; inheritance

Genetic analysis of complex traits

Genetic human diseases are classified into two categories: (i) Mendelian diseases, and (ii) complex diseases. Mendelian diseases are rare and generally caused by variations in a single gene (monogenic). They present a perfect correlation between genotype and phenotype. A complex disease is the result of an interaction between genetic (often polygenic) and non-genetic factors (Sørensen *et al*, 1988; Strachan and Read, 2002). Dissecting the genetic component of a complex disease is not, however, a trivial task. Understanding the genetic basis of susceptibility to these frequent diseases could have a profound impact on public health. Examples of complex diseases with a known genetic component include infectious disorders such as leprosy (Mira *et al*, 2003, 2004; Ranque *et al*, 2005, 2007; Mira, 2006; Moraes *et al*, 2006; Alcais *et al*, 2007), tuberculosis (Fieschi *et al*, 2003; Remus *et al*, 2004; Baghdadi *et al*, 2006), and oral diseases such as periodontitis (de Brito Junior *et al*, 2004; Souza *et al*, 2005) and dental decay (Finn and Caldwell, 1963; Beck and Drake, 1975; Boraas *et al*, 1988; Conry *et al*, 1993; Bonecker and Cleaton-Jones, 2003).

Genetic analysis of complex diseases takes into consideration all genetic and non-genetic factors that influence disease development. Generally, the first step is to determine whether or not there is a genetic component influencing the disease development (Figure 1). At this stage, the main strategies are experimental: application of animal models and controlled crossbreeding, and observational: applicable to human populations, such as familial aggregation and twin studies. Typically, animal studies use inbred strains presenting extreme known phenotypes to analyze the result of controlled crossing, correlating genotypes and phenotypes (Kanamoto *et al*, 1994). Familial aggregation analysis investigates the clustering of disease cases in large pedigrees resulting from excessive sharing of genetic and/or environmental variants (Burton *et al*, 2005; Hopper *et al*, 2005). When conducting such type of genetic analysis, no attempt is made to determine the cause of aggregation; the sole objective is to observe clusterization. The rationale for twin studies is straightforward: monozygotic twins (MZ) share 100% of genes and dizygotic twins (DZ) share 50% of their genes. Importantly, twins normally share the same habits and environment during their first years, thus, any

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Received 25 September 2009; revised 3 December 2009; accepted 21 December 2009

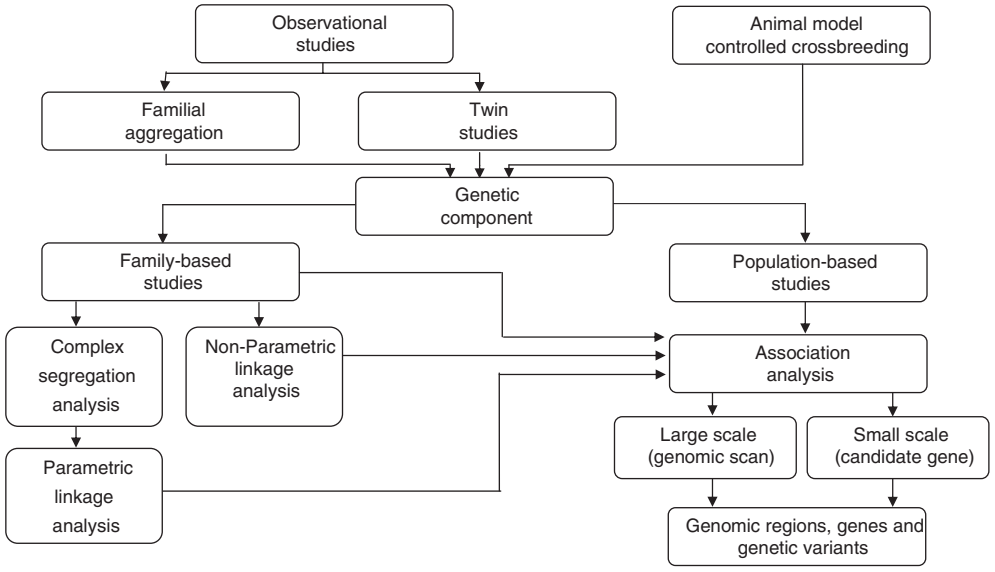

Figure 1 Flowchart – strategies for genetic epidemiologic investigation

epidemiologic influence should be minimized (Shuler, 2001; Brathall and Hänsel, 2005; Townsend *et al*, 2008). A sophistication of the design is to use twins reared apart, generating even more powerful results (Boraas *et al*, 1988; Conry *et al*, 1993). These studies compare intra-class correlation between MZ and DZ twin pairs and/or calculate the heritability (the proportion of the phenotypic variability due to genetic variance).

To obtain genetic parameters not detected when conducting observational studies, a complex segregation analysis (CSA) can be developed. CSA is a specific familial aggregation analysis, focusing on the pattern of aggregation within families (Burton *et al*, 2005). CSA includes an analysis of pedigrees (Hassel, 1995), and is generally carried out before and to justify costly molecular studies. It is defined as a statistical methodology to identify the transmission of inherited traits for a particular phenotype using family data, and to elucidate genetic effects (Elston and Stewart, 1971). In a CSA, information regarding pedigrees and phenotype data is analyzed using the maximum likelihood test. The analysis includes and compares genetic mechanisms (monogenic/polygenic; dominant/recessive), allelic frequencies and penetrances, in order to obtain the best likelihood among all variables and tested models (Thomas, 2004; Burton *et al*, 2005). The parameters of the genetic model defined by a CSA are potentially useful in subsequent genetic mapping studies. However, the method does present some limitations, for instance it cannot produce information regarding the exact genetic nature of all genes and sequence variations involved.

DNA-based strategies for genetic epidemiology studies usually involve linkage and/or association analysis.

Linkage analyses are family-based studies designed to locate chromosome regions that may contain genes related to the study disease (Dawn and Barrett, 2005). The goal is to find non-random segregation of chromosomal loci and phenotypes of the disease being mapped. If genetic and disease markers co-segregate, regions containing candidate genes for the disease are identified (Figure 2). The two methods of conducting linkage analysis are parametric (model-based) and non-parametric (model-free). For parametric analysis, it is necessary to specify parameters of the genetic model involved, obtained from the CSA (Burton *et al*, 2005). Non-parametric linkage analysis is performed when it is not possible to infer a genetic model by CSA. Statistical significance is defined by the logarithm odds (LOD) score method, reaching significance with a score of greater than 3.0 for candidate region analysis, and 3.3 for genome-wide studies (Thomas, 2004). Linkage analysis is a powerful tool to locate genomic regions exerting strong but not moderate or weak effects over the phenotype (Risch, 2000). The advantage of this approach is the fact that it allows for a genome-wide

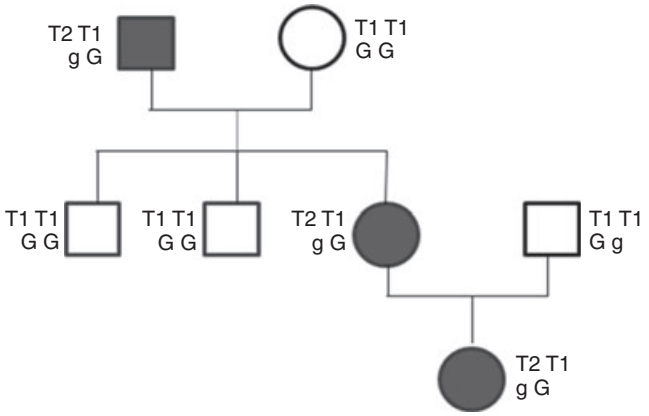

Figure 2 Linkage analysis – non-random segregation between the disease locus (T2) and marker which location is known (g)

search with a relatively small number of markers (approximately 400). The main limitation of linkage analysis is that it normally locates a chromosomal region spanning across several megabases and a number of genes. In order to pinpoint the precise gene and the variants causing the genetic effect, methods with greater sensitivity, such as association analysis are required.

Association analysis (family or population-based) is applied to identify the precise genetic variants related to disease development (Cordell and Clayton, 2005). The objective of genetic association analysis is to identify differences of allele frequencies across affected and unaffected population samples. When positive association is detected, three distinct possibilities are presented: (i) the causal allele was found; (ii) the associated allele is itself associated with the causal allele, a phenomenon known as linkage disequilibrium (LD); in this case, the effect of the latter can be indirectly tested by genotyping the former; (iii) association is sporadic due to chance or population stratification, i.e. the existence of cryptic differences in the genetic background of the population sample. To overcome population stratification bias, association analysis using a family-based design can be applied. The objective of this method is to monitor disease allele transmission using family trios composed by the two parents and one affected child. Association is identified when an allele is over or under-transmitted from heterozygous parents to the affected offspring, as detected by the transmission disequilibrium test (TDT, Figure 3). Association analysis is the ideal approach for identifying genes that generate moderate to small effects, and can be conducted both in a small scale candidate gene study, and a large scale, genome-wide association study (GWA). In candidate gene analyses, genes are selected from functional and positional-cloning studies. In large-scale, hypothesis-generating studies, new, unsuspected genes can be found. However, these studies demand approximately 500 000 markers to cover the entire genome, as well as a large population sample. To date, the challenge of GWA is to find an adequate approach for handling and analyzing the data and for discrimination of true association from signals generated by chance, due to the tremendous number of tests applied. Replication of findings on a series of independent populations has become an increasingly accepted strategy to validate results from GWA studies.

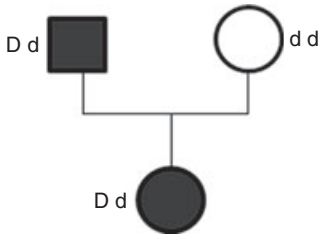

Figure 3 Transmission disequilibrium test (TDT) observes the number of times the heterozygous father Dd transmits the allele D or d to her daughter

Dental caries as a complex trait

Dental caries is a complex, chronic, multifactorial disease (Fejerskov, 2004; Department of Health and Human Services, 2005) and one of the most common diseases in Dentistry, together with periodontal disease and malocclusion (Fejerskov, 2004; Brathall and Hänsel, 2005). Dental decay has an important role in the manifestation of tooth pain and loss, and has been associated with problems in school and absenteeism in the workplace (Fejerskov, 2004; World Health Organization, 2004), leading to a decrease in quality of life (Petersen, 2003). Moreover, oral health presents a close association with the individual's general health, and may be a risk factor for several diseases (Petersen, 2003).

The index recommended by the World Health Organization (WHO) (World Health Organization, 2004) for caries estimation is the Decayed, Missing and Filled Teeth (DMFT) index (Klein and Palmer, 1940; Petersen, 2003). The DMFT index has been generally decreasing over the last few years in developed and developing countries. According to WHO (World Health Organization, 2004), the DMFT index in children of 12 years of age was higher than 3.0 in 49% of the 184 reporting countries in 1980. In 2000, this value for children of the same age was equal to or less than 3.0 in 68% of the same countries studied (World Health Organization, 2004), reflecting a decline of the disease over the last 20 years, even in the developing countries (Peterson, 2005). Although such information may appear satisfactory for health professionals, according to the WHO, this disease still affects from 60% to 90% of children at school age as well as the majority of adults and is the most prevalent mouth disease in many countries (World Health Organization, 2004).

Numerous studies have shown that the global decline in prevalence of dental caries is occurring non-homogeneously throughout many countries (Bonecker and Cleaton-Jones, 2003; World Health Organization, 2004). Although strategies such as the use of topical fluoride, sealants and diet control have been developed to prevent dental decay, their effectiveness in eliminating the disease is not well established (Petersen, 2003; Dye *et al*, 2007). Dental caries, early tooth loss and edentulism seem to concentrate in specific groups of individuals. This phenomenon, termed *polarization* (Pine, 2005), has been exhaustively discussed, but its cause still remains obscure.

Since the 1960s, dental decay has been suggested to be the result of the interaction of four major factors: biofilm, diet, time, and host (Keyes, 1960, 1962; Evans *et al*, 1993). When biofilm is exposed to highly fermentable carbohydrates, cariogenic bacteria are selected, modifying the biofilm composition (Cury *et al*, 2000; Nobre dos Santos *et al*, 2002). The main reported cariogenic bacteria are *Streptoccocus mutans (S. mutans), S. sobrinus* and some species of *Lactobacillus* (Keyes, 1962; Feathrstone, 2004). Continuous exposure to acids produced by these bacteria could lead to dental decalcification (Feathrstone, 2004). Thus, when a more cariogenic biofilm occurs and the host buffer capacity cannot compensate

the acid attack of the bacteria, dental cavities may appear. Also, salivary flow and saliva composition are important to the biofilm etiopathogenicity (Lenander-Lumikari and Loimaranta, 2000). Moreover, for the past four decades, different authors have described gender, ethnicity and age as additional risk factors for the disease progress (Evans *et al*, 1993; Antunes and Peres, 2006).

Environmental factors, such as behavioral habits (Fejerskov, 2004), may also influence the development of dental decay. Low socioeconomical status (SES) is a non-biological risk factor which is often related to educational level, the perception of the individual about his/her own health, life style, dietary composition, and access to dental care (Antunes and Peres, 2006; Bastos *et al*, 2007). All these factors play a role in the development of dental caries. Hygiene habits are also correlated with the educational level and SES (Adair *et al*, 2004), and the frequency of tooth brushing has been shown to influence the amount of caries (Chesters *et al*, 1992). Access to fluoridated water is another variable contributing to the decline of tooth decay (Krasse, 1996; Antunes and Peres, 2006; Griffin *et al*, 2007). Family size can be considered a risk factor for dental decay: individuals from large families have a greater probability of presenting high DMFT values (Evans *et al*, 1993).

However, the combination of all the factors mentioned above does not entirely explain disease outcome. Individuals exposed to the same levels of environmental risk factors present differences in the DMFT index (Pine, 2005). Those differences may be due to the fact that environmental factors can be more cariogenic for some than for others, suggesting an influence of genetic factors in the etiopathogenesis of dental caries.

Genetic analysis of dental caries

Bacteria genetic studies

The question about the genetic influence in dental decay has been discussed since the 1920s (Bachrach and Young, 1927). From the 1970s through the 1990s the great majority of studies concerning genetic aspects of caries searched for gene variants in cariogenic bacteria (Macrina *et al*, 1990). The involvement of *S. mutans* and its different genotypes in susceptibility to dental decay has been widely studied, and many *S. mutans* strains have already been identified as having influence on the disease (Napimoga *et al*, 2005). One example involves results obtained from two studies with *S. mutans*, in which the authors identified genetic changes able to encode the proteins involved in biofilm development (Wen and Burne, 2002; Cheryl *et al*, 2005). These polymorphisms were associated with an increment of biofilm virulence, with impact on dental decay risk.

In a more recent approach, innate host-related variables have been added to this complex scenario. For example, the relationship among human leukocyte antigen (HLA) class II and *TNFA* alleles, levels of oral bacteria that play a role in the etiology of dental caries, and the DMF surface (DMFS) index was investigated in Afro-American women (Acton *et al*, 1999). The results support the hypothesis of an association between host HLA class II and *TNFA* genetic profile with colonization of *S. mutans*, *L. casei*, and *L. acidophilus*.

Host genetic studies

Genetic studies have been conducted to better comprehend the genetic component associated with the individual susceptibility to dental decay development. The approaches which have been used include: (i) experimental studies involving animal models and controlled crossbreeding (Hunt *et al*, 1944, 1955; Kanamoto *et al*, 1994); (ii) observational studies involving human populations, such as familial aggregation analysis (Garn *et al*, 1976a) and twin studies (Finn and Caldwell, 1963; Boraas *et al*, 1988; Conry *et al*, 1993; Sofaer, 1993; Hassel, 1995; Shuler, 2001), and (iii) linkage and association studies (Slayton *et al*, 2005; Bagherian *et al*, 2008; Deeley *et al*, 2008; Patir *et al*, 2008; Vieira *et al*, 2008).

Experimental studies in animals

Studies with different mice strains support the hypothesis that differences in susceptibility to caries could be due to hereditary factors (Steggerda and Hill, 1936). The observation that some mice from genetically heterogeneous populations differed in the disease experience under the same environmental conditions suggested the existence of dental decay susceptibility (Hunt *et al*, 1944) and resistance (Hunt *et al*, 1955). Animals strains are selected and crossed based on their level of susceptibility/resistance to disease so that the trait can be traced over the next generations. In the classic Hunt–Hoppert studies (Hunt *et al*, 1944, 1955), 35 days were necessary for the development of carious lesions in the susceptible strain as opposed to 505 days for the same effect in the resistant strain. These results strongly suggest the influence of genetic differences between mice strains in controlling caries progression. In a Harvard study also comparing susceptible and resistant lines, caries lesions were almost ten times more extensive in susceptible mice than in resistant animals at the age of 110 days (Willett *et al*, 1958). However, when the mice were exposed to a high cariogenic diet, the difference between the two lines decreased (Larson, 1965; Larson *et al*, 1968). In the 1990s, Kanamoto *et al* (1994) also demonstrated the genetic influence in caries scores of molar teeth, when comparing four inbred strains of male mice which were inoculated with *S. mutans* and fed with a cariogenic diet. Other studies started to identify genomic regions and polymorphisms related to susceptibility and/or resistance variations (Kurihara *et al*, 1991; Quivey *et al*, 2005). Research on mice showed linkage between dental decay and chromosome 2, 8 (Nariyama *et al*, 2004) and 17 (Suzuki *et al*, 1998), where the mice MHC complex is localized. Matsumoto *et al* suggested that the *E2f1* gene, which mutation cause a decreased volume of saliva production and protein production rate, affected susceptibility for oral biofilm formation by streptococci (Matsumoto *et al*, 2004). A study with *Aqp–/–* knockout mice showed a relationship between this gene and the reduction of salivary flow, as

well as an increase in caries, mainly in buccal and succal surfaces (Culp *et al*, 2005). Finally, another study comparing MRL/l and MRL/n strain mice, for which the former possess a lymphoproliferative gene inducing swelling of systemic lymph nodes, investigated whether the salivary immune response caused by *S. mutans* infection prevented dental caries in the two strains. The results showed a difference between strains, indicating that the salivary immune response may be an important factor in regulating dental decay development (Maeda *et al*, 1995).

Observational studies

Familial aggregation studies. Several studies of familial aggregation in caries have been reported since the 1930s, as observed in Table 1. As a result, a solid body of evidence was created indicating familial clustering of caries experience and allowing for speculation whether or not there are genetic factors controlling the disease. One of the first studies, reported in 1938, investigated dental decay correlation in siblings (Klein and Palmer, 1938). Students at 10 years old were classified into two groups: no caries and having a DMFT of six or more. The siblings of caries-free children had lower average caries scores than the siblings of the susceptible children. A larger study involving 16 000 sibpair participants in the Ten-State Survey was conducted. DMFT correlation was estimated and gender, ethnicity and age (ranging from 7 to 18 years old) were used for stratification. Sibling correlation was found ($r = 0.23$ to 0.41), presenting higher correlations between black and older sibpairs, however no evidence was described for ethnicity influence (Garn *et al*, 1976a).

Investigations were also conducted to observe familial aggregation and the relationship to caries between parents and children. A study in 1953 collected data from caries-free and no caries-free males enlisted for military service and related individuals. Parents and siblings of the caries-free subjects had a significantly lower caries index than the parents and siblings of the non caries-free group (Book and Grahnen, 1953). In a natural fluoride area, children reflected the parents' caries experience in a study including 5400 individuals. Even with the fluoride exposure, both groups (high and low level of caries) had differences in the degree of susceptibility, demonstrating that fluoride does not decrease the genetic risk (Klein, 1946). A recent study conducted in Quebec with mother-child pairs composed of 6039 dentate and 264 edentulous mothers showed that children from the latter were more likely to experience caries on both primary and permanent dentitions when compared with children of dentate mothers. In the same study, environmental factors (socio-economic status, age, gender, and children's

Table 1 Evidence for genetic influence to dental decay susceptibility through observational studies

Reference	*Study population* (N)	*Findings*
Klein and Palmer, 1938	Siblings (4416)	Similarity in caries rate between siblings
Klein, 1946	Parents and Children (5400)	Offspring dental disease quantitatively related to parents experience
Klein, 1947	Parents and Children (-)	Similarity in caries rate between parents and children
Book and Grahnen, 1953	Parents and Siblings (317)	Correlation between siblings and parents of caries-free individuals
Garn *et al*, 1976b	Parents and Children (6580)	Mother-child similarities in the DMFT scores are systematically higher than father-child
Garn *et al*, 1976a	Siblings (16000)	Positive siblings correlation
Garn *et al*, 1977	Spouse Pairs (1800)	Positive spouses DMFT correlation
Maciel *et al*, 2001	Mothers and Children (-)	Positive mother and children correlation in relation to patterns of sweetness preference and caries experience
Bedos *et al*, 2005	Mother and Child (-)	Positive correlation between edentulous mother and their children
Bachrach and Young, 1927	MZ (130) DZ (171)	No differences between the MZ and DZ twin pairs
Horowitz *et al*, 1958	MZ (30) DZ (19)	MZ more alike caries experience than DZ twin pairs
Mansbridge, 1959	MZ (96) DZ (128)	MZ twin pairs with greater similarity in caries experience
Goodman *et al*, 1959	MZ (19) DZ (19)	Intrapair variance of DZ greater than MZ
Finn and Caldwell, 1963	MZ (35) DZ (31)	MZ and DZ differences greater for smooth surface caries in anterior teeth.
Bordoni *et al*, 1973	MZ (17) Unrelated Controls (-)	Greater similarity in tooth morphology and eruption timing in primary teeth between MZ than unrelated controls.
Gao, 1990	MZ and DZ (280)	Higher correlation in MZ, but not statistically significant
Conry *et al*, 1993	MZ (46) DZ (22) reared apart	MZ with greater within-pair similarity than DZ pairs for: teeth present, teeth present excluding third molars, teeth restored, teeth restored index, surfaces restored, surfaces restored index and surfaces restored or carious, in reared apart twin pairs
Boraas *et al*, 1988	MZ and DZ (44) reared apart	Resemblance within MZ for number of teeth present, percentage of teeth and surfaces restored, percentage of teeth and surfaces restored or carious, tooth size, and malalignment
Liu *et al*, 1998	MZ and DZ (82)	Strong evidence of genetic influence to third molar presence, tooth size, arch size, and upper lateral incisor malformation
Bretz *et al*, 2005a	MZ (142) DZ (246)	For surface-based caries prevalence rates the heritability was strong - 76.3; for lesion severity the heritability was also strong – 70.6
Bretz *et al*, 2005b	MZ (112) DZ (202)	For surface-based caries prevalence rates the heritability was moderate ($H = 30.0$) and greatest for the oldest groups ($H = 46.3$); for lesion severity the heritability was also moderate ($H = 36.1$) and greatest for the youngest group ($H = 51.2$)

Table 2 Evidence for genetic influence to dental decay susceptibility through linkage and association study

Reference	*Study population (N)/type of study*	*Candidate region(s)/gene(s)*	*Findings*
Slayton *et al* (2005)	Children dmfs > 4 (92) and dmfs = 0 (343)/Case-Control	*AMELX, AMBN, TUFT1, ENAM, TFIP11, and KLK4*	Tuftelin gene and high level of *S. mutans*, associated with susceptibility to dental decay
Pehlivan *et al* (2005)	Children caries-free (40) and with carious teeth (42)/Case-Control	MBL	No significant difference between two groups and genotypes distribution.
Zakhary *et al* (2007)	Adult Caucasians (60); Children of: Caucasian Parentage (89), African-American Parentage (96), and Mixed Parentage (23)/Case-Control	PRH1 locus (Db)	Db-negative Caucasians had significantly more caries
Bagherian *et al* (2008)	ECC children (44) and Caries-free children (35)/Case-Control	HLA-DRB1 and HLA-DQB1	HLA-DRB1*04 was associated with ECC susceptibility
Deeley *et al* (2008)	DMFT ≤ 2 (44) and DMFT ≥ 3 (66)/ Case-Control	*AMELX, AMBN, TUFT1, ENAM*, and *TFIP11*	Strong association of AMELX with DMFT ≥ 20 and increased age-adjusted
Patir *et al* (2008)	dmfs ≥ 4 (91) and dmfs = 0 (82)/ Case-Control	*AMELX, AMBN, TUFT1, ENAM*, and *TFIP11*	TUFT1 overrepresentation of T allele and AMELX overrepresentation of the C allele
De Soet *et al* (2008)	5 groups: caries free (53); full dental treatment (75); extraction only (66); ART filling only (77); and no treatment (77)/Case-Control	*CD14-260*	Protection effect of the *CD14*-260 TT genotype for AFF in children with dmft + DMFT ≥ 4 at baseline
Peres *et al* (2009)	Children (245) caries free and with caries Case-Control	*CA6*	Positive association between buffer capacity and the rs2274327 (C/T) polymorphism
Vieira *et al* (2008)	46 families/Genome-Wide Linkage Analysis	–	Five suggestive *loci* were identified: – 3 for low caries susceptibility (5q13.3, 14q11.2, and Xq27.1) – 2 for high caries susceptibility (13q31.1 and 14q24.3)

oral-health-related behaviors) were also assessed but did not show significant influence (Bedos *et al*, 2005).

In contrast, the observation that spouse-pairs share similar DMFT scores suggests that variables such as household, diet and environmental stress are also determinants for the outcome of caries disease (Garn *et al*, 1977).

Twin studies. Numerous twin studies for caries have been reported, as observed in Table 1. High concordance rates between MZ twins for several dental phenotypes such as dental decay, tooth size, dental arch dimensions, intercuspal distances and occlusal traits have been described (Townsend *et al*, 2003, 2008). Horowitz *et al* (1958), using matched pairs of MZ and DZ twins, concluded that MZ twins had greater caries concordance ($P < 0.001$). Fairpo (1979), working with 100 MZ and 120 DZ twin pairs, concluded that there was genetic influence in the susceptibility to dental decay development in both deciduous and permanent teeth. In 2005, two studies using twin pairs estimated heritability, which measures the percentage of the phenotypic variance that is the result of genetic factors. Bretz *et al* (2005a) studied 388 twin pairs and heritability was estimated in 70% for the dental decay. The same population was studied once more after 12 months and the heritability value was again significant (H = 30% for the younger and 46.3% for the older twin pairs) (Bretz *et al*, 2005b). The higher concordance and heritability between MZ than DZ twins may demonstrate that there is a genetic factor influencing dental decay development; however, these results do not discard the influence of environmental factors. An alternative approach in twin studies in order to dissect the environmental component of heritability is to study twins who have been reared apart. This allows a more precise assessment of the inherited component controlling the phenotype. In caries, two twin studies using twin pairs reared apart demonstrated that MZ twins had higher similarity in incidence of dental decay than DZ twins, despite the fact that the individuals have been raised in different families, communities and/or even countries, a strong argument in favor of the existence of a genetic contribution (Boraas *et al*, 1988; Conry *et al*, 1993). Another advantage of these studies was that the patients' average age was over forty years old and all pairs had been separated shortly after birth.

In contrast, there are twin studies for which the results do not show significantly higher concordance rates for caries occurrence between MZ versus DZ pairs. Comparison between 82 pairs of female-female twins from 6 to 12 years showed strong evidence of genetic influence controlling third molar presence, tooth size, arch size, and upper lateral incisor malformation; while a weak heritability was seen in tooth eruption and caries (Liu *et al*, 1998). Bordoni *et al* (1973) worked with a sample of 17 MZ twins and 17 unrelated controls and concluded that a genetic component is more important in tooth morphology and eruption timing than caries. In addition, a study with 280 pairs of twins demonstrated a higher concordance rate for caries in MZ twins, but the results were not statistically significant (Gao, 1990).

Linkage and association analysis

Animal studies. More recently, the aim of host genetic studies using animal models has shifted towards the identification of the polymorphisms associated with susceptibility and/or resistance (Kurihara *et al*, 1991; Quivey *et al*, 2005), and several candidate genomic regions and genes have been identified. Genetic linkage between dental decay and *loci* of chromosomes two, eight (Nariyama *et al*, 2004) and 17 (Suzuki *et al*, 1998), where mice major histocompatibility complex (MHC) is localized, was identified. A comparison between wild type and knockout mice for the *Mrl, a* lymphoproliferative gene that induces systemic swelling of lymph nodes, showed higher levels of caries disease in the knockout animals, indicating that the immune response may be an important factor in regulating dental decay development (Maeda *et al*, 1995). Caries-free monkeys showed increased serum antibody titres and proliferation of T lymphocytes when stimulated with *Streptococcus* (Lamb *et al*, 1980). Moreover, in caries resistant subjects, a lower dose of *Streptococcus* is necessary to stimulate T-helper activity (Lehner *et al*, 1981). The low-dose feature was associated with the specificity HLA-DRw while the high-dose was associated with HLA-DR4.

Human studies. A polygenic nature for the genetic control of caries disease has been discussed since the 1970s. Muhlemann (1972) suggested that a set of genes could influence the enamel resistance while a different set could influence the saliva composition and host response to infection. Nevertheless, only recently linkage and association studies have begun to be conducted in an attempt to identify genomic regions and polymorphisms related to dental caries (Table 2).

The first linkage analysis for dental decay was carried out in 2008. A genome-wide scan (392 markers) was performed aiming to identify genomic regions that might contain genes related to dental decay. The population was composed of 46 families (624 individuals), living in the same area in the Philippines. Five suggestive *loci* were identified: three for low caries susceptibility (5q13.3, 14q11.2, and Xq27.1) and two for high caries susceptibility (13q31.1 and 14q24.3) (Vieira *et al*, 2008). The authors highlight the presence of genes related to saliva flow control and diet preferences in regions 13q31.1, 14q24.3 and 14q11.2. Unfortunately, the authors applied parametric linkage analysis using parameters from a CSA for which details were not included in the report.

Although studies associating dental decay with genetic polymorphisms were initiated during the 1980s using animal models such as monkeys and mice, only in 2005 the first studies involving humans were published.

Genes related to enamel development and mineralization: *amelogenin (AMELX)*, *ameloblastin* (*AMBN*), *tuftelin* (*TUFT1*), *enamelin* (*ENAM*), *tuftelin-interacting protein* (*TFIP11*), and *kallikrein 4* (*KLK4*) have been investigated (Slayton *et al*, 2005). Markers of these candidate genes were tested for association following a case-control design using a sample of children from 3 to 5 years old. No evidence for association between caries occurrence and any independent investigated gene was observed. However, when performing a multivariate analysis, the effect of the *TUFT1* gene combined with the effect of high level of *S. mutans* increased the susceptibility to dental decay (Slayton *et al*, 2005). Another study also investigated *AMELX, AMBN, TUFT1, ENAM, and TFIP11* for association with caries in a population from Guatemala. Strong evidence ($P = 0.0000001$) for association was found for one *AMELX* marker with higher DMFT (DMFT $\geq$ 20) and increased age-adjusted caries experience (Deeley *et al*, 2008). The same gene was studied in a population sample from Istanbul and the findings confirmed the previous study (Patir *et al*, 2008). The authors concluded that the best-fitting model for increased dmfs is composed of a combined overrepresentation of specific alleles of a marker of *TUFT1* and a marker of *AMELX* in the case group.

A study using a population-based design investigated the influence of *mannose-binding lectin* (*MBL*) gene, which plays a critical role in the immune response in early childhood. Decreased blood levels of MBL may cause predisposition to infections and autoimmune diseases (Pehlivan *et al*, 2005). Polymorphisms in the *MBL* gene were analyzed and the overall genotype distribution did not significantly differ between caries-free and children with carious teeth. A different study investigated the association of HLA-DRB1 and HLA-DQB1 alleles with susceptibility to early childhood caries (ECC), a type of caries that affects the deciduous teeth of infants and toddlers. The authors found significant association between HLA-DRB1*04 and ECC (Bagherian *et al*, 2008). Yu *et al* (Yu *et al*, 1986) found an association between DMFS increase and saliva levels of a specific *proline-rich protein* (PRPs), a saliva component that influences the attachment of bacteria. A subsequent study investigating a gene related with the PRPs observed an association between dental decay and the Db allele, one of the three alleles of *PRH1* gene (Zakhary *et al*, 2007). The same study showed that Db negative Caucasians had significantly more caries than Afro-American Db negative patients, demonstrating the importance of ethnicity associated with genetic information. Another study involved the carbonic anhydrase VI gene (*CA6*), that encodes an enzyme that catalyzes the hydration of carbon hydroxide in saliva and other body fluids. The authors found no association between the alleles and genotype distribution of three polymorphisms in the coding sequences of *CA6* gene with caries experience. However, positive association between buffer capacity and the rs2274327 (C/T) polymorphism was found (Peres *et al*, 2009). Finally, a study involving a population sample of children, tested for an association between abscess or fistula formation (AFF), which may be caused when caries progresses into pulpal inflammation, with a polymorphism in the bacterial ligand *CD14* (–260), an immune factor

responsible for modulating the immune response. A *CD14* genotype was significantly associated with the presence of 4 or more carious lesions (De Soet *et al*, 2008).

Genetic analysis of dental decay - future perspectives

Several biological and environmental risk factors for dental decay development have been identified in the last few years. It is well known that dental decay is primarily determined by environmental factors; however, despite the use of different strategies to control these factors and prevent disease, caries is far from being controlled as a public health problem: dental decay continues to affect children and adults in both developed and developing countries, being one of the most important and prevalent mouth diseases (Burt, 1998; World Health Organization, 2004; Brathall and Hänsel, 2005). The characterization of high-risk individuals (Pine, 2005) may indicate that dental decay outcome can also be influenced by additional variables, such as the genetic background of the host.

When studying genetics of complex diseases, usually the first goal is to characterize the existence of a genetic component controlling phenotypes of the disease so that further comprehensive studies can be developed (Haile *et al*, 1985; Abel and Demenais, 1988; Wagener *et al*, 1988; Abel *et al*, 1995; Feitosa *et al*, 1995). Classic experimental studies using animal models have been effectively employed to demonstrate a genetic influence on caries outcomes, with differences between susceptible and resistant strains (Hunt *et al*, 1944, 1955). Observational studies in humans have identified a genetic impact over dental decay. Reports on individuals from the same family show that the correlation of the dental decay index between parents and siblings, as well as between sibpairs, follows a pattern; therefore, the disease does aggregate in families (Garn *et al*, 1976a,b, 1977). Twin studies have demonstrated that the concordance rate for caries occurrence increases as grows up the proportion of genome sharing between two individuals (Bordoni *et al*, 1973; Bretz *et al*, 2005a; b), even when the twins are reared apart (Boraas *et al*, 1988; Conry *et al*, 1993). All of these experimental and observational studies strengthen the hypothesis of a genetic influence in dental decay development. However, in the era of molecular genetics and genome-wide association studies, few advances have been made towards the dissection of the exact nature of the genetic component controlling susceptibility to dental caries. For example, to date, no clear heritage pattern has been estimated for dental caries. In this context, a well conducted CSA seems imperative to provide the first formal set of data to define the genetic inheritance model for caries, and to determine the best approaches for further genetic studies. Moreover, this genetic model could be included in parametric linkage analysis, a powerful hypothesis-generating tool that could indicate the genomic location of major loci controlling susceptibility to disease. Finally, a CSA would include not only genetic factors but also covariables such as SES, dental hygiene, dietary composition, which for a disease as complex as caries, may be of extreme importance.

To date, one single study has used both parametric and non-parametric linkage analysis to detect genomic regions containing candidate genes for dental caries. Unfortunately, the authors did not include the results of the CSA used for parametric linkage analysis in the report (Vieira *et al*, 2008). Nonetheless, for a protocol widely accepted today, additional studies have yet to be conducted to replicate these first findings in different populations.

More recently, case-control association studies have been conducted to investigate candidate genes that may influence dental caries susceptibility and resistance. These studies have mainly focused on genes influencing enamel formation, saliva composition, and immune response. Nevertheless, the great majority of these findings were still not replicated. In this context, family-based association could also be used, aiming to avoid bias caused by population stratification that may remain unnoticed in a case-control design. As the most powerful tool to identify genes associated with disease available nowadays, GWA could also be applied to dental decay phenotypes, using information of very large sets of markers covering the entire genome, selected from the 6 million SNPs available at public databases (NCBI, 2009).

Identifying the genes that play a role in controlling caries susceptibility is essential for a full understanding of the molecular basis of the disease pathogenesis, and would have potential impact on the development of new preventive and therapeutic strategies – such as molecular vaccines and even gene therapy. Clinicians would be able to screen and identify susceptible patients, adopting individual, tailor-made intervention with a potential high impact over maintenance and preservation of individual oral health. Finally, the identification of genetic risk factors for caries would help reduce costs associated with treatment and prevention of one of the most frequent oral diseases.

Another discussion that should not be overlooked regarding etiopathological risk factors for dental caries is the definition of the ideal phenotype. Are the current methods of caries identification suitable for genetic studies? Further studies should be conducted in order to compare the existing and new methods of caries diagnosis to make the phenotype more precise. Moreover, the selection of individuals with high susceptibility (for example, having high DMFT and low sucrose consumption) and with low susceptibility to caries (for instance, having low DMFT and high sucrose consumption) could contribute to a more precise phenotype, and likely have stronger genetic influence than the general population.

Acknowledgments

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