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Original Article

Potential risk factors and genetic variants associated with dental caries incidence in Appalachia using genome-wide survival analysis

Tianyu Zou[1], Katherine Neiswanger[2], Eleanor Feingold[1,3], Betsy Foxman[4], Daniel W McNeil[5], Mary L Marazita[1,2,6], John R Shaffer[1,2]

[1]Department of Human Genetics, School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; [2]Center for Craniofacial and Dental Genetics, Department of Oral and Craniofacial Sciences, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Biostatistics, School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; [4]Center for Molecular and Clinical Epidemiology of Infectious Diseases, Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI, USA; [5]Department of Community Dentistry and Behavioral Science, College of Dentistry, University of Florida, Gainesville, FL, USA; [6]Clinical and Translational Sciences, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

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Abstract: Objective: The aim of this study was to identify the potential risk factors and genetic variants associated with dental caries incidence using survival analysis. Methods: The Center for Oral Health Research in Appalachia recruited and prospectively followed pregnant women and their children. A total of 909 children followed from birth for up to 7 years were included in this study. Annual intra-oral examinations were performed to assess dental caries experience including the approximate time to first caries incidence in the primary dentition. Cox proportional hazards models were used to assess the associations of time to first caries incidence with self-reported risk factors and 4.9 million genetic variants ascertained using a genome-wide genotyping array. Results: A total of 196 of 909 children (21.56%) had their first primary tooth caries event during follow-up. Household income, home water source, and mother's educational attainment were significantly associated with time to first caries incidence in the stepwise Cox model. The heritability (i.e., proportion of variance explained by genetics) of time to first caries was 0.54. Though no specific genetic variants were associated at the genome-wide significance level (P < 5E-8), we identified 14 loci at the suggestive significance level (5E-8 < P < 1E-5), some of which were located within or near genes with plausible biological functions in dental caries. Conclusion: Our findings indicate that household income, home water source, and mother's educational attainment are independent risk factors for dental caries incidence. We nominate several suggestive loci for further investigation.

Keywords: Risk factors, genetics, bioinformatics, genomics, epidemiology, child dentistry

Introduction

Dental caries is the most common chronic disease of childhood affecting 21.4% of US children by age 5 years and 50.5% by age 11 years [1]. Dental caries can lead to chronic pain, loss of teeth, poor school performance and decreased success later in life [2, 3]. A better understanding the etiology of dental caries incidence in the primary dentition is needed to improve caries prevention at both individual and population levels.

Dental caries is a complex disease influenced by multiple factors. Environmental risk factors, such as low socioeconomic indicators and inadequate fluoride exposure, have been identified to be associated with caries prevalence [4, 5]. Behavioral risk factors related to dental caries include poor oral hygiene [6] and cariogenic diet [7], especially sugar intake, which is a key component influencing caries development [8, 9]. Moreover, biological factors including the oral microbiome [6, 10] and genetic factors are associated with caries experience.

Risk factors specifically for caries incidence - that is the proportion of new cases of dental caries across a defined period of time - in the primary dentition include parental income, education, and occupation [11, 12], previous caries experience, consumption of sweetened foods/drinks [13-15], *Streptococcus mutans* level [12, 16], fluoridation in drinking water [11], and tooth brushing frequency [17]. However, most longitudinal risk factor studies have coded caries incidence in the primary dentition as binary traits (e.g., presence or absence of caries) and/or incremental increase of decayed, missing, and filled teeth or surfaces (dmft/dmfs) between two time points, which does not fully capture timing information. By contrast, survival analysis retains "time at risk" information and has been used to identify risk factors for time-to-event of caries incidence including the time to caries incidence in both primary and permanent molars [18], the time to caries increment [19] and the time to first caries in permanent teeth [20]. However, the small sample sizes and/or short follow-up periods of these studies limit their power to detect and model the effects of risk factors.

Genetic studies have reported a strong genetic component to dental caries in the primary dentition, with genetic factors explaining 30%-76% of variation in the dental caries traits [21-25]; the wide range is estimates may be due to differences in study design, populations, sample sizes, or dental caries phenotyping methods. Several "hypothesis-free" genome-wide association studies (GWASs) of dental caries in the primary dentition have been performed over the past decade to investigate clinical phenotypes including caries affection status (affected vs. unaffected) and quantitative caries indices (e.g., counts of the number of affected teeth or tooth surfaces), in samples ranging from approximately 100 to 19,000 individuals [26-30]. These studies have identified several association signals located near genes with plausible biological roles in dental caries, however, to date, few of these associations reached the genome-wide significance threshold or have been rigorously replicated. Moreover, the SNP-based heritability (i.e., the proportion of the phenotypic variance explained by the set of common genetic variants) of caries in the primary dentition was much lower than previously reported family-based heritability estimates. For instance, a consortium GWAS with the largest combined sample size (n = 19,003) identified only one genome-wide significant association, in *ALLC*, using a binary phenotype. The consortium-wide estimated heritability was only 1% [28], in stark contrast to the aforementioned family-based estimates, and lower than the individual heritability estimates observed in the constituent studies that formed the consortium. In addition, prior GWASs used phenotypes collected at a single time point, thus were not able to evaluate the genetic basis of time-to-caries incidence. As mentioned above, survival analysis takes time information into account and its application in GWAS is an approach that may broaden our understanding of the genetic underpinnings of dental caries onset. Therefore, we used the time to first caries incidence in the primary dentition as the outcome in order to identify its potential risk factors and associated genetic variants using survival analysis.

Methods

Study sample

The study design and recruitment descriptions of the Center for Oral Health Research in Appalachia, cohort 2 (COHRA2) have been reported before [31]. Briefly, COHRA2 is a longitudinal study and has been recruiting and prospectively following pregnant white women from Pennsylvania and West Virginia and their children starting in 2011. This cohort has information on genetic, microbial, behavioral, and environmental factors involved in oral health. A subset of 909 children with available data on potential risk factors and genetics was included in this analysis. Each participant was followed annually from birth with 7 years being the longest follow-up, to date, in this ongoing study. Annual intra-oral examinations were performed to assess dental caries experience. Parental consent was provided for all participants, and Institutional Review Boards and Ethics Committees approved all aspects of the study.

Phenotype and genotyping

The participants were phenotyped based on intra-oral examinations according to caries criteria that distinguished cavitated from non-cavitated lesions (i.e., white spots/incipient

lesions). The time to first caries incidence was defined as the time (in years) from birth to the first visit when one or more carious lesions were observed. If no carious lesions were present by the end of follow-up period, time to first caries incidence was coded as censored at the last available visit. For example, if the child had carious lesions at the third visit (3 years-old), then his/her survival time is 3 years and the outcome was 1 (i.e., with carious lesions); if no carious lesions were observed during the 7 years, then survival time is 7 years and the outcome was 0 (i.e., without carious lesions).

Participants were genotyped using the Infinuim Multi-Ethnic Global-8 v1.0 Array. Ungenotyped single nucleotide polymorphisms (SNPs) and sporadic missing data of genotyped SNPs were imputed using the Michigan Imputation Server (Minimac 3 for autosomal chromosomes and Minimac 4 for chromosome X), and Haplotype Reference Consortium Phase 1 as the reference panel. Variants were excluded if they had low imputation quality (INFO score < 0.3), departed from Hardy-Weinberg equilibrium (P < 1E-6), or had minor allele frequencies < 5%. A total of 4.9 million genetic variants were included in this study after filtering.

Risk factor modeling

Demographic, behavioral and environmental factors that putatively relate to oral health were collected including sex (male or female), recruitment site (Pennsylvania [PA] or West Virginia [WV]), household income (< $50,000 per year or ≥ $50,000 per year), home water source (water company or well), home fluoride level (ppm, measured in a water sample), mother's educational attainment (< high school, high school or some college, or college degree or more), mother's tooth brushing frequency (> 1/day, 1/day, or < 1/day), breastfeeding status at baseline (yes or no), and breastfeeding duration (months). The correlations among factors were calculated using Pearson's correlation method. There were no strong correlations (**Figure 1**). Univariate Cox proportional hazards regression models with one risk factor included at a time were used to evaluate the relationships between risk factors and the time to first caries incidence. To identify the independent risk factors, bidirectional stepwise (with iterations between the "forward" and "backward" steps) Cox modeling was performed via the R package "My.stepwise". First, all potential risk factors were put on the "variable list" to be selected, the significance levels for entry into and for retention in the model were set at 0.15. Then, the final Cox model was obtained by dropping the variables with *P* value > 0.05 one at a time until all included variables were significant at alpha level of 0.05. Potential multicollinearity among the variables was evaluated using variance inflation factor (VIF). VIF > 10 in continuous variables or VIF > 2.5 in categorical variables was considered indicative of a multicollinearity issue among variables. In the final model, VIF values of selected variables (household income, home water source, and mother's educational attainment) were 1.45, 1.01, 1.43, respectively, indicating no multicollinearity problem. Because previous studies have shown that stepwise regression can result in poor model fit out-of-sample [32], we also performed regularized Cox modeling as a second variable selection procedure (see Supplementary Materials for methods details of regularized Cox modeling) as confirmation of the variables included in the final model. The results of the regularized Cox model were the same as the stepwise Cox model (Supplementary Figure 1).

Survival GWAS and candidate SNP replication

Cox proportional hazards regression models were used to assess the associations between time to first caries incidence and genetic variants in the R package "gwasurvivr" [33], while adjusting for household income, home water source, and mother's educational attainment (i.e., the three risk factors that were significantly associated with time to first caries incidence in the final Cox model), and the first 6 principal components (PCs) of ancestry derived from a principal component analysis of the genome-wide genetic data. The number of PCs sufficient to capture the population structure was determined based on the scatterplots and scree plots of PCs. The threshold for genome-wide significance was *P*-value < 5E-8, and for suggestive significance was *P*-value < 1E-5. The results were summarized and visualized using Manhattan plots and quantile-quantile (Q-Q) plots created in the R statistical environment (R Foundation for Statistical Computing, Vienna, AU). The regional association plots showing

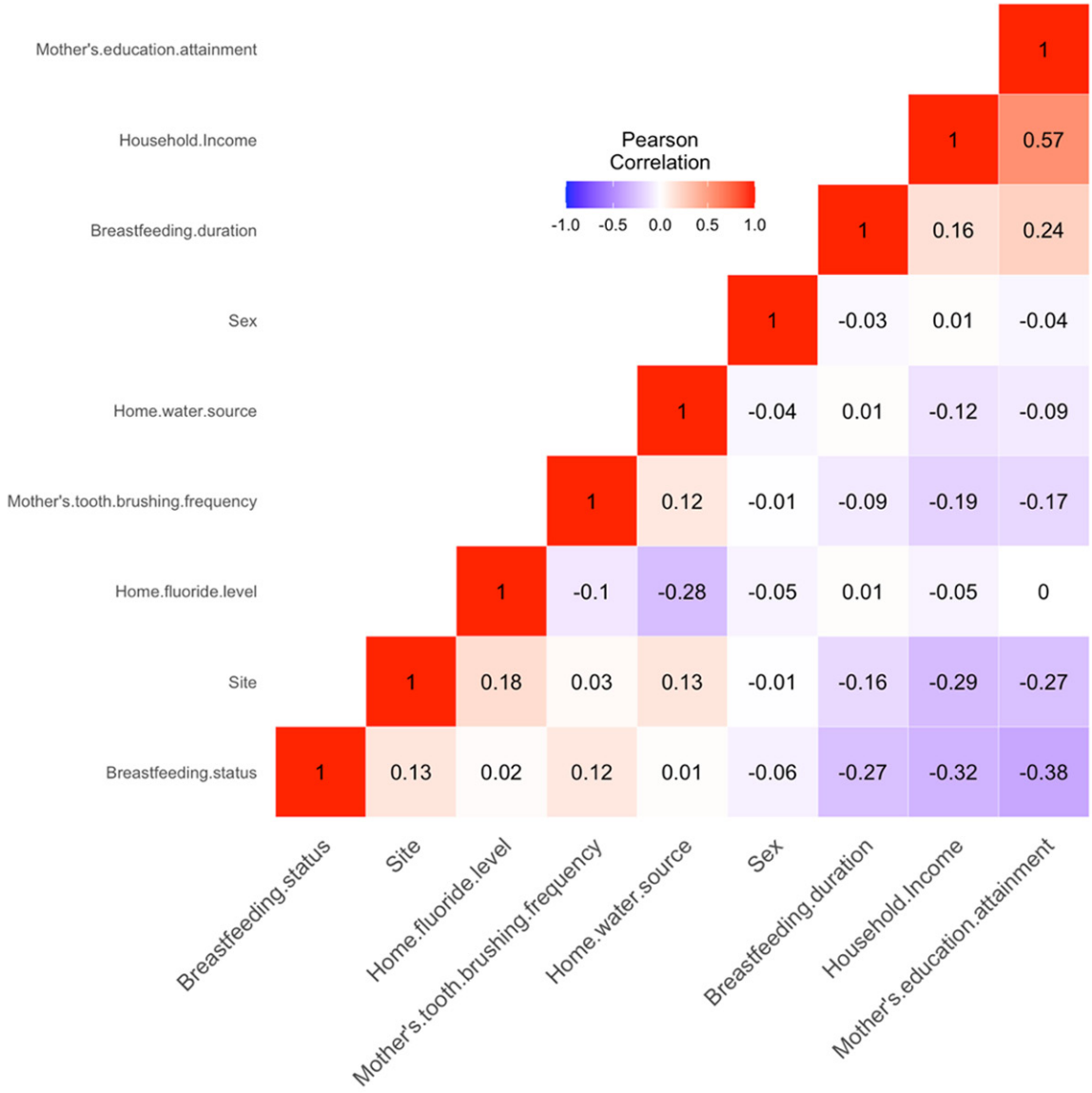

Figure 1. Pearson's correlations among environmental risk factors. The number in each block indicates the correlation coefficient between two factors. The color shows the degree of the correlation (red represents positive correlation and blue represents negative correlation).

mapped genes were visualized using LocusZoom [34].

In addition to the GWAS approach, we conducted a candidate SNP replication study to test the associations between time to first caries incidence and 23 genetic variants that were reported to be associated with dental caries traits in previous GWASs [28, 35-38] at the genome-wide significant level using the same Cox models and covariates as described above (methods details described in the Supplementary Materials). The significance level for candidate SNP replication was set at *P*-value < 0.0022 (i.e., 0.05/23, the Bonferroni correction for 23 tested variants).

Gene-based and gene set enrichment analyses

We performed a gene-based analysis using MAGMA [39] that uses multiple linear principal components regression model to compute the gene-level *p*-values and a gene set enrichment analysis using Genomic Regions Enrichment of Annotations [40] with 14 independent SNPs in

Table 1. Basic characteristics of study sample

Variables	n (%) or mean ± SD
Sex	
Male	504 (55.45)
Female	405 (44.55)
Recruitment site	
PA	483 (53.14)
WV	426 (46.86)
Annual household income	
< $50,000	375 (43.60)
≥ $50,000	485 (56.40)
Home water source	
Water company	766 (93.87)
Well	50 (6.13)
Home fluoride level (ppm)	0.79 ± 0.29
Mother's educational attainment	
< High school	171 (18.98)
High school-Some college	260 (28.86)
College degree or more	470 (52.16)
Mother's tooth brushing frequency	
> 2/day	661 (73.36)
1/day	216 (23.97)
< 1/day	24 (2.66)
Breastfeeding status at baseline	
Yes	733 (83.58)
No	144 (16.42)
Breastfeeding duration (months)	3.66 ± 5.48

Note: PA: Pennsylvania; WV: West Virginia; SD: standard deviation.

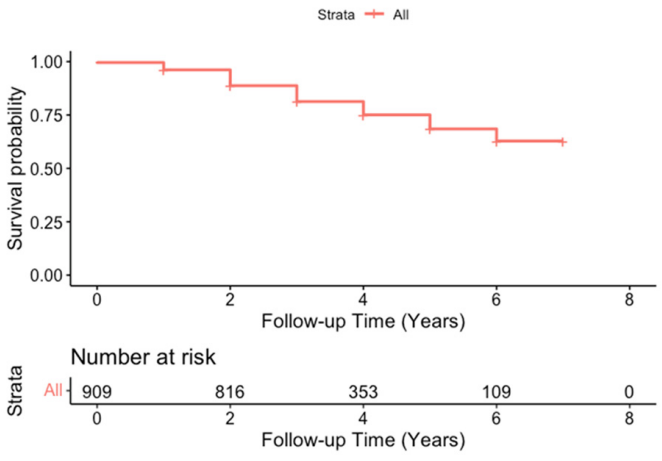

Figure 2. Kaplan-Meier survival curve, using first decayed or filled primary tooth surface as the event. Number at risk on the bottom means the number of individuals that are caries-free (in the risk of having the first caries) at the follow-up time. Our follow-up period was 7 years, therefore there were no children at risk at 8 years.

suggestive signals (P < 1E-5) as input. The gene-based analysis aggregates genetic variants at the gene level and tests the joint association of aggregated variants with the phenotype. In the gene set enrichment analysis, genes are aggregated to gene groups based on biological functions, which can provide evidence of specific pathways involved in the phenotype. The advantages of aggregation include reduced number of tests and more power to detect weak associations.

Heritability estimation

We calculated the SNP-based heritability estimate (i.e., the proportion of the phenotypic variance explained by a given set of genetic variants) of time to first caries incidence trait using SumHer [41], which used the summary statistics from the survival GWAS as input and reference linkage disequilibrium scores from the HapMap3 reference panel using the linkage disequilibrium-adjusted kinship method.

Results

Sample summary

The basic characteristics of the study sample are summarized in **Table 1**. A total of 196 of 909 children (21.56%) had their first primary tooth caries event during the follow-up period. The caries-free survival rates were 99.6% at 1-year follow-up, 96.1% at 2-year follow-up, 88.7% at 3-year follow-up, 81.3% at 4-year follow-up, 75.1% at 5-year follow-up, 68.5% at 6-year follow-up, and 62.8% at 7-year follow-up (**Figure 2**).

Risk factor modeling

In the univariate Cox models, household income, home water source, mother's educational attainment, mother's tooth brushing frequency, breastfeeding status at baseline, and breastfeeding duration were individually significantly associated with time to first caries incidence ($P < 0.05$, **Table 2**). However, in the final stepwise Cox model, only household income, home water source, and mother's educational attainment were retained. Higher household income

Table 2. Univariate Cox models of risk factors and time to first caries incidence

Factors	Reference group	Alternative group	Estimate	SE	P	HR (95% CI)
Sex	Male	Female	-0.041	0.144	0.773	0.959 (0.724-1.271)
Recruitment site	PA	WV	0.207	0.143	0.147	1.230 (0.929-1.629)
Household income	< 50,000/year	≥ 50,000/year	-0.813	0.149	< 0.0001	0.443 (0.331-0.594)
Home water source	Water company	Well	0.989	0.244	< 0.0001	2.689 (1.666-4.339)
Home fluoride level		per ppm	0.069	0.249	0.782	1.072 (0.657-1.747)
Mother's educational attainment	< High school	High school or some college	-0.596	0.182	0.001	0.551 (0.385-0.788)
Mother's educational attainment	< High school	College degree or more	-1.259	0.177	< 0.0001	0.284 (0.200-0.402)
Mother's tooth brushing frequency	< 1/day	1/day	0.066	0.169	0.698	1.068 (0.767-1.488)
Mother's tooth brushing frequency	< 1/day	> 1/day	1.216	0.316	< 0.0001	3.375 (1.818-6.265)
Breastfeeding status	Yes	No	0.723	0.173	< 0.0001	2.061 (1.468-2.893)
Breastfeeding duration		per month	-0.036	0.015	0.017	0.964 (0.936-0.994)

Note: PA: Pennsylvania; WV: West Virginia; SE: standard error; HR: hazard ratio; CI: confidence interval.

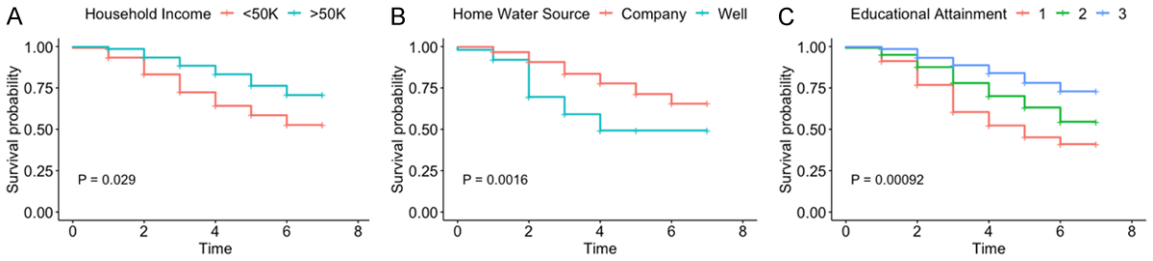

Figure 3. Survival curves for time to first caries incidence stratified by significant risk factors in the final stepwise Cox model. The x-axis is the follow-up time in years. The y-axis is the probability of remaining caries free. The lines represent survival curves of stratified groups. A. Stratified by household income. B. Stratified by home water source. C. Stratified by mother's educational attainment (1 = < high school, 2 = high school or some college, 3 = college degree or more).

group and higher levels of mother's educational attainment were significantly associated with lower hazards for time to first caries incidence (Hazard ratio [HR] = 0.65 and 0.66, P = 0.029 and 0.00092, respectively), while well water as the home water source was significantly associated with greater hazards for time to first caries incidence (HR = 2.28, P = 0.0016, **Figure 3**) compared to municipal water. These results indicate that household income, home water source, and mother's educational attainment may have independent (though not necessarily causal) effects on time to first caries incidence. These three final risk factors were included in the following survival GWAS.

Heritability estimation, survival GWAS, and candidate SNP replication

The SNP-based heritability estimate of the time to first caries incidence was 0.54 (standard deviation [SD]: 0.41) indicating that genetics may have a large role in the timing or pace of the cariogenic processes. **Figure 4** shows the Manhattan plot of the survival GWAS results for time to first caries incidence. There was no evidence of genomic inflation (genomic inflation factor was 1.00; Supplementary Figure 2). Though there were no specific genetic variants associated with time to first caries incidence at the genome-wide significance level (P < 5E-8), 14 loci at the suggestive level were identified (P < 1E-5). **Table 3** shows the 14 suggestive loci and their nearby genes and annotation. Notably, some lead SNPs of the suggestive association signals were within or near genes with biologically plausible roles in dental caries including rs34201252 (P = 1.77E-7, located near the gene *COL5A1*), rs7503428 (P = 2.10E-6, located in the gene *ASIC2*), and rs35508695 (P = 7.30E-6, located in the gene *ESR1*). In addition, the lead SNP rs28567072 on chromosome 8 (P = 5.36E-6) is located near the gene *ABRA*, which was previously reported to be associated with dental caries in the permanent dentition at the suggestive significance

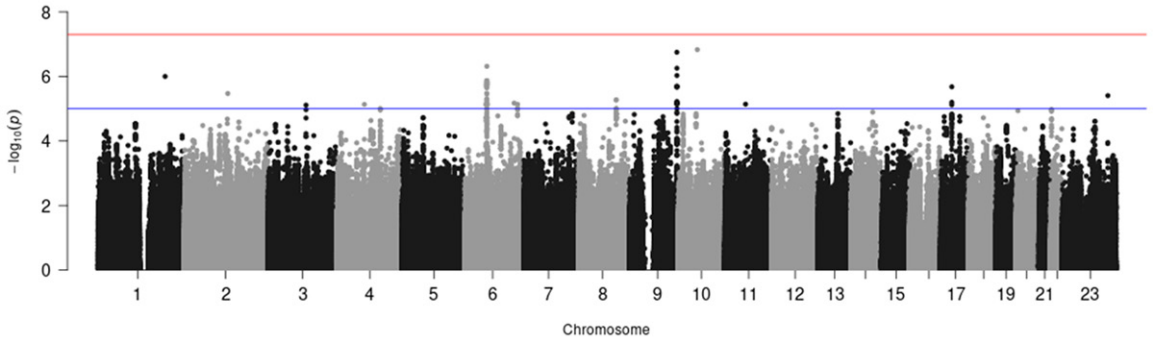

Figure 4. Manhattan plot for the survival GWAS of time to first caries incidence. The red line indicates the genome-wide significance threshold (P = 5E-8), and the blue line indicates the suggestive significance threshold (P = 1E-5). The y-axis is the -$\log_{10}$-transformed *P* value, the x-axis is the physical position of each variant organized by chromosome.

level [42]. The regional association plots for these 4 loci near or in the genes with potential roles in dental caries are provided in **Figure 5**.

We also tested genetic variants that have been previously reported to be associated with dental caries traits in the primary or permanent dentitions at the genome-wide significance level (P < 5E-8). There were 23 tested variants, however, none of them showed association with time to first caries incidence in this analysis (Supplementary Table 1).

Gene-based genetic association, and gene set enrichment analyses

In the gene-based genetic association analysis, the SNPs from the survival GWAS were mapped to 18,942 protein coding genes. The genome-wide significance threshold for gene-based analysis was *P*-value < 2.74E-6 (0.05/18942). There were no genes reaching genome-wide significance threshold. The gene with the smallest *P*-value was *TBX1* (P = 4.49E-5). There was no significant enrichment in the gene set enrichment analyses.

Discussion

Dental caries is a complex disease that can be impacted by environmental, behavioral and genetic factors. All the factors are important in the etiology of dental caries. We have a cohort with relatively larger sample size and longer follow-up period, which provides statistical power to detect risk factors and genetic factors for dental caries incidence. It is beneficial to determine behavioral/environmental factors first because these significant risk factors could explain some non-genetic variation. Accounting for such non-genetic sources of variance would in turn improve our ability to detect genetic effects.

In this study, we identified that household income, home water source, and mother's educational attainment were significantly associated with caries incidence in the primary dentition. Household income and education are commonly used as indicators of the socioeconomic status (SES) of research participants. It is well-established that populations with lower SES have higher caries prevalence [4], both within specific communities and across communities, worldwide. Our results provided further support that SES level was associated with time to first caries incidence. Higher family SES may reduce children's caries incidence through promoting healthy living conditions, such as better access to dental care, promotion of positive oral health behaviors including regular tooth brushing, and reduced exposure to environmental stressors and risk factors including lower or less frequent consumption of sugary foods and beverages.

Regarding the observed association with home water source, we speculate that this association could be explained by well water differing in fluoridation level from municipal water [11] and/or that participants living in homes serviced by well water reside in more rural areas and may experience decreased access to dental care [43]. Further studies are needed to investigate the role of home water source in caries incidence and disentangle its potentially

Table 3. Survival GWAS results for loci showing suggestive evidence of association with time to first caries incidence

SNP	CHR	BP	EA/RA	MAF	P	HR (95% CI)	Gene nearby and biological relevance
rs35324031	10	55529375	G/A	0.060	1.48E-7	0.41 (0.30-0.58)	*PCDH15:* is associated with Usher type 1 syndrome involved in congenital hearing loss [69]
rs34201252	9	137426383	G/A	0.054	1.77E-7	0.40 (0.28-0.56)	*COL5A1*: is associated with Ehlers-Danlos syndrome presenting with dental pathology [70] and canine agenesis [71] *RXRA:* encodes retinoid X receptor α involved in retinoic acid-mediated gene activation
rs4710384	6	63554552	G/A	0.155	4.84E-7	0.56 (0.44-0.70)	NA
rs112019823	1	194344257	C/T	0.114	1.00E-6	0.53 (0.41-0.68)	NA
rs7503428	17	32372318	G/C	0.362	2.10E-6	0.61 (0.50-0.75)	*ASIC2:* encodes a member of acid-sensing ion channels involved in the perception of sour taste [56] and is associated with gingival inflammation [58]
rs35643512	2	126373265	G/T	0.162	3.38E-6	0.56 (0.44-0.72)	NA
rs12834574	23	131692337	C/T	0.073	3.94E-6	0.57 (0.44-0.72)	NA
rs28567072	8	107883740	A/G	0.094	5.36E-6	0.52 (0.39-0.69)	*ABRA:* encodes a muscle specific actin-binding protein involved in skeletal muscle hypertrophy [72] and arteriogenesis [73] *OXR1:* is an important regulator of neuronal survival in response to oxidative stress [74]
rs9399396	6	142564217	C/T	0.201	6.69E-6	0.59 (0.47-0.75)	*VTA1*: encodes a protein involved in trafficking of the multivesicular body and is associated with arthrogryposis [75] *ADGRG6*: is associated with earlobe attachment [76] *GJE1*: is associated with hearing loss [77] *NMBR*: a member of the mammalian bombesin receptor family involved in several physiological effects [78]
rs11230097	11	59646339	T/C	0.182	7.26E-6	0.60 (0.48-0.75)	*TCN1*: is associated with colorectal cancer [79] *OOSP* family: is associated with postfertilization development *CBLIF*: encodes a glycoprotein *MRPL16*: is associated with colorectal tumors [80] *STX3*: participates in granule-granule fusion and is associated with Sjögren's syndrome [81] *OR10V1*: encodes olfactory receptor involved in smell perception *MS4A3*: is associated with myeloid differentiation in human hematopoiesis [82]
rs35508695	6	152377709	G/A	0.071	7.30E-6	0.51 (0.38-0.69)	*ESR1*: encodes estrogen receptor alpha and is associated with periodontitis [61], and dental fluorosis [62] *SYNE1*: encodes spectrin repeat containing protein associated with spinocerebellar ataxia [83]
rs3111790	4	80558720	G/T	0.387	7.39E-6	0.63 (0.52-0.77)	NA
rs78891138	3	109679425	T/C	0.055	7.75E-6	0.46 (0.33-0.65)	NA
rs139737462	4	126756165	G/A	0.056	9.85E-6	0.45 (0.32-0.64)	NA

Note: EA: effect allele; RA: reference allele; MAF: minor allele frequency; HR: hazard ratios; NA: intergenic (gene desert).

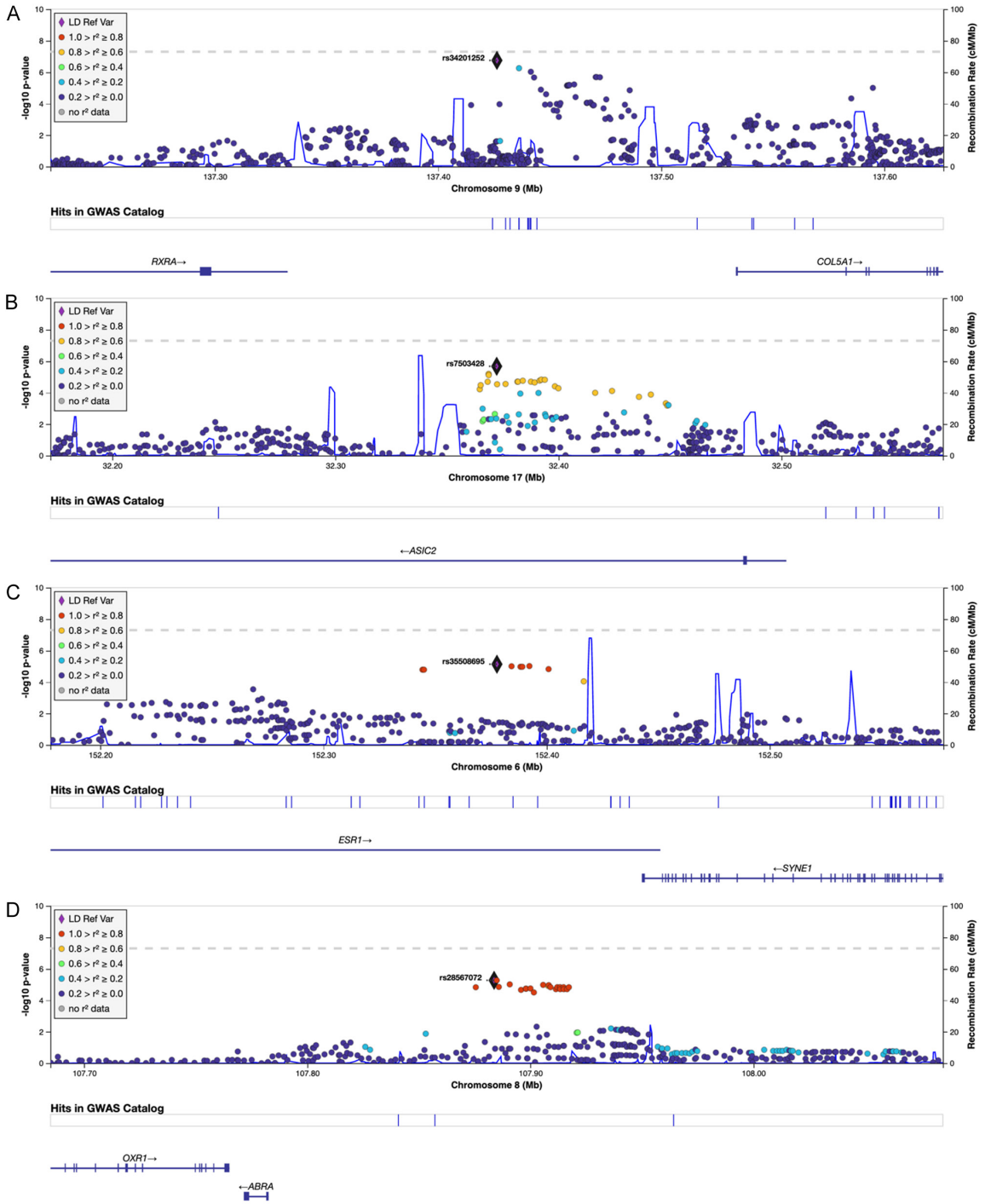

Figure 5. Regional association plots for the 4 loci located in or near genes with plausible function in dental caries. A. rs34201252 (chr9:137426383) near the gene *COL5A1*; B. rs7503428 (chr17:32372318) in the gene *ASIC2*; C. rs35508695 (chr6:152377709) in the gene *ESR1*; D. rs28567072 (chr8:107883740) near the gene *ABRA*. Each point represents the significance (-$\log_{10}$-transformed *P* value; left Y-axis) of a SNP. The purple diamond point with rsID label is the lead SNP in the association region. The color of the points represents the linkage disequilibrium (i.e., correlation [r^2]) of the SNP with the lead SNP. Right Y-axis (recombination rate) provides information about the LD structure of region. The rug plot labeled "Hits in GWAS Catalog" below the plot marks the positions of SNPs that have been previously reported in the GWAS Catalog to be associated with a trait. Genes near the association signal are shown on the bottom.

causal effects from confounding indicators of rurality. Moreover, despite the caries-preventive benefit of water fluoridation [44], we did not find a significant association between home fluoride level and caries incidence, which is likely due to the small variance of home fluoride level in our sample.

There are mixed results regarding the association between breastfeeding practices and dental caries in previous studies. Some studies showed that bottle feeding [45] and prolonged breastfeeding [46, 47] increased caries risk, while others showing no associations for breastfeeding practices [48-50]. In our study, breastfeeding and longer breastfeeding duration decreased the hazards for caries incidence in univariate models, but they were not significant in the final stepwise model. The correlations between breastfeeding practices and SES variables that were retained in our model make it difficult to independently assess the effect of breastfeeding practices.

Here we also present the first survival GWAS to date for caries incidence in the primary dentition. The SNP-based heritability estimate of time to first caries incidence was 0.54 (SD: 0.41), which is similar to the heritability estimates of caries scores measured at a single point in time in the primary dentition (0.54-0.70) from a family study [21], slightly higher than the heritability estimates of the incremental increase in caries scores (0.30) from a twin study [22], and slightly higher than the SNP-based heritability estimate of caries status (yes or no) in the primary dentition (0.28 [95% CI: 0.09-0.48]) from a GWAS [28]. The SNP-based heritability estimate in our study only reflects the genetic contributions of common variants; other genetic components (e.g., low frequency and rare variants, copy number variants, structural variants, or genetic interactions) were not considered. In addition, the relatively large SD of the heritability estimate indicates the uncertainty of heritability modeling. One explanation for the uncertainty is that our sample size (under 5000) was too small to calculate the precise estimate, hence the large SD. Future studies with larger sample size are needed to estimate the heritability more precisely and further explore the genetic contributions of caries incidence.

Of the 14 identified loci at the suggestive significance level, 3 loci are located within or near genes with plausibly biological functions in dental caries (*COL5A1*, *ASIC2*, and *ESR1*). *COL5A1* encodes type V collagen which is a dominant regulator of collagen fibrillogenesis [51]. Mutations in *COL5A1* have been reported to be associated with Ehlers-Danlos syndrome (EDS) [52]. EDS patients experience a higher prevalence of oral problems including caries [53], tooth fractures, and periodontal disease [54], as well as pulp calcification and abnormal tooth roots [55]. *ASIC2* encodes acid-sensing ion channel 2 detected in the nerves supplying the taste buds of zebrafish [56] and in human odontoblasts implying it has a role in tooth mechanosensitivity [57]. Its association with severe gingival inflammation has been identified in a GWAS [58] and its involvement in the inflammation process has been shown in knockout mice [59]. *ESR1* encodes estrogen receptor alpha that is expressed in dental tissues and cells, such as ameloblasts, odontoblasts, and dental pulp and is involved in enamel formation [60]. Associations with *ESR1* have been identified for several dental traits including chronic periodontitis [61], dental fluorosis [62, 63], and tooth size [64]. Though these genes have been shown to be related to dental traits, it is currently unknown whether they are involved in genetic susceptibility to dental caries, specifically. Notably, the locus at chromosome 8 is located near gene *ABRA* which has been reported to be associated with dental caries in the permanent dentition at the suggestive significance level ($P \leq 10E\text{-}5$) in a prior GWAS [42]. However, there were no significant associations between time to first caries incidence and previously reported SNPs associated with caries traits at the more liberal significance level in the candidate SNP replication analysis. Failure to replicate these previous genetic associations may be due to the different phenotypes between this time-to-event study and prior studies, population differences, low statistical power to detect weak effects, or prior false positive results.

Though there were no genome-wide significant genes associated with caries incidence in the gene-based analysis, we found the top gene *TBX1* is a major candidate gene for 22q11.2 Deletion Syndrome, which causes craniofacial malformations including dental defects and

cleft palate [65]. *TBX1* encodes a member of the T-box gene family involved in tooth morphogenesis by regulating the proliferation, differentiation, and maturation of ameloblasts [66]. In vivo, microdontia with decreased stem cell proliferation was observed in *Tbx1* conditional knockout mice, while increased dental epithelial progenitor cells was shown in *Tbx1* overexpressed mice [67]. Given the role of *TBX1* in tooth development, it is plausible that this gene has effects on dental caries onset by influencing the timing of tooth eruption.

There are some limitations in this study. First, the sample size was very small for a GWAS. Any variants with small effect sizes or low frequency may not have been detectable in this survival GWAS. Second, external study samples with the necessary data (i.e., longitudinal caries assessments and genotyping data) are not currently available to replicate the identified variants in this study. Thus, independent studies with larger sample size are needed in the future. Third, timing of tooth eruption, which is variable and has been shown to be influenced by genetic factors [68], impacts the time that primary teeth are at-risk, and thus may affect caries incidence. Although we do not have data on the timing of tooth eruption across the dentition, and therefore we cannot incorporate this into our models of caries incidence, we tested the associations between caries incidence and tooth eruption-related SNPs that were reported in previous studies. No tooth eruption-associated SNPs were significantly associated with caries incidence after Bonferroni correction (Supplementary Table 2). In addition, we calculated the association and genetic correlation between caries incidence and timing of the eruption of the first tooth using the subset of our sample with such data (n = 469). Note, the first tooth to erupt is the only tooth for which we collected timing data; this is most often a mandibular central incisor, a very low-risk tooth and likely not to exhibit incident dental caries, but the timing of its eruption may serve as an indicator of the initiation of dental eruption, in general. Strictly speaking, the timing of the first tooth eruption was not associated with caries incidence (HR: 0.22 [95% CI: 0.05-1.04], P = 0.06), however, the trend was suggestive of a protective effect (i.e., later tooth eruption is protective). A weak genetic correlation (Rg: 0.22, SD: 1.09) was observed, indicating that the timing of initiation of dental eruption, while possibly contributing, does not fully explain the heritability of caries incidence, suggesting other mechanisms are at play.

In conclusion, we reported household income, home water source, and mother's educational attainment as risk factors for caries incidence and nominated several genes with biologically plausible roles in caries incidence for future investigations. Understanding the risk factors and genetic contributions to caries incidence may provide the foundation for better early detection, risk assessment and personalized interventions, and thereby improving the overall oral health.

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Disclosure of conflict of interest

None.

Address correspondence to: Mary L Marazita and John R Shaffer, Department of Human Genetics, School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA. E-mail: marazita@pitt.edu (MLM); john.r.shaffer@pitt.edu (JRS)

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Supplementary Materials

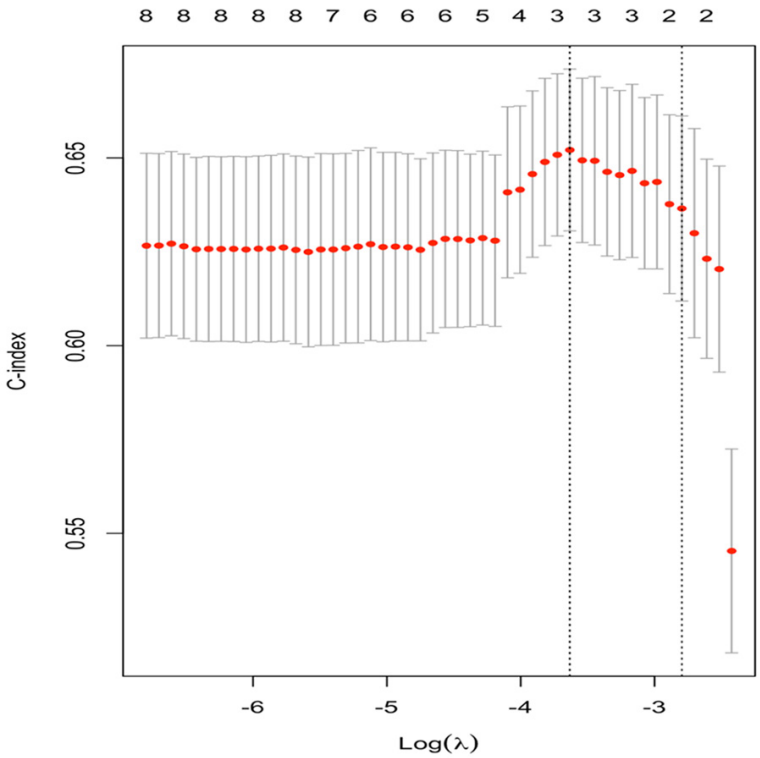
Methods

Risk factor modeling

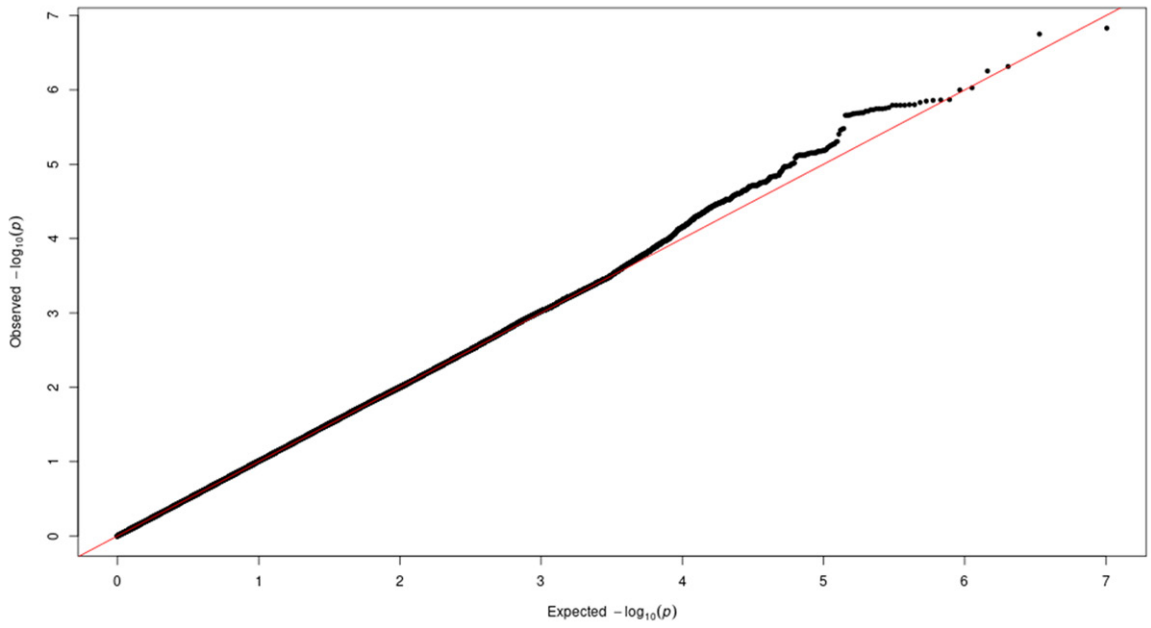
To verify the variable selection results, we performed a second procedure, regularized Cox modeling, using the R package glmnet [1]. Glmnet penalizes the negative log of the partial likelihood with an elastic net penalty and uses cyclical coordinate descent (i.e., optimizing function over each parameter with others fixed successively and cycling repeatedly until convergence). We first used the function "glmnet" to compute the solution path under default settings including all covariates, then performed cross-validation using the Harell C index to evaluate model performance. Higher C index indicates better performance. We chose the model with highest C index as the best regularized Cox model. The three variables selected in the best regularized Cox model were household income, home water source, and mother's educational attainment, which was the same set of variables as in the stepwise Cox model.

Candidate SNP replication

We tested the associations between time to first caries incidence and variants reported to be associated with dental caries traits at the genome-wide significance level ($P < 5E\text{-}8$) in previous GWASs. In total, 25 variants were reported in 5 prior GWASs [2-6]. Among these 25 variants, 2 variants had minor allele frequencies of 0% and 3 variants had no genotyping information in our data. We tested proxy SNPs based on high LD ($r^2 \geq 0.75$) for the 3 variants without genotyping information. Thus, 23 variants were tested in this study.



Supplementary Figure 1. The C-index generated from cross-validation under different values of λ. The y-axis is the C-index, the x-axis is the natural logarithm of λ, which is the tuning parameter controlling the overall strength of the penalty. The integers above the plot indicate the number of variables included in the Cox model at the current Log (λ). The left vertical line shows the highest C-index indicating the best Cox model. The 3 variables included in the best Cox model were household income, home water source, and mother's educational attainment. The results of regularized Cox model were the same as the stepwise Cox model.



Supplementary Figure 2. Quantile-quantile plot for the survival GWAS of time to first caries incidence. The genomic inflation factor was 1.00.

Supplementary Table 1. Associations between time to first caries incidence with previously reported variants associated with dental caries in both primary and permanent dentitions

SNP	Chr	BP	EA/RA	MAF	P	HR (95% CI)	Associated traits in previous GWASs
rs3896439	1	4668670	G/A	0.062	0.58	1.13 (0.74-1.72)	Partial DMFS[a] [2]
rs399593	10	30912030	T/G	0.134	0.4	1.14 (0.84-1.55)	
rs7607421	2	220792320	C/T	0.381	0.742	1.04 (0.84-1.28)	DMFS [3]
rs10790497	11	99033861	A/G	0.349	0.144	1.18 (0.95-1.46)	
rs17236529	3	160226307	T/C	0.018	0.451	1.46 (0.54-3.95)	PF.dfs[b] [7]
rs190395159[f]	7	105964857	A/G	0.001	0.586	1.73 (0.24-12.42)	DMFT [4]
rs138769355	3	151367491	A/G	0.002[e]	0.009	2.97 (1.31-6.70)	DMFS [4]
rs138642966	1	235695611	T/C	0.02	0.588	1.25 (0.56-2.82)	
rs116717469	10	62237360	C/T	0.001[e]	0.485	0.50 (0.07-3.55)	
rs71381322	17	48900824	G/A	0.024	0.82	1.08 (0.56-2.09)	
rs16946661	18	26946870	T/C	0.001[e]	0.343	0.39 (0.05-2.77)	
rs1594318	2	3733944	C/G	0.4	0.764	1.03 (0.84-1.26)	Binary in pimary[c] [5]
rs7738851	6	11241788	A/T	0.147	0.566	1.09 (0.81-1.47)	Binary in permanent[d] [5]
rs4971099[f]	1	155155731	A/G	0.426	0.358	1.10 (0.90-1.35)	DMFS [6]
rs121908120	2	219755011	T/A	0.028[e]	0.675	0.89 (0.53-1.51)	
rs1122171	5	134509987	T/C	0.439	0.738	0.97 (0.79-1.18)	
rs1482698	5	44539453	G/C	0.385	0.754	1.03 (0.84-1.27)	
rs9366651	6	26336696	T/G	0.47	0.974	1.00 (0.82-1.22)	
rs10987008[f]	9	128661600	A/G	0.36	0.464	1.08 (0.88-1.33)	
rs72748935	15	63639416	T/C	0.472	0.172	0.87 (0.71-1.06)	
rs10048146	16	86710660	A/G	0.168	0.607	1.07 (0.82-1.41)	
rs28822480	18	57924823	G/A	0.287	0.496	0.93 (0.75-1.15)	
rs11672900	19	49220323	A/G	0.496	0.398	1.09 (0.89-1.33)	

Note: Chr: chromosome; BP: base pair position; EA: effect allele; RA: reference allele; MAF: minor allele frequency; HR: hazard ratios; DMFS: decayed, missing, filled surfaces in the permanent dentition; DMFT: decayed, missing, filled teeth in the permanent dentition. [a]DMFS in mid-dentition (pre-molars and canines). [b]dfs in Pit-and Fissure surfaces. [c]binary trait (Yes/No affected) in the primary dentition. [d]binary trait (Yes/No affected) in the permanent dentition. [e]MAF < 0.05, excluded in the survival GWAS. [f]No genotyping information in our data. We listed the original rsID from previous GWASs in the table and tested their closest proxies ($r^2 \geq 0.75$). The proxy of rs190395159 is rs375025137 ($r^2 = 0.75$, distance = 854 bp). The proxy of rs4971099 is rs4971100 ($r^2 = 0.91$, distance = 123 bp). The proxy of rs10987008 is rs10987017 ($r^2 = 1$, distance = 7115 bp).

Supplementary Table 2. Associations between time to first caries incidence with previously reported variants associated with tooth eruption

SNP	Chr	BP	EA/RA	MAF	P	HR (95% CI)	Associated traits in previous GWASs
rs8079702	17	68190826	A/G	0.445	0.682	0.96 (0.78-1.17)	Age at first tooth eruption and/or number of teeth at 12 months [8]
rs4844096	23	68805318	G/A	0.463	0.311	1.09 (0.93-1.28)	
rs5936487	23	68892916	A/G	0.452	0.380	1.07 (0.92-1.26)	
rs10506525	12	65783378	C/T	0.393	0.618	1.05 (0.86-1.29)	
rs9674544	17	47084711	A/G	0.497	0.956	0.99 (0.82-1.21)	
rs1956529	14	68788924	T/C	0.387	0.031	0.80 (0.65-0.98)	
rs12424086	12	66364509	T/C	0.214	0.390	0.90 (0.71-1.14)	Number of permanent teeth [9]
rs4491709	2	217894756	T/C	0.271	0.702	0.96 (0.76-1.2)	
rs2281845	1	201081943	C/T	0.401	0.596	0.95 (0.78-1.15)	
rs7924176	10	76295789	A/G	0.429	0.714	1.04 (0.85-1.26)	
rs10932688	2	217863481	C/G	0.238	0.772	1.04 (0.81-1.32)	Age at first tooth eruption and/or number of teeth at 12 months [10]
rs6568401	6	106188818	T/C	0.269	0.778	1.03 (0.83-1.29)	
rs1799922	7	128415195	T/G	0.397	0.244	0.89 (0.72-1.09)	
rs10740993	10	18442482	C/T	0.456	0.188	0.87 (0.72-1.07)	
rs7924176	10	76295789	A/G	0.429	0.714	1.04 (0.85-1.26)	
rs4937076	11	125826702	A/G	0.472	0.990	1.00 (0.82-1.23)	
rs12229918	12	65762058	G/C	0.365	0.801	1.03 (0.84-1.26)	
rs17101923	12	66338202	G/T	0.246	0.240	0.88 (0.7-1.09)	
rs9316505	13	51390598	A/G	0.437	0.619	0.95 (0.78-1.16)	
rs997154	14	23464482	G/A	0.222	0.511	0.92 (0.73-1.17)	
rs17563	14	54417522	G/A	0.464	0.996	1.00 (0.81-1.23)	
rs1994969	17	47080431	T/G	0.474	0.819	0.98 (0.8-1.19)	
rs412000	17	56709058	C/G	0.458	0.675	0.96 (0.78-1.17)	
rs8080944	17	68185586	A/G	0.421	0.437	0.92 (0.75-1.13)	
rs11796357	23	68798703	A/G	0.210	0.708	0.96 (0.8-1.16)	

Note: Chr: chromosome; BP: base pair position; EA: effect allele; RA: reference allele; MAF: minor allele frequency; HR: hazard ratios.

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