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Fine mapping of locus Xq25.1-27-2 for a low caries experience phenotype

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Abstract

Objective—The purpose of this study was to fine map the *locus* Xq25.1-27-2 in order to identify genetic contributors involved in low caries experience.

Design—Seventy-two families from the Philippines were studied. Caries experience was recorded and genomic DNA extracted from peripheral blood was obtained from all subjects. One hundred and twenty-eight polymorphisms in the locus Xq25.1-27-2, a region that contains 24 genes, were genotyped. Association between caries experience and alleles was tested using the transmission disequilibrium test (TDT). This initial analysis was followed by experiments with DNA samples from 1,481 subjects from Pittsburgh, 918 children from Brazil, and 275 children from Turkey in order to follow up the results found in the Filipino families. Chi-square or Fisher's exact tests were used. Sequencing of the coding regions and exon-intron boundaries of *MST4* and *FGF13* were also performed on 91 women from Pittsburgh.

Results—Statistically significant association with low caries experience was found for 11 markers in Xq25.1-27-2 in the Filipino families. One marker was in *MST4*, another marker was in *FGF13*, and the remaining markers were in intergenic regions. Haplotype analysis also confirmed these results, but the follow up studies with DNA samples from Pittsburgh, Brazil, and Turkey showed associations for a subset of the 11 markers. No coding mutations were identified by sequencing.

Conclusions—Our study failed to conclusively demonstrate that genetic factors in Xq25.1-27-2 contribute to caries experience in multiple populations.

Introduction

Caries prevalence differences between sexes have been reported in some populations.[1–3] Hypotheses have been proposed to explain this phenomenon, such as: (1) female sex hormones and associated physiological factors can affect cavity formation, (2) women produce less saliva than men, (3) women have food cravings, and (4) women have variations in immune response.[4,5]

Variation between females and males in the expression of genes in the X chromosome could also explain differences in caries experience between sexes. Amelogenin is a gene involved in enamel formation and is located at chromosome X (Xp22.3-p22.1). Our group demonstrated an association between markers in this gene and caries experience.[6–9] The recent genome-wide association studies did not suggest associations between markers in the X chromosome and caries.[10,11]

Our previous genome-wide linkage scan for caries provided evidence of the involvement of the locus Xq27.1 in low caries experience.[12] A nonparametric LOD score p-value of 0.0005 was found when the analysis considered low caries experience rates as the phenotype. Therefore, the aim of this study was to fine map the locus Xq25.1-27-2 in order to identify genetic contributors involved in low caries experience.

Methods

Studied Population

We studied 3,151 individuals from six population data sets, including samples from the Philippines, USA, Brazil, and Turkey.

The Filipino sample set consisted of DNA samples from 477 subjects (224 females and 253 males) from 72 families living in the Cebu Island. The mean age of the individuals was 25.8 years and ages ranged from one to 82 years. The mean DMFT/dmft score was 9.7 and scores ranged from 0 to 32.

The sample from Pittsburgh, USA consisted of 1,481 (715 males and 766 females) unrelated subjects who sought treatment at the University of Pittsburgh and were part of the Dental Registry and DNA Repository project. The mean age of the individuals was 40.9 years and ages ranged from six to 92 years. The mean DMFT/dmft score was 15.9 and scores ranged from 0 to 28.

From Brazil, two sample data sets were available for this study. The first consisted of DNA samples from 598 unrelated children and teenagers (313 males and 285 females) that sought treatment at the Federal University of Rio de Janeiro. The mean age of the children was 9.0 years and ages ranged from two to 18 years. The mean DMFT/dmft score was 2.5 and scores ranged from 0 to 17. The second sample set included DNA samples of children from Nova Friburgo. The city of Nova Friburgo is located in the northern mountainous region of the Rio de Janeiro state, 136 km from downtown Rio de Janeiro. Children (n=320, 158 males and 162 females) were from eight daycare centers in Nova Friburgo. The mean age of the children was 3.5 years and ages ranged from one to six years. The mean dmft score was 1.4 and scores ranged from 0 to 16.

From Istanbul, Turkey, two sample data sets were also available for this study. The first sample was from a study originally designed as a case-control study and consisted of 172 unrelated children (93 females and 79 males) from three to six years of age. Ninety children (mean age 4.82 years) had a dmft score of four or more and 82 children (mean age 5.99 years) were caries free.[7] The second sample was designed as a cohort study and included 103 children (45 males and 58 females). The mean age of the children was 5.0 years and ages ranged from four to six years. The mean dmft score was 2.5 and scores ranged from 0 to 9.

These samples were used with the approval of the University of Pittsburgh Institutional Review Board and each Institutional Review Board at the original sites where the samples were obtained, and appropriate written informed consent was obtained from all participants. Age appropriate assent documents were used for children between seven and 14 years and informed written consent was obtained from the child, as well as from the parents.

Determination of caries experience

Caries was diagnosed using a modified World Health Organization protocol recommended for oral health surveys.[13] Teeth lost to trauma or primary teeth lost to exfoliation were not

included in the final DMFT/dmft scores. When records indicated that teeth were extracted for orthodontic reasons or periodontal disease, or treatments were performed on sound teeth, these situations were not included in the final DMFT/dmft scores. The studies developed in Turkey included white spot lesions as evidence of caries. For all studies, carious lesions were recorded as present when a break in enamel was apparent on visual inspection. All of the examiners carried out the clinical examination after being calibrated by an experienced specialist. Details about the determination of caries experience were previously described. [6,7,11,12,14]

In this study, the populations were classified as either 'low caries experience' or 'high caries experience,' based on DMFT/dmft distribution in each cohort (DMFT/dmft mean and standard deviation) and subject's age. The criteria used here for classification of caries experience took age into consideration, since it is expected that caries experience will increase in the general population with age.[1] Table 1 presents caries experience definitions for Filipino and US cohorts. For the Turkish and Brazilian cohorts (which included only children), subjects that had a DMFT/dmft score between 0–2 were classified as 'low caries experience.' The subjects that had a DMFT/dmft score 3 or higher were classified as 'high caries experience.'

Single Nucleotide Polymorphism (SNP) Genotyping

The Xq25.1-27.2 locus was fine mapped based on our previous genome-wide linkage results.[12] This region covers approximately fourteen million base pairs and has 24 genes. For the selection of genetic markers, we used data from the International HapMap Project on Whites and Chinese (www.hapmap.org), viewed through the software Haploview.[15] Based on pairwise linkage disequilibrium and haplotype blocks, we selected 128 single nucleotide polymorphism (SNPs; table 2; locations and minor allele frequencies obtained from dbSNP 138) in the region and genotyping was performed by polymerase chain-reactions with the Taqman method with the real-time PCR system ABI PRISM® 7900HT Sequence Detection System (Foster City, CA, USA). Probes were supplied by Applied Biosystems (Foster City, CA, USA).

Hardy-Weinberg equilibrium was evaluated using the chi-square test within each SNP in each population and only the results that were in Hardy-Weinberg equilibrium were further analyzed. In the first step of analysis, we evaluated the 128 selected SNPs in the Filipino families. The association between caries experience and the SNPs was tested with the transmission disequilibrium test (TDT) within the program Family-Based Association Test (FBAT)[16] in the "sex chromosome model." An alpha of 0.0004 (0.05/128) was used to indicate statistical significance. In the second step of the genotyping analysis, the eleven SNPs selected from the original 128 SNP panel with p-values of 0.05 or lower were selected for follow-up studies. The data sets from the US, Brazil, and Turkey were used for the follow up experiments. The differences in genotype and allele frequencies between 'high' and 'low' caries experience groups were tested using the PLINK software package[17] with sex as a covariate in the model for sex chromosomes. An alpha of 0.0045 (0.05/11) was used to indicate statistical significance (Bonferroni correction). Haplotype analysis was also performed.

Sequence Analysis

We sequenced exons, exon-intron boundaries, and untranslated regions (UTRs) of *MST4* and *FGF13*, since associations were found in these genes. The primers for the amplification of these regions were designed using the software PRIMER3.[18] Primer sequences and PCR conditions are presented in table 3. Samples were sent to Functional Biosciences, Inc. (Madison, WI, USA) for purification and sequencing. Sequence contigs were verified against a consensus sequence obtained from the UCSC genome browser with the software Sequencher 5.1 (Gene Codes Corporation, Anna Harbor, MI, USA).

Results

Association Results in the Filipino Families

Out of 128 SNPs used for fine mapping the target chromosomal region and tested for association with low caries experience, eleven had nominal p-values of 0.05 or lower. These results are presented in Table 2. Similar results could also be seen between low caries experience and the haplotypes of these markers (Table 4).

Association Results in the Follow-up Populations

Follow-up studies showed similar nominal results for some markers in the US, Brazilian, and Turkey data sets, which are presented in Table 5.

Sequence data

For sequence analyses, we selected women from the Pittsburgh dataset with low caries experience. Samples from 91 subjects were selected for sequencing. Nine subjects presented a heterozygous mutation in the base pair position 131,208,595 of chromosome X, previously reported as rs5933061, and four subjects presented a mutation in the base pair position 131,208,596, previously reported as rs995249. Both mutations are in the 3′ UTR region of *MST4*. In *FGF13*, two subjects were heterozygous in the base pair position 137,713,678 downstream of the gene, variant previously described as rs17539045.

Discussion

Epidemiological and clinical studies have demonstrated that females have higher caries prevalence than males.[19] The mechanisms underlying the reasoning for this trend can possibly be explained by an investigation of the genes present on the X chromosome. In this work we investigated the locus Xp22.3-p22.1, which we found linked to low caries experience in our previous genome-wide linkage study. This region contains 24 genes and some of them can be articulated as related to caries experience. With the exception of the Filipino data that show slightly higher caries experience in females than males,[1] our data from Brazil, USA, and Turkey did not show differences in caries experience between females and males. It is possible that genetic associations can be more readily detectable in groups where females are more severely affected than males.

This fine map study failed to determine the presence of association between markers in Xp22.3-p22.1 and low caries experience. At the nominal level, markers in *FGF13* and *MST4* showed trends for association with low caries experience.

The trends found for *FGF13* and low caries experience are exclusive for the population datasets comprised of adults (Philippines and Pittsburgh). The sequence analyses showed two subjects with a variant previously identified and described as rs17539045. The minor allele frequency of this variant is 2.2% in the dbSNP build 138 database, the same as our study. FGF13, when mutated, affects hair growth and also causes dental anomalies,[20] but our results do not clearly implicate this gene in caries experience. We biased the sample selection to females only to increase the number of chromosomes evaluated since the hypothesis was that rare variants not possibly detected by association could be involved in caries. Since males are hemizygous, they would provide half of the number of chromosomes that could have been assayed.

Previous studies uncover FGF signaling as a major regulator of lumen formation during salivary gland development.1[21–24] The flow rate and composition of saliva in the host oral environment impacts caries susceptibility. Saliva plays a protective role in the oral cavity through its buffering, mechanical washing, antimicrobial, and remineralization activities. In addition, the flow rates of saliva and compositional analysis have been shown to be generally less protective in women than in men.[17]

MST4 is a member of the GCK group III family of kinases, which are a subset of the Ste20-like kinases. The protein codified by *MST4* is localized in the Golgi apparatus and is specifically activated by binding to the Golgi matrix protein.[25] A trend for association between markers in MST4 and low caries experience was found in the Filipino dataset only. These results are modest and may indicate a false-positive association.

It is possible that our study did not have enough statistical power to detect an association between Xq25.1-27-2 and low caries experience. The effects of this locus on caries may be so small that only several thousand samples may be able to detect. Being concerned with multiple testing, we applied the strict Bonferroni correction to not increase type II erros. However our own data from previous projects demonstrate that under Bonferroni correction we can miss true associations.[26] Hence, here we report our modest results and nominal p-values to avoid publication bias favoring only positive genetic association results. Another limitation of this study is the phenotype definition that relies on a one time assessment of caries experience. Longitudinal approaches may be more suitable to detect relationships between caries and genes since the pathogenesis of the disease is multifactorial. DMFT/dmft scores represent a snapshot of the consequence of the disease but they are not informative in regards to the mechanisms involved in the disease severity. Other definitions of disease, including clinical findings related to plaque accumulation, saliva composition, and fluoride exposure, may facilitate the identification of specific genes and gene mechanisms contributing to caries.

Conclusion

This study failed to demonstrate an association between low caries experience and Xq25.1-27-2.

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Table 1

Definitions of caries experience used in the Filipino and US data sets based on age and DMFT/dmft scores.

Philippines			**Pittsburgh (USA)**		
Caries Experience Level	n	DMFT/dmft	Caries Experience Level	n	DMFT/dmft
Children [under to 12 yrs of age]			***Children and teeneger [from 6 to 19 yrs of age]***		
			Low caries experience	73	0–3
Low caries experience	26	0–2	High caries experience	138	4 or higher
High caries experience	89	3 or higher	***Young Adults [from 20 to 39 yrs of age]***		
Teenagers [from 13 to 19 yrs of age]			High caries experience	215	0–10
			High caries experience	241	11 or higher
Low caries experience	44	0–5	***Middle age [from 40 to 59 yrs of age]***		
High caries experience	60	6 or higher	Low caries experience	152	0–15
Adults [20 yrs of age and older]			High caries experience	384	16 or higher
			Elderly [60 yrs of age and older]		
Low caries experience	99	0–8	Low caries experience	95	0–20
High caries experience	159	8 or higher	High caries experience	183	21 or higher

Table 2

Studied single nucleotide polymorphisms and p-value of the association between caries experience group in the Filipino families.

Location	Marker number	Base Change	Gene Symbol	Gene Name	p-value
chr. X 129088939	rs5977183	C/G			0.085
chr. X 129202285	rs6637685	C/T	*ELF4*	E74-like factor 4 (ets domain transcription factor)	0.160
chr. X 129232391	rs209990	A/G			0.663
chr. X 129607422	rs6529430	A/G			0.389
chr. X 129623643	rs17305502	G/T			0.405
chr. X 129749449	rs6634795	C/T	*ENOX2*	ecto-NOX disulfide-thiol exchanger 2	0.132
chr. X 130193804	rs4074535	A/G	*ARHGAP36*	Rho GTPase activating protein 36	0.521
chr. X 130331814	rs5932861	A/G			0.832
chr. X 130404369	rs6637822	C/G	*IGSF1*	immunoglobulin superfamily, member 1	0.512
chr. X 130413442	rs10521765	C/G			0.101
chr. X 130488944	rs5932901	C/T			0.473
chr. X 130492895	rs2475412	C/T			0.649
chr. X 130554291	rs12556610	A/G			0.779
chr. X 130669439	rs4829728	A/T	*OR13H1*	olfactory receptor, family 13, subfamily H, member 1	0.483
chr. X 130746838	rs707254	A/G			0.454
chr. X 130949827	rs4830231	C/T	*LOC286467*	hypothetical LOC286467	0.512
chr. X 131154139	rs2748729	A/G	*MST4*	serine/threonine protein kinase MST4	**0.034**
chr. X 131820147	rs858618	A/G	*HS6ST2*	heparan sulfate 6-O-sulfotransferase 2	0.341
chr. X 131894744	rs7881124	A/G			0.177
chr. X 131947668	rs5977761	G/T			0.122
chr. X 132285877	rs242143	C/T			0.844
chr. X 132547106	rs5977872	A/G	*GPC4*	glypican 4	0.513
chr. X 132566456	rs11096369	A/G			0.263
chr. X 132638098	rs2106472	A/G			0.616
chr. X 132942023	rs1908817	C/G	*GPC3*	glypican 3	0.219
chr. X 133030056	rs2284125	C/T			0.232
chr. X 133150848	rs12558772	A/G			0.780

Location	Marker number	Base Change	Gene Symbol	Gene Name	p-value
chr. X 133242671	rs5977959	A/G			0.800
chr. X 133280911	rs2312983	C/T			0.311
chr. X 133561242	rs6638230	A/G	*PHF6*	PHD finger protein 6	0.227
chr. X 133571373	rs2097778	A/G			0.427
chr. X 133699960	rs1982	G/T	*PLAC1*	placenta-specific 1	0.994
chr. X 134176178	rs5975493	C/G			0.733
chr. X 134473551	rs933383	A/G	*ZNF449*	zinc finger protein 449	0.601
chr. X 134553458	rs5930702	C/G			0.847
chr. X 134584301	rs6528247	C/T			0.699
chr. X 135148483	rs903143	C/T			**0.033**
chr. X 135262632	rs7061270	C/T	*FHL1*	Four and a half LIM domains 1	0.400
chr. X 135268469	rs5975695	C/T			0.650
chr. X 135431358	rs5930933	C/T	*GPR112*	G protein-coupled receptor 112	0.677
chr. X 135445357	rs5974594	C/T			0.672
chr. X 135673047	rs5930964	A/C			0.983
chr. X 135706075	rs5930970	C/G			0.747
chr. X 135837778	rs661426	C/T	*ARHGEF6*	Rac/Cdc42 guanine nucleotide exchange factor(GEF)6	0.565
chr. X 135842871	rs476774	A/C			0.758
chr. X 136053904	rs1190738	A/C			0.591
chr. X 136225349	rs5931073	C/T			0.156
chr. X 136248929	rs5931088	A/C			0.856
chr. X 136396877	rs5929821	C/G			0.188
chr. X 136495205	rs2840672	A/G			0.805
chr. X 136571724	rs5931158	C/T			**0.029**
chr. X 136600502	rs6635446	C/T			0.183
chr. X 136699584	rs12687601	C/T			**0.017**
chr. X136738181	rs4829893	A/G			**0.012**
chr. X 136816568	rs1324156	A/T			**0.043**
chr. X 137038855	rs5929862	A/G			0.356

Location	Marker number	Base Change	Gene Symbol	Gene Name	p-value
chr. X 137086064	rs882448	A/G			0.933
chr. X 137214310	rs12556287	C/G			0.335
chr. X 137273537	rs5931325	C/T			0.152
chr. X 137306790	rs5974725	C/T			0.354
chr. X 137329745	rs5931353	C/T			0.390
chr. X 137396573	rs1361551	A/G			0.391
chr. X 137409895	rs5931378	A/G			0.265
chr. X 137471351	rs1487918	G/T			0.517
chr. X 137848601	rs5931483	A/G	*FGF13*	fibroblast growth factor 13	0.584
chr. X 137927172	rs12838463	A/G			0.883
chr. X 137951029	rs5931506	C/T			0.606
chr. X 137996323	rs5931514	A/T			0.954
chr. X 138045552	rs10856566	A/T			0.272
chr. X 138057626	rs4520317	G/T			0.764
chr. X 138155474	rs5931566	G/T			0.925
chr. X 138236517	rs5931572	A/G			**0.045**
chr. X 138266294	rs4829963	C/T			0.095
chr. X 138270349	rs5974804	C/T			0.063
chr. X 138308908	rs6634045	C/T			**0.035**
chr. X 138615521	rs371000	C/T	*F9*	coagulation factor IX	0.711
chr. X 138722200	rs5907607	A/G	*MCF2*	MCF.2 cell line derived transforming sequence	0.811
chr. X 138967742	rs2485724	G/T			0.869
chr. X 139185642	rs1886366	A/G			0.724
chr. X 139295472	rs439883	C/T			0.328
chr. X 139492869	rs6634148	A/G			0.328
chr. X 139685339	rs6634180	A/G			0.794
chr. X 139757252	rs5954063	A/T			0.71
chr. X 139827513	rs11095831	A/T			0.413
chr. X 140027664	rs5907093	A/C			**0.045**

Location	Marker number	Base Change	Gene Symbol	Gene Name	p-value
chr. X 140055547	rs1565843	T/C			0.097
chr. X 140230896	rs1016824	T/C			0.223
chr. X 140275485	rs5907830	C/G	*LDOC1*	leucine zipper, down-regulated in cancer 1	0.303
chr. X 140313609	rs1099501	A/G			0.548
chr. X 140371091	rs845163	G/A			0.568
chr. X 140407317	rs5907882	T/C			0.673
chr. X 140511790	rs6636302	A/G			0.239
chr. X 140544892	rs5907945	G/A			0.589
chr. X 140555282	rs6654428	G/A			0.531
chr. X 140828207	rs916354	C/G			0.617
chr. X 140895389	rs5908052	A/G			0.545
chr. X 141033351	rs6636538	A/G			0.199
chr. X 141127038	rs5908097	A/G			0.564
chr. X 141166965	rs7056485	C/T			0.799
chr. X 141181280	rs5907230	C/G			1.000
chr. X 141322841	rs11796500	A/G			0.467
chr. X 141488796	rs5908311	C/T			0.757
chr. X 141579534	rs5954679	C/T			0.721
chr. X 141616460	rs1040474	A/G			0.744
chr. X 141697312	rs7891458	A/G			0.116
chr. X 141765482	rs6529043	A/G			0.762
chr. X 141802685	rs5908399	A/G			0.963
chr. X 141923733	rs7065033	G/T			0.727
chr. X 141944963	rs5907355	A/C			0.362
chr. X 142087501	rs5908491	C/T			0.855
chr. X 142093305	rs5908499	A/T			0.346
chr. X 142125008	rs4825150	A/G	*SPANXN4*	SPANX family, member N4	0.219
chr. X 142241362	rs5908569	A/G			0.295
chr. X 142271947	rs5908582	C/T			0.954

Location	Marker number	Base Change	Gene Symbol	Gene Name	p-value
chr. X 142420719	rs2865521	C/T			0.751
chr. X 142434889	rs5955002	A/G			0.947
chr. X 142456822	rs5908648	C/T			0.561
chr. X 142490526	rs5955016	C/T			0.177
chr. X 142517697	rs5907426	C/G			**0.009**
chr. X 142566176	rs5955034	C/T			0.114
chr. X 142637455	rs2207580	A/C			0.718
chr. X 142692263	rs2073252	G/T			**0.027**
chr. X 142720238	rs12156770	C/T	*SLITRK4*	SLIT and NTRK-like family, member 4	0.426
chr. X 142813456	rs5908778	C/T	*SPANXN2*	SPANX family, member N2	0.217
chr. X 142898144	rs5953891	A/C			0.971
chr. X 142966181	rs237514	C/T	*UBE2NL*	ubiquitin-conjugating enzyme E2N-like	0.325
chr. X 142994731	rs237537	A/T			0.063
chr. X 143031283	rs1077314	C/T			0.184

Note: Markers with no gene identification are in an intergenic area. Bold form indicates p≤0.05.

Table 3

Primers used for sequence analysis.

Gene	Region	Primer sequences (5′ 3′)- Forward	Primer sequences (5′ 3′)- Reverse	Amplicon size (bp)	Annealing Temperature
MST4	Exon 1	GCGCCAGAAAGGTAGACTGA	ACACCAGGAAACACCTCCAG	371	58°C
	Exon 2	TGGGGAGTCAGAGGAGTTTG	GATTCTGCCTGGAAGCCATA	486	54°C
	Exon 3	GCTCCCACAAATAAATGAGAACA	AATGCCAGCAACATGTTCAA	381	53°C
	Exon 4	GGAACCTCAGAATTTGAAGCCTA	TTGGCAGCTAGGAGGAAAAA	483	54°C
	Exon 5	TTGGAATGGAGTTTCAAGGTG	AACCAGGTTCCTGGATGGAT	420	52°C
	Exon 6	TTGGAATGGAGTTTCAAGGTG	CCCTACTGCAGGAAAAGCTG	372	52°C
	Exon 7	TTTTCCTACAGCATTTTAGTTGTTC	CAAGCACACTGGAGCATAGC	377	51°C
	Exon 8	AGTATTCGCCTGAAGCTGAGA	AGTGAGGCAGACAGGCATTT	367	55°C
	Exon 9 and 10	TTTTGTTTTCAGTAGGGGATTCA	TTGGAATGGTTGAATCAAAGG	482	51°C
	Exon 11 and UTR1	TGTGAAATCAGATGCAGAAGG	CATCCTCGTAACTTAAGAAATAGGG	497	53°C
	UTR2	TTTGTGATGGCGTTTATCATTT	CCAACTTGGTAGAAGAGTCAATACTTT	400	51°C
	UTR3	CAGGTCTTTCAAAGTCATTCTCAA	GCTCTCCAAATCCAAGCAGA	483	53°C
	UTR4	GGCAATTTTACCCTTATTTCACA	TTTGTGTGGACAGAAATCATCC	389	51°C
	UTR5	TTTGCCATGGTAATAAATTGTCC	GGATGTAATGGCCACTTTGAC	469	51°C
	UTR6	TGGGGCTGTATTTCAGTAGTTG	TTGGGATGCAATTGTGTTGT	498	53°C
	UTR7	TTGTGGTGTTGTATGCCAAGA	TGAAAAGTATCTTCTGGTTTCCA	498	52°C
FGF13	Exon 1	CAAACGCAGGCTAAGGAGAC	TGTTACGGAGGCAAGAAAGC	484	56 °C
	Exon 2	TTGTTTTCTTTCTTCCAGACACC	TTCAGGATTTAGGAAAGCGTATTT	353	53 °C
	Exon 3	GCAGTCCTGCTGGAAACATT	AAAATGCTTGCTTCAAATAACTG	380	53 °C
	Exon 4	CCTAAAAGGCAATACCACATGA	ACCACCTGTACCCCCAAAAC	370	52 °C
	Exon 5	TCATTTAGGTGTGTGGAATAGAACA	TGCTTGGCATTCTTATGCAC	456	54 °C
	UTR1	GGTGTGTGGAATAGAACACCTACA	GAACTCTGCCTGTTTGTTTGG	363	55 °C
	UTR2	CAAATCCATGAGCCACAATG	ATTTTCTTGGTGGGAGAGCA	373	53 °C
	UTR3	TCTGAGAGAAGGACTGCCAAA	CTTGAGCCTGAGAAGCCAAT	390	55 °C
	UTR4	TGTTGTTTTCTTGGCTTGATG	TGCCAAATACAATGACAGGAAG	500	59 °C
	UTR5	CCTCAAGTGTAGATTTCTCTTAATTCC	TGTCCACTTTTGGAACACAGAT	386	54 °C
	UTR6	CTGGTTTTCCCTCAACAACA	CCTTTGTACCAATCTGAAGTGC	500	54 °C

Table 4

Summary results of the haplotype analyses in the Filipino families.

Haplotype	n	Alleles	p-value
rs2748729- rs903143	29	G-C	0.018
rs903143- rs5931158	4	T-C	0.003
rs5931158- rs12687601	13	C-C	0.006
rs12687601- rs4829893	18	C-A	0.008
rs4829893- rs1324156	15	A-2	0.043
rs5931572- rs6634045	24	A-T	0.004
rs6634045- rs5907093	17	T-A	0.035
rs5907093- rs5907426	12	C-C	0.017
rs5907426- rs2073252	27	G-G	0.001

Notes: n=number of informative families

Table 5

Markers association results in five populations.

Region	Markers	Pittsburgh (USA) Cohort		Rio de Janeiro (Brazil) Cohort		Nova Friburgo (Brazil) Cohort		Istanbul (Turkey) Case-control		Istanbul (Turkey) Cohort	
		n=1,481		n=598		n=320		n=172		n=103	
		p-value		p-value		p-value		p-value		p-value	
		Allele	*Genotype*	*Allele*	*Genotype*	*Allele*	*Genotype*	*Allele*	*Genotype*	*Allele*	*Genotype*
MST4	rs2748729	0.58	0.54	0.55	0.23	-	-	0.45	0.82	0.63	0.65
Intergenic	rs903143	**0.05**	0.16	0.60	0.42	**0.02**	**0.03**	0.53	0.70	0.80	0.87
Intergenic	rs5931158	0.16	0.37	0.24	0.23	0.92	0.98	0.89	0.75	0.52	0.75
Intergenic	rs12686701	0.88	0.68	-	-	0.29	0.51	0.50	0.79	0.56	0.77
Intergenic	rs4829893	-	-	0.73	0.77	**0.01**	**0.01**	0.36	0.70	0.64	0.65
Intergenic	rs1324156	0.14	**0.03**	-	-	**0.05**	0.11	-	-	0.95	0.94
FGF13	rs5931572	**0.01**	**0.03**	0.25	0.54	0.16	0.36	0.52	0.35	0.78	0.32
Intergenic	rs6634045	0.52	0.78	0.34	0.59	0.93	0.53	0.92	0.67	-	-
Intergenic	rs5907093	0.38	0.68	**0.01**	**0.04**	**0.05**	0.08	0.09	0.10	0.69	0.87
Intergenic	rs5907426	0.06	0.19	-	-	0.02	0.02	0.97	0.91	0.83	0.96
Intergenic	rs2073232	0.67	0.01	0.68	0.81	0.56	0.72	0.85	**0.05**	0.32	0.53

Note: p-values represent the comparisons performed between low and high caries experience for allele and genotype distribution for each marker; bold form indicates p≤0.05. “-” indicates all individuals were homozygous for the wild type allele.

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