JDR
JOURNAL OF DENTAL RESEARCH®
Featuring Critical Reviews in Oral Biology & Medicine

VOLUME 84 • NUMBER 11 • NOVEMBER 2005

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RESEARCH REPORTS
Clinical

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J Dent Res 84(11):1047-1051, 2005

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ABSTRACT

The role of genetic and environmental factors on dental caries progression in young children was determined. A detailed caries assessment was performed in 2 examinations on 314 pairs of twins initially 1.5 to 8 years old. Surface-based caries prevalence rates (SBCPR) and lesion severity (LSI) were computed. Heritability estimates were calculated by SOLAR software. Analyses were performed on all ages combined and by age group (1.5-< 4; 4-6; > 6). Overall heritability estimates (H) of net increments SBCPRs were H = 30.0 ($p < 0.0001$), and were greatest for the youngest (H = 30.0) and oldest groups (H = 46.3). Overall LSI heritability estimates [H = 36.1 ($p < 0.0001$)] were also greatest for the youngest (H = 51.2) and oldest groups (H = 50.6). Similar findings were found for net increments of occlusal surfaces and deep dentinal lesions SBCPRs (H = 46.4-56.2). These findings are consistent with a significant genetic contribution to dental caries progression and severity in both emerging primary and permanent dentitions.

KEY WORDS: genetics, dental caries, epidemiology, twins, longitudinal.

Received February 24, 2005; Last revision July 20, 2005; Accepted July 22, 2005

INTRODUCTION

Dental caries is a chronic, complex, multifactorial disease for which myriad etiologic host and environmental factors have been proposed. Identification and elucidation of the relative contributions of factors important in caries initiation and progression have been hampered because of the difficulties in controlling the effects of potential modifying factors, such as fluoride exposure and access to professional dental care, that alter disease onset and presentation. The natural history of caries development and the relative contributions of genetic and environmental factors to caries of the primary dentition have been poorly characterized. As a result, certain crucial features of caries onset and progression have been difficult to model.

In an attempt to quantify the relative contributions of genetic and environmental factors to the initiation and progression of early childhood caries, we performed a longitudinal assessment of dental caries in a cohort of 314 twin pairs aged 1.5-8 yrs. A large proportion of these children were found to be caries-active, with unmet dental treatment needs. The majority of the twins studied did not have access to optimally fluoridated water or professional dental care, providing a model for natural caries. In this study, we tested the hypothesis that incident dental caries has a heritable component in very young children, and that environmental factors play a more pronounced role in dental caries onset and development with increasing age.

MATERIALS & METHODS

Demographic Characteristics of the Study Population

Consent was obtained according to the University of Pittsburgh and Universidade Estadual de Montes Claros Institutional Review Boards' guidelines. Twins were from families of a low socio-economic level who resided in the urban setting of the city of Montes Claros, State of Minas Gerais, Brazil. Water supplies of the city have fluoride levels of < 0.2 ppm, and parents reported 91% of the children having never visited a dentist.

The sample consisted of 314 pairs of twins (n = 628) who, at baseline, were, on average, 4.2 ± 2.0 yrs old (range, 1.5 to 8 yrs old). Assessment of zygosity revealed 112 pairs of monozygotic (MZ) twins and 202 pairs of dizygotic (DZ) twins.

Zygosity Assessment

DNA was extracted from peripheral venous blood leukocytes with use of the QIAmp blood kit (Valencia, CA, USA), and individuals were genotyped for polymorphic DNA-marker loci by standard methods with an ABI-377 fluorescent sequencer (Bretz *et al.,* 2005). Amplification products were analyzed by GENESCAN 2.1 (Applied Biosystems, Foster City, CA, USA) (Zhang *et al.*, 2001). We determined zygosity by genotyping all individuals for 8 highly polymorphic DNA loci (on chromosomes 2, 7, 11, 17, and 20). Individuals discordant for one or more markers were considered dizygotic.

Dental Caries Examination

Prior to the dental caries examination, twins had their teeth thoroughly brushed and flossed. Two examinations were performed 12 mos apart, and a net increment dental caries outcome (Δ) was computed.

Two examiners conducted dental caries examinations according to NIDCR criteria, modified to distinguish caries lesions of chalky whitish/yellowish opaque appearance, with no clinically detectable loss of substance (white-spot lesions), from cavitated caries lesions. Caries examinations were complemented by the use of digital imaging fiber-optic trans-illumination (DIFOTI, Irvington, NY, USA) for interproximal surfaces.

Once a lesion was detected, its severity was determined as follows: 1 - white-spot lesions; 2 - enamel lesions with loss of intact surface; 3 - dentinal lesions at initial stages of breakdown; and 4 - lesions > 2-3 mm into dentin.

Table 1. Net Increment of Dental Caries Traits over a 12-month Period by Age Group in Twins

Dental Caries Phenotypes	Visit/Δ Change	Age Groups 1.5 < 4 yrs old# (n = 133)	4-6 yrs old# (n = 291)	> 6 yrs old# (n = 204)
Overall Caries Rates				
SBCPR	Baseline	2.4 ± 5.5[a]	9.1 ± 9.0	14.4 ±10.0
	12-month	6.1 ± 8.4	10.8 ± 9.7	14.6 ± 9.6
	Δ Change	3.8 ± 7.2	1.6 ± 5.7	0.3 ± 6.8
LSI	Baseline	0.05 ± 0.1	0.2 ± 0.2	0.3 ± 0.3
	12-month	0.1 ± 0.2	0.3 ± 0.1	0.3 ± 0.3
	Δ Change	0.08 ± 0.2	0.04 ± 0.3	- 0.01 ± 0.2
Caries Rates by Surface				
SBCPR	Baseline	1.2 ± 2.2	4.3 ± 3.0	6.2 ± 3.0
Occlusal	12-month	2.7 ± 3.0	4.5 ± 3.2	6.1 ± 3.2
	Δ Change	1.6 ± 2.5	0.3 ± 2.2	- 0.02 ± 2.6
SBCPR	Baseline	0.2 ± 0.8	1.2 ± 2.1	2.0 ± 2.4
Mesial	12-month	0.7 ± 1.5	1.4 ± 2.2	1.5 ± 1.9
	Δ Change	0.5 ± 1.4	0.2 ± 1.4	- 0.5 ± 1.9
SBCPR	Baseline	0.1 ± 0.4	1.0 ± 1.8	1.8 ± 2.2
Distal	12-month	0.4 ± 1.2	1.3 ± 2.0	1.6 ± 1.9
	Δ Change	0.4 ± 1.1	0.2 ± 1.5	- 0.2 ± 1.5
SBCPR	Baseline	0.7 ± 2.0	1.7 ± 2.7	2.5 ± 3.0
Buccal	12-month	1.4 ± 3.0	2.0 ± 2.8	2.7 ± 2.8
	Δ Change	0.8 ± 2.9	0.4 ± 2.5	0.2 ± 2.6
SBCPR	Baseline	0.3 ± 1.8	1.0 ± 1.6	1.9 ± 2.2
Lingual	12-month	0.9 ± 1.8	1.6 ± 1.9	2.6 ± 2.3
	Δ Change	0.6 ± 2.0	0.6 ± 1.6	0.7 ± 2.2
Caries Rates by Severity				
SBCPR	Baseline	0.8 ± 1.9	2.3 ± 2.6	3.2 ± 3.3
White-spot	12-month	2.0 ± 3.6	2.4 ± 3.3	3.3 ± 3.8
	Δ Change	1.2 ± 3.8	0.1 ± 3.5	0.04 ± 3.8
SBCPR	Baseline	0.8 ± 2.2	2.8 ± 2.9	4.4 ± 3.1
Initial Enamel	12-month	2.5 ± 3.4	3.9 ± 3.7	6.1 ± 4.0
	Δ Change	1.7 ± 3.2	1.1 ± 3.1	1.7 ± 3.3
SBCPR	Baseline	0.6 ± 1.9	2.5 ± 3.4	3.7 ± 3.6
Initial Dentin	12-month	0.8 ± 1.9	2.0 ± 2.7	2.1 ± 2.5
	Δ Change	0.2 ± 2.2	-0.5 ± 2.5	- 1.6 ± 2.9
SBCPR	Baseline	0.2 ± 0.7	1.6 ± 3.5	3.1 ± 4.4
Deep Dentin	12-month	0.8 ± 3.6	2.4 ± 4.7	3.2 ± 4.3
	Δ Change	0.7 ± 3.1	0.8 ± 2.6	0.1 ± 3.5

Age at baseline; [a]mean ± SD.
SBCPR, surface-based caries prevalence rates; LSI, lesion severity.

Dental Caries Variables

Summary variables were generated as follows: (1) surface-based caries prevalence rate (SBCPR) - 100*(total number of decayed surfaces/total number of surfaces present) (Bretz *et al.*, 1992); (2) SBCPRs based on surface location (SBCPR for occlusal, mesial, distal, buccal, and lingual surfaces; (3) SBCPRs according to lesion severity (SBCPR for white-spot, initial enamel, initial dentin, and deep dentinal lesions); and (4) Lesion Severity Index (LSI) - a weighted outcome of caries severity derived by the following formula: $(N_1 + 2N_2 + 3N_3 + 4N_4)$/total number of surfaces present, where N_1-N_4 represent the number of lesions at stages 1-4. This index permits true attack rates to be assessed, but it is weighted to reflect the severity of the lesions.

Statistical Analysis

We performed descriptive analysis by using SPSS statistical software (SPSS Inc., Chicago, IL, USA). We used the Kappa statistic to assess inter- and intra-examiner reliability for dental caries in 10% of the participants. (The Kappa test was for both exercises above 0.80.) We computed heritability using a variance component model implemented in the SOLAR (AAVSO, Cambridge, MA, USA) computer analysis package (Almasy and Blangero, 1998). Variance component models of the type implemented in the SOLAR package do not simply take the difference in MZ and DZ twins correlations and multiply by 2.0 to get an estimate of heritability, but rather model the covariation in twin-pair trait values as a function of kinship coefficient in a random effects linear model, where one random effect (*i.e.*, a variance component term) corresponds to the effect of kinship (the kinship coefficient of MZ twins is 1.0, and that of DZ twins is 0.5). This linear model also allows for covariate effects. The inclusion of covariates in the model, and the unique way in which variance component models assess heritability, can create heritability estimates different from those produced by overly simplistic estimates obtained from, *e.g.*, differences in MZ and DZ correlations unadjusted for covariate effects. Twin-based variance component models do assume bivariate normality of the phenotype and, in our case, were used to estimate narrow-sense heritability. Although differences in kinship that account for variation and covariation in a trait are likely to reflect genetic effects, there is the possibility that the differences in kinship that appear to influence variation and covariation in the trait reflect the effect of something merely correlated with kinship differences (*e.g.*, some shared cultural or environmental effect). Thus, our estimates of heritability may to some extent reflect familiality.

RESULTS

Average dental caries rates (at baseline and after 12 mos) and mean net increments by age group are described (Table 1). Twins who were 1.5 - < 4 yrs old developed, over a 12-month period, an average of 3.8% new decayed surfaces, whereas 4- to 6-year-olds and those over 6 yrs old developed an average of 1.6% and 0.3% new decayed surfaces, respectively. Analysis of SBCPR net increments by location revealed comparable trends for all surfaces for the 3 age groups, with the exception of lingual surfaces, which had comparable rates for all age groups. The occlusal surfaces had the highest SBCPR net increments in 1.5 - < 4-year-olds, followed by smooth surfaces and interproximal surfaces. No particular trends were observed for the intermediate age group. Negative SBCPR net increments in some surfaces were observed for the older group, probably as a result of changes in dentition status. Net increments for lesion severity (LSI) were positive and higher for the younger group and negative for the older group. Dissection of lesion severity showed higher SBCPR net increments for white-spot lesions and initial dentinal lesions for the younger group compared with the older groups.

The net increments for all age groups had a significant genetic contribution, *i.e.*, 30% ($p < 0.0001$) (Table 2). Stratification of age groups showed a significant genetic contribution to SBCPRs for the younger and older groups, and a non-significant contribution for the intermediate age group. SBCPR net increments for occlusal and lingual surfaces had the highest heritability estimates for all ages. Lower, yet significant, heritability estimates were found for the distal and buccal surfaces. Upon age stratification, the younger cohort had significant heritability estimates for the occlusal and distal surfaces. With the exception of SBCPR net increments for the lingual surfaces, all other surface net increments appeared to be modulated by the environment for the intermediate group. In the older group, significant net increments for the occlusal surface were found and were among the highest for the smooth surfaces.

For the LSI, overall net increment rates had a significant genetic contribution. Again, for the younger and older cohorts, about 50% of lesion severity variability was explained by genetic factors. Dissection of lesion severity revealed a significant genetic contribution to SBCPR net increments for deep dentinal lesions for all ages and for individual age groups. Only SBCPR net increments for white-spot lesions in the older cohort appeared to have a significant genetic contribution.

DISCUSSION

Studies have evaluated the incidence and lesion progression of caries in adolescents and adults; however, longitudinal studies designed to elucidate the relative contributions of genetic and environmental factors to the natural progression of dental caries have not been performed, particularly in young children. Results of the present study indicate that the highest incidence of

Table 2. Longitudinal Analysis of Heritability Estimates for Dental Caries Rates

Dental Caries Phenotypes	All* (n = 628)	Age Groups 1.5 < 4 yrs old# (n = 133)	4-6 yrs old# (n = 291)	> 6 yrs old# (n = 204)
Overall Caries Rates				
Δ SBCPR	30.0[a]	30.0	13.3	46.3
	3.6[b]	3.1	0.0	0.0
	-1472.4[c]	-325.9	-650.3	-486.3
	< 0.0001[d]	0.0296	NS[e]	0.0003
Δ LSI	36.1	51.2	12.2	50.6
	4.2	0.0	0.0	0.0
	867.2	183.3	446.1	255.2
	< 0.0001	0.0004	NS	< 0.0001
Caries Rates by Surface				
Δ SBCPR	21.3	35.2	7.8	23.7
Occlusal	5.0	0.0	0.0	0.0
	- 858.4	-186.2	-375.7	-290.4
	0.0018	0.0101	NS	0.0284
Δ SBCPR	3.6	16.2	0.0	14.0
Mesial	4.0	2.7	0.0	0.0
	- 607.7	-111.7	-244.6	-232.7
	NS	NS	NS	NS
Δ SBCPR	18.2	37.4	17.6	10.3
Distal	2.8	0.0	0.0	0.0
	- 519.5	- 77.5	-253.2	-180.9
	0.0120	0.0073	NS	NS
Δ SBCPR	17.4	12.8	9.1	43.8
Buccal	0.0	3.7	0.0	0.0
	- 919.3	-206.6	-415.1	-291.9
	0.0085	NS	NS	0.0039
Δ SBCPR	38.5	5.9	44.4	54.0
Lingual	0.2	2.8	0.0	0.0
	- 694.8	-155.8	-263.3	-255.1
	< 0.0001	NS	< 0.0001	< 0.0001
Caries Rates by Severity				
Δ SBCPR	17.7	21.0	9.3	27.4
White-spot	0.0	0.0	0.0	0.0
	-1127.5	-243.3	-508.5	-374.5
	0.0093	NS	NS	0.0438
Δ SBCPR	0.0	0.0	0.0	3.0
Initial Enamel	0.0	3.3	0.0	0.0
	-1044.4	-219.5	-475.3	-344.6
	NS	NS	NS	NS
Δ SBCPR	0.0	2.6	0.0	0.0
Initial Dentin	7.9	0.0	0.0	3.0
	- 903.5	-170.1	-416.5	-313.4
	NS	NS	NS	NS
Δ SBCPR	45.5	56.2	33.3	46.4
Deep Dentin	0.0	0.0	0.0	0.0
	- 981.1	-207.3	-412.3	-348.4
	< 0.0001	< 0.0001	0.0003	< 0.0001

* Heritabilities adjusted by gender and age.
\# Heritabilities adjusted by gender.
[a] Heritability estimate.
[b] % explained by covariate.
[c] Log likelihood estimate.
[d] Significance.
[e] NS, not significant.
SBCPR, surface-based caries prevalence rates
LSI, lesion severity.

dental caries occurs at an early age, and may correlate with eruption of the primary dentition. This conclusion is based on the observed overall net increments for SBCPRs, which were the highest in the younger cohorts (1.5 yrs - < 4 yrs old). This finding is consistent with the notion that newly erupted surfaces may be more prone to caries development. Net increments for SBCPR at all locations, *i.e.*, occlusal, smooth, and interproximal surfaces were highest in the youngest cohorts. The observed dental caries susceptibility in younger individuals may be the result of host factors such as salivary composition, environmental insults (*e.g.*, diet or microbial), or more surfaces 'at risk'. Occurrence of the lowest overall net increments for SCBPRs and for surface-specific rates in the older cohort may reflect that 'at risk' surfaces had already experienced decay.

Caries incidence rates vary by surface, with occlusal surfaces reported to be the most susceptible to caries development (Sheiham, 1997). The reason for the higher incidence of occlusal caries is unknown, although both environmental and host factors have been proposed (Psoter *et al.*, 2003). A major goal of this twins study was to evaluate caries incidence and progression longitudinally. The current twins study found that occlusal surfaces presented with the highest net increment rates, followed by smooth and interproximal surfaces.

Net increments for lesion severity (LSI) were highest for the younger group (1.5 - < 4 yrs old), findings that are consistent with our observations for SBCPRs and surface-specific rates. Categorization of lesion severity showed higher net increments for white-spot lesion rates and dentinal lesion rates for the younger group, and for white-spot lesions for the older cohort group (> 6 yrs old).

Heritability estimates for overall SBCPR net increments suggest a significant genetic contribution. Stratification of age groups indicated that the genetic contributions to SBCPR net increments were greatest for the younger (1.5 yrs - < 4 yrs) and older (> 6 yrs) groups. These findings partially contradict our initial hypothesis, that dental caries onset and development may have a heritable component in infancy, and that environmental factors may have a more pronounced role on dental caries onset and development with increasing age. The fact that the older cohort (> 6 yrs) had the lowest SBCPR net increments, yet a significant genetic contribution to net increments, has led us to hypothesize that dental caries onset in emerging dentitions may be under the influence of genetic factors.

In the only longitudinal study of dental caries heritability where the twins model was used (Finn and Caldwell, 1963), 8- to 16-year-old twins (32 MZ pairs, 29 DZ pairs) received 2 dental caries examinations over a two-year period. The average number of decayed, missing, and filled teeth and surfaces (DMFT/S) and the average intra-pair differences at each examination were computed. The mean incremental DMFT intra-pair differences over the two-year period were twice as great for DZ twin pairs as for MZ twins, consistent with a genetic contribution to dental caries progression. Similar findings for incremental intra-pair DMF surfaces were also demonstrated. Beyond the consistency that heritable factors may be important in caries experience in both studies, it is difficult to compare results of this study with those of our study, due to differences in the ages of participants and the incongruent methodologies related to study design, caries measurement, and analysis.

Heritability of dental caries net increments by surface location has not been previously reported. We observed that the SBCPR net increments for occlusal surfaces resembled similar patterns for the overall and age-stratified SBCPR net increments, where emerging primary and permanent dentitions exhibited significant heritability. Some occlusal surface characteristics that could potentially influence caries development may be under genetic influence. These include occlusal surface topography, fissure depth, and fissure wall inclination. These features are likely genetically determined, but the numbers of genes and the identification of the specific genes responsible are unknown. Alterations of enamel—such as increased enamel porosity, decreased mineral content, and structural alterations of enamel crystals—are reported for syndromic diseases, and responsible genes are known in many cases (Kirkham *et al.*, 2000; Ravassipour *et al.*, 2000; Wright *et al.*, 2003). However, these tend to be inherited as simple Mendelian conditions that cause major disruption of enamel and have profound clinical pathology. Whether genetic variants of these genes affect a more moderate phenotypic variance that influences caries susceptibility in non-syndromic cases is unknown.

Lesion severity net increment rates (LSI) exhibited a significant heritable component. We previously reported, in cross-sectional observations, that lesion severity had a high and (> 70%) significant genetic contribution in a large twins cohort (Bretz *et al.*, 2005), consistent with findings in this longitudinal analysis. In the current study, the overall net-increment caries and surface-specific rates for both the younger and older cohorts showed that approximately 50% of lesion severity variance may be explained by genetic factors. Categorization of lesion severity revealed a significant heritable component of SBCPR net increments for deep dentinal lesions, for combined age groups and for individual age groups. The progression of dentinal caries involves both demineralization of dentin and degradation of the organic matrix (Michelich *et al.*, 1980; Goldberg and Keil, 1989; Clarkson *et al.*, 1991; Butler *et al.*, 1992). It has been well-established that odontoblasts are capable of responding to dentin injury, and can up-regulate their secretory activity, leading to a dentin repair response characterized by the deposition of reactionary dentin (Smith *et al.,* 1994). Growth factors sequestered within the dentin matrix may be released following tooth injury associated with caries lesions, and may initiate or modulate reparative responses, leading to odontoblast-like cell differentiation and possibly vascularization responses (Roberts-Clark and Smith, 2000; Tziafas *et al.*, 2000; Smith and Lesot, 2001). Host MMPs may also be involved in caries progression (Tjäderhane *et al.*, 1998; Sulkala *et al.*, 2001), and may play a role in early alterations of non-collagenous organic matrix during caries progression in dentin (Lormee *et al.*, 1986). For example, dentin-bound MMP20 released by caries progression may function in a protective host response to caries lesions (Tjäderhane *et al.*, 1998; Sulkala *et al.,* 2001). The role of genetic susceptibility in dentinal degradation remains to be determined. However, innate characteristics of the host dentin cannot be excluded from modulating caries progression.

In conclusion, findings in this longitudinal twins study model are consistent with previous findings, that a significant proportion of the caries variance in young children is heritable, indicating a genetic contribution (Bretz *et al.*, 2005). Current

findings also indicate that, in longitudinal assessments of site-specific incidence rates, and in lesion progression, heredity may play a significant role. Heritability estimates were greatest at the age ranges 1.5 - < 4 yrs and > 6 yrs. These findings—that risk for caries incidence and progression may be greatest when the dentitions are emerging—have important clinical implications for the timing of dental caries interventions.

ACKNOWLEDGMENTS

This study was supported by NIH/NIDCR grants DE15351 and DE14528.

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