Effects of enamel matrix genes on dental caries are moderated by fluoride exposures

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SUPPLEMENTAL MATERIAL

Supplemental tables are provide which show the full linear model results for tests of genetic association and pairwise linkage disequilibrium between SNPs.

Recruitment and data collection in parent studies

Center for Oral Health in Appalachia, cohort 1 (COHRA1): COHRA1 was designed as a collaborative initiative between the University of Pittsburgh and West Virginia University in order to investigate the oral health problems facing rural Appalachian communities of western Pennsylvania and northern West Virginia (Polk et al, 2008). COHRA1 focuses on exploring multiple levels of factors that may influence oral health disparities, including factors pertaining to the community, the family, and the individual. Recruitment for COHRA1 sampled communities by household, with eligible households required to include at least one biological parent-child pair. All members of an eligible household were then invited to participant without regard to their oral health status or their biological or legal relationships. Intra-oral examinations were performed on each study participant by a licensed dentist or research dental hygienist. Inter- and intra-rater agreement for dental assessments was high (Polk et al. 2008; Wendell et al. 2010). DNA samples were collected from blood, buccal swab, mouthwash, or saliva samples via DNA Genotek Oragene kits (www.dnagenotek.com). Written informed consent was obtained from all adult participants. Written parental consent was obtained for all assenting child participants. All communications, procedures, protocols, and forms were approved by Institutional Review Boards (IRBs) at the University of Pittsburgh and West Virginia University.

Iowa Head Start (IHS) Study: Low-income children (aged 3 to 5 years) who participated in the federally subsidized Iowa Head Start programs were recruited into the IHS study (Slayton et al., 2005). Dental caries experience was assessed by standardized in-field dental examinations performed by trained research dental hygienists. DNA samples (buccal swabs or saliva samples) were collected using Oragene kits from DNA Genotek (www.dnagenotek.com). Participants provided assent with parental or legal guardian consent in order to participate in the study. All protocols and procedures were approved by the IRB at the University of Iowa.

Iowa Fluoride Study (IFS): The IFS was designed as a longitudinal study to follow newborns from participating Iowa postpartum wards from birth through adulthood (Wang et al 2012). The goal of the study was to investigate the relationships between extensive measures of fluoride exposures and dental-related phenotypes. Trained dentists performed dental assessments during field examinations for children at ages 4 to 6 years. DNA was obtained from blood, buccal swab, or saliva samples as part of an ancillary genetics study. All parents provided informed written consent, and all children provided verbal assent. All study questionnaires, procedures, and protocols were approved by the IRB at the University of Iowa.

Dental Strategies Concentrating On Risk Evaluation (SCORE): The Heart SCORE study was designed to investigate the factors contributing to disparities in cardiovascular risk factors across racial and socioeconomic groups (Aiyer et al 2007a, b). Participants were recruited from the Pittsburgh area. The Dental SCORE study was an ancillary project, which included a subset of the original Heart SCORE participants. Dental SCORE participants received dental assessments by trained research dental hygienists following the same protocols as the COHRA1 study. DNA from saliva samples was obtained using DNA Genotek Oragene kits (www.dnagenotek.com). All participants provided informed consent. All protocols were approved by the University of Pittsburgh IRB.

Dental Registry and DNA Repository (DRDR): The DRDR was initiated to serve as a data warehouse for facilitating interdisciplinary dental research at the University of Pittsburgh. Invitation to participate was offered to patients seeking dental treatment at the University of Pittsburgh School of Dentistry, and all participating patients were enrolled without regard to their oral health or medical status. DNA samples were provided via saliva samples taken in Oragene kits from DNA Genotek

(www.dnagenotek.com), which were linked to patient dental records. Participants provided written consent for the use of their genetic data and dental records in future research studies. The DRDR was approved by the IRB at the University of Pittsburgh.

Center for Education and Drug Abuse Research (CEDAR): The CEDAR study was designed to investigate substance use risk factors in the offspring of fathers with and without substance use disorder. Nuclear families were recruited and their participating offspring of the proband fathers were followed from initial ages of 10 to 12 years through age 30 years (Tarter and Vanyukov, 2001). The Substance Abuse and the Dopamine System Genes (Vanyukov et al, 2004) (Study 12 of the NIDA Genetics Consortium: https://zork5.wustl/ nida/study_description) recruited a subsample of the CEDAR cohort for genetic studies, which included dental assessment conducted at the University of Pittsburgh School of Dental Medicine by trained and calibrated dental hygienists. Lymphoblast cells lines established from blood samples at the NIDA Center for Genetic Studies (https://zork5/wustl/nida) provided DNA for the present study. Study questionnaires and protocols were approved by the University of Pittsburgh IRB. All parents provided informed consent along with assent from children.

Post-hoc power calculations

Though we acknowledge that there exists considerable controversy regarding the utility of post-hoc power calculations, we present the minimal effect sizes that can be detected in our tests for genetic association.

		Phenotype	Minimal Detectable Effect Size*		
	N	SD	MAF=0.4	MAF=0.2	MAF=0.1
Children					
COHRA whites	608	4.6	0.22	0.27	0.36
COHRA blacks	81	5.1	0.58	0.72	0.96
IHS whites	41	8.2	0.79	0.97	1.29
IHS blacks	23	8.1	0.99	1.21	>1.5
IFS whites	136	3.9	0.46	0.56	0.75
meta white kids	785	-	0.19	0.24	0.32
meta all kids	889	-	0.18	0.22	0.3
Adults					
COHRA whites	994	8.2	0.17	0.21	0.28
COHRA blacks	86	9.8	0.57	0.7	0.61
Dental SCORE whites	277	7.8	0.33	0.4	0.53
Dental SCORE blacks	225	7.5	0.36	0.44	0.59
DRDR whites	702	13.6	0.21	0.25	0.34
DRDR blacks	173	13.8	0.41	0.5	0.67
CEDAR whites	173	4.8	0.41	0.5	0.67
CEDAR blacks	68	4.2	0.63	0.78	1.02
meta white adults	2146	-	0.12	0.14	0.19
meta all adults	2698	-	0.1	0.13	0.17

* minimal detectable effect size is in units of phenotype SD for 80% power at alpha = 0.003.
MAF = minor allele frequency
SD = standard deviation

References:

Aiyer AN, Kip KE, Mulukutla SR, Marroquin OC, Hipps L Jr, Reis SE. Predictors of significant short-term increases in blood pressure in a community-based population. Am J Med. 2007a Nov;120(11):960-7.

Aiyer AN, Kip KE, Marroquin OC, Mulukutla SR, Edmundowicz D, Reis SE. Racial differences in coronary artery calcification are not attributed to differences in lipoprotein particle sizes: the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) Study. Am Heart J. 2007b Feb;153(2):328-34.

Polk DE, Weyant RJ, Crout RJ, McNeil DW, Tarter RE, Thomas JG, Marazita ML. Study protocol of the Center for Oral Health Research in Appalachia (COHRA1) etiology study BMC Oral Health 2008; 8:18

Slayton RL, Cooper ME, Marazita ML. Tuftelin, Mutans Streptococci and dental caries susceptibility. Journal of Dental Research 2005;84: 711-714.

Tarter RE and Vanyukov MM. Theoretical and operational framework for research into the etiology of substance use disorders. Journal of Child and Adolescent substance Abuse 2001;10:1-12.

Vanyukov MM, Maher BS, Devlin B, Tarter RE, Kirilova GP, Yu LM, Ferrell RE. Haplotypes of the monoamine oxidase genes and the risk for substance use disorders. Am J Med Genet Part B: Neuropsychiatric Genetics 2004;125B(1):120-125.

Wang X, Willing MC, Marazita ML, Wendell S, Warren JJ, Broffitt B, Smith B, Busch T, Lidral AC, Levy SM. Genetic and environmental factors associated with dental caries in children: The Iowa Fluoride Study. Caries Res. 2012 Apr 13;46(3):177-184.

Wendell S, Wang XJ, Brown M, Cooper ME, DeSensi RS, Weyant RJ, Crout R, McNeil DW, Marazita ML. Taste genes associated with dental caries. Journal of Dental Research 2010;89: 1198-1202.