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Genetic Analyses of Enamel Hypoplasia in Multiethnic Cohorts

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AUTHOR CONTRIBUTIONS

R.N.A. generated the data set for the analyses. R.N.A. analyzed data. R.N.A., M.L.M., J.R.S., S.M.W. defined the outcomes to be studied. R.N.A. wrote the first draft of the manuscript. M.LM, J.R.S, S.M.W, B.J.H, L.M.U, and K.N. critically reviewed the manuscript. R.N.A., M.L.M. designed the study. R.N.A. interpreted data. M.L.M., C.S., F.W.B.D., K.N., C.P.R., C.V.R., F.A.P., I.M.O., C.J.B., G.L.W., J.R.S., S.M.W., J.C.M., R.E.L., A.R.V., L.M.U., collected and interpreted data. R.N.A. and M.L.M. generated final draft of the manuscript. All authors reviewed final manuscript.

STATEMENT OF ETHICS

Study approval was granted by the University of Pittsburgh Institutional Review Board (coordinating center approval number CR19030367–003, Pittsburgh site approval numberCR19080127–00). All participants provided written informed consent for themselves and for children younger than 18 years, informed consent was obtained from their parents or their legally authorized representative. Local ethical approval was obtained at each site and all methods in this study were performed in accordance with the Institutional Review Board policies and guidelines of the University of Pittsburgh and all of the other sites. See also Table S2 in the supplemental materials.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest with respect to the publication of this article.

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Abstract

Introduction: Enamel hypoplasia causes a reduction in the thickness of affected enamel and is one of the most common dental anomalies. This defect is caused by environmental and/or genetic factors that interfere with tooth formation, emphasizing the importance of investigating enamel hypoplasia on an epidemiological and genetic level.

Methods: A genome-wide association of enamel hypoplasia was performed in multiple cohorts, overall comprising 7,159 individuals ranging in age from 7–82 years. Mixed-models were used to test for genetic association while simultaneously accounting for relatedness and genetic population structure. Meta-analysis was then performed. More than 5 million single-nucleotide polymorphisms were tested in individual cohorts.

Results: Analyses of the individual cohorts and meta-analysis identified association signals close to genome-wide significance ($P < 5\times10^{-8}$), and many suggestive association signals ($5\times10^{-8} < P < 5\times10^{-6}$) near genes with plausible roles in tooth/enamel development.

Discussion/Conclusion: The strongest association signal ($P = 1.57\times10^{-9}$) was observed near *BMP2K* in one of the individual cohorts. Additional suggestive signals were observed near genes with plausible roles in tooth development in the meta-analysis, such as *SLC4A4* which can influence enamel hypoplasia. Additional human genetic studies are needed to replicate these results and functional studies in model systems are needed to validate our findings.

Keywords

Enamel Hypoplasia; Dental anomalies; GWAS; Multiethnic; Genetics

INTRODUCTION

Tooth development (odontogenesis) is a complex process that starts early during embryogenesis and requires signaling between mesenchymal and epithelial tissues. Any

disturbances during this complicated process in signaling pathways or mutations in any of the regulatory genes might cause dental anomalies, including changes in tooth morphology, structure, size and number [1, 2]. Dental anomalies, such enamel hypoplasia, are assumed to be caused by interactions between environmental, genetic, and epigenetic factors during odontogenesis [3].

Tooth enamel is formed by ameloblasts, cells that produce and secrete enamel matrix proteins during the secretory stage of amelogenesis. There are two important groups of enamel proteins that are involved in the development of the enamel: the first group is non-amelogenins, which include enamelin (*ENAM*), ameloblastin (*AMBN*), tuftelin (*TUFT1*), and tuftelin-interacting protein 11 (*TFIP11*); the second group includes amelogenin (*AMELX*) [4].

Enamel hypoplasia is a quantitative defect that causes a reduction in the enamel thickness. This defect usually occurs during the secretory stage of amelogenesis and can affect both primary and permanent dentitions [5]. The clinical signs of enamel hypoplasia include or shallow fossa (i.e. depression on the tooth surface) with either horizontal or vertical grooves and sometimes could lead to a partial or complete absence of enamel. Teeth affected with enamel hypoplasia could have an increased risk of dental caries [6, 7]. This is because enamel hypoplasia can create an enhanced environment for colonization, adhesion, and retention of cariogenic bacteria. Jointly with other dental caries risk factors, such as poor oral hygiene and cariogenic diet, dental caries might progress more rapidly [6]. Additionally, hypoplastic enamel has higher acid solubility than normal enamel, which makes it more susceptible to the acid secretions of cariogenic bacteria [8].

Several factors cause variation in the prevalence of enamel hypoplasia, such as: the method of examination, population studied, specific teeth, and dentition. The reported prevalence of enamel hypoplasia ranges between 0.8% to 17% in permanent dentition, while the prevalence of enamel hypoplasia in primary dentition ranges between 0.6% to 15.1% [9–12]. Note that these variations in the prevalence of enamel hypoplasia may result from populations or dentition differences and/or the sample size available in each of these studies.

Genetic factors may contribute to the development of enamel hypoplasia, but to date there have been no large-scale gene-mapping attempts. Therefore, in an effort to better understand the genetic architecture of this relatively common dental trait, the aim of this study is to conduct genome-wide association studies (GWAS) to identify novel variants associated with enamel hypoplasia in four independent studies cohorts and in all four cohorts combined via meta-analysis.

MATERIAL AND METHODS

Study Sample

The participants for this study included individuals age 7 years and older recruited through four studies: the Pittsburgh Orofacial Cleft Study (POFC, N= 3,579); the Center for Oral Health Research in Appalachia (COHRA1, N= 1,837; COHRA2, N=1,195), a joint study of

the University of Pittsburgh and West Virginia University; and the Pittsburgh Dental SCORE project (N= 548). The following is a basic description of each cohort.

POFC—The first cohort for this study (N= 3,579) comes from the Pittsburgh Orofacial Cleft Study (POFC). The POFC study populations were recruited from multiple cleft centers in the United States, including Colorado, Iowa, Pennsylvania, Texas, and Puerto Rico, and internationally, from Argentina, the Philippines, Colombia, Guatemala, and Hungary for the purpose of studying orofacial clefts. Recruitment of participants and the collection of the dental data for POFC started in 2006 and finished in 2016. The same data collection protocols were used for every site. This cohort included 1,392 control individuals, including control probands, their parents, and siblings and 2,187 unaffected relatives of individuals with orofacial clefts. Enamel hypoplasia data were collected via in-person dental exams, performed by trained and calibrated dentists or dental hygienists, and/or intraoral photographs scored by one of three calibrated dentists. The Modified Developmental Defects of Enamel Index [13] was used to diagnose and identify enamel hypoplasia and distinguish it from other enamel defects. Enamel Hypoplasia can be defined as a quantitative defect in enamel was used for the identification of enamel hypoplasia. A visual dictionary was provided to every rater for the intraoral photographs, see supplemental file 1. The visual dictionary also included definition and photographs of other dental anomalies, such as hypocalcification, to help the rater distinguish enamel hypoplasia from the other dental anomalies. Enamel hypoplasia was recorded as present or absent for each tooth. Training and calibration for the intra-oral photos and the in-person dental exam was done at the University of Pittsburgh for all sites before the start of data collection. The three photo raters (BJH, LMU, and ARV), each rated 15 randomly selected participants twice for calibration. Calibration was measured by comparing the enamel hypoplasia results from the ratings by LMU and ARV against the gold-standard rater, BJH (BJH's intra-rater reliability (kappa) = 0.95)). The Inter-rater reliability (kappa) across the 3 raters ranged between 0.91 and 0.93. Data from 15 participants who had both intra-oral photos and in-person dental exams were used to assess the reliability between those modalities, and the results showed good agreement between the two modes of assessment (kappa >80%)[14].

COHRA1—Our second cohort (N= 1,837) comes from the Center for Oral Health Research in Appalachia (COHRA), which was designed to address oral health disparities in the Appalachian region in the US. Appalachia has the largest burden of oral health problems per capita in the United States. The participants in COHRA1 were recruited from rural Appalachia in West Virginia and Western Pennsylvania by using a household-based recruitment protocol and using the same data collection protocols for every site. The recruitment and data collection process, including the dental data, started in 2003 and ended in 2009. Training and calibration for the periodontal and dental exam was done at the University of Pittsburgh for all sites at the beginning of the data collection and periodically during the collection of the data. The dental examination was performed by trained and calibrated dental examiners (either a dentist or dental hygienist plus an assistant). The assessments were calibrated at the start of the study and during the course of data collection. The mean inter-and intra-rater reliability of the dental assessments, which included enamel hypoplasia, were excellent, i.e. 0.83 and 0.98 respectively [15].

COHRA2—The third cohort (COHRA2, N=1,195) recruited women from West Virginia and southwest Pennsylvania who were in their first and second trimesters of pregnancy and followed them and their offspring until the children reached age six. Data were collected through multiple in-person interviews, periodic telephone questionnaires, samples for DNA extraction, and dental assessment. The recruitment and data collection process, including the dental data, started in 2011 and is still ongoing. The dental examination was performed by trained and calibrated dental examiners (either a dentist or dental hygienist). The same definition and index of enamel hypoplasia in POFC, Modified Developmental Defects of Enamel Index [13] was used here and enamel hypoplasia was recorded as absent or present for each tooth. The calibration and training on caries assessment was done before the beginning of the data collection and on a regular basis. The intra-rater reliability of dental caries assessments ranged between 46.1 to 80.6. The inter-rater reliability of dental caries assessments showed a good agreement with kappa =87.8 [16].

Dental SCORE—The fourth cohort (Dental SCORE, N= 548) is a subset of a larger project, Heart SCORE, which is an ongoing cardiovascular disease study of 2,000 middle aged White and African Americans in the Pittsburgh region of Pennsylvania [17]. The recruitment and data collection process, including that of the dental data, started in 2007 and ended in 2010. In Dental SCORE, an extended dental and periodontal examination was performed following the same protocols as in COHRA1 study. The dental examination was performed by trained and calibrated dental examiners (either a dentist or dental hygienist plus an assistant).

Table 1 provides a basic description of each cohort in regard to age, sex, enamel hypoplasia, and the mean score of dental caries assessed by counts of decayed and filled teeth due to caries (DFT) index. More details regarding data collection are provided in Table S1 in the supplementary materials.

Enamel Hypoplasia Phenotypes

Trained and calibrated dental examiners (dentists or dental hygienists) performed the dental examination for the participants. Enamel hypoplasia in each study was scored for each tooth in the entire dentition except for the wisdom teeth. We looked at the distribution of which teeth were affected with enamel hypoplasia and found that the maxillary incisors (central and lateral) are the most affected teeth with enamel hypoplasia, see Table 1 for complete details. For the purpose of this study, enamel hypoplasia was analyzed as a binary trait, with values “0” when no teeth are affected with enamel hypoplasia, and “1” where one tooth or more is affected with enamel hypoplasia. The calibration and training information are discussed in the previous section.

DNA Collection, Genotyping and quality control

All the sites in POFC and the COHRA2 studies used Oragene kits for DNA saliva sample collection. For COHRA1 and Dental SCORE, blood samples were the priority, however saliva (Oragene), cheek swab (Oragene), or mouthwash buccal cell samples were taken if blood samples were not possible to collect.

Genotyping for all cohorts was performed by the Johns Hopkins University Center for Inherited Disease Research (CIDR, https://cidr.jhmi.edu/). The genotyping chip used in COHRA1 was the Illumina Human610-Quadv1_B BeadChip (Illumina, San Diego, CA, USA) [18], while COHRA2, Dental SCORE, and a subset of COHRA1 were genotyped on the Illumina Multi-Ethnic Genotyping Array (MEGA) chip. For the POFC study the Illumina HumanCoreExome array with added custom content was used for genotyping [19].

The genetic data was imputed using IMPUTE2 [20] to a comprehensive imputation panel. The reference panel for POFC was the phase 3 reference panel from the 1000 Genomes Project. For COHRA the 1000 Genomes project phase 1 version 2 release was used [21]. Genotype data went through an extensive process of cleaning, imputation, and quality assurance, performed by CIDR and/or the CIDR Genetics Coordinating Center at the University of Washington. Standard quality-control criteria were applied to filter the SNPs. SNPs were excluded if they had (i) a missing call rate of ≥5% in cases or controls; (ii) >1 discordant calls; (iii) SNPs with MAF < 1% in the population; (iv) significant deviation from Hardy–Weinberg equilibrium (P-values less than 10^{-4}); (v) >25 Mendelian errors; (vi) imputed genotypes were filtered out if their probability was < 0.9; [22] SNPs with INFO score < 0.5; and (viii) more than 1 HapMap replicate error [23, 24, 19]. The total of SNPs that were available to analyze after applying the standard quality control measures were 6,025,027 for COHRA1, 5,375,488 for COHRA2, 5,375,490 for Dental SCORE, and 7,168,909 for POFC. Table S2 in the supplemental materials provides a summary of the DNA collection, storage, genotyping and dbGaP accession numbers for each project.

Statistical Methods

All descriptive and statistical analyses were performed using the R statistical analysis environment version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria. https://www.Rproject.org/). Associations between SNPs and the binary enamel hypoplasia trait were assessed using mixed-models with adjustment for age, age^2, sex, and recruitment site, carried out using Efficient Mixed-Model Association eXpedited (EMMAX) software [25]. EMMAX was selected to account for relatedness and population structure [26].

A GWAS of enamel hypoplasia was conducted in each of the four independent cohorts and followed up with a meta-analysis. SNPs strongly associated with enamel hypoplasia were further evaluated for their likely function based on publicly available genomic annotation databases. In the meta-analysis, the results obtained from the four individual GWAS were combined, using Stouffer's p-value-based meta-analysis method in the software package METAL [27]. The METAL heterogeneity test (I^2) was used to find which observed effect sizes are heterogeneous across the four cohorts.

To account for multiple testing in GWAS results, a genome-wide significance threshold of $P < 5 \times 10^{-8}$ was applied, assuming 1 million independent tests. The p-value threshold for suggestive significance was set to $5 \times 10^{-8} < P < 5 \times 10^{-6}$. R was also used to calculate the genomic inflation factor, lambda (λ), and to create Manhattan plots to visualize the association results. More details regarding the statistical methods are available in the supplementary materials.

Functional annotation—Identification of genes of interest were based on physical proximity using ±500 kb windows from the lead SNP at each of the loci associated with enamel hypoplasia. These genes were queried for corroborating biological connections to tooth development, oral health and/or craniofacial development in different online databases, including the National Center for Biotechnology Information (NCBI) databases, specifically the Gene, PubMed, and OMIM. In addition, HaploReg v4.1 was used to acquire information on chromatin interaction, expression quantitative trait loci (eQTL), gene regulation, and linkage disequilibrium (LD) of the lead SNPs [28].

GWAS summary statistics from the meta-analysis were entered into the Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) platform, a downstream GWAS analysis tool, using default parameters to prioritize, annotate, and interpret the genomic variants and genes from the meta-analysis results [29].

RESULTS

In the four cohorts combined, the majority of the participants were white_ (63.8% vs. 35.2% other races), there were more female participants than male participants (65.1% vs. 34.9%), and the mean age was 37.6 years. The overall prevalence of enamel hypoplasia significantly differed across the cohorts ($P < 0.00001$), with 7.9% of participants in POFC (N=3,579), compared with 2.6% in COHRA1 (N=1,837), 6.7% in COHRA2 (N=1,195), and 17.7 % in Dental SCORE (N= 548). The prevalence of enamel hypoplasia among white participants across cohorts ranged from 2.3% in COHRA1 to 10.6% in Dental SCORE ($P < 0.00001$). According to previous studies that investigated enamel hypoplasia in comparable population to ours (Brazil, Spain, and Australia), prevalence of enamel hypoplasia ranges from 0.6% to 11% in the primary dentition and from 0.8% to 17% in the permanent dentition. Thus, the prevalence of enamel hypoplasia in the current study is within the previously reported ranges [9, 10, 12].

GWAS

This section summarizes the GWAS results in each cohort and across all the cohorts combined by meta-analysis. A number of association signals were observed in the individual cohorts, both at the genome-wide and suggestive significance levels. Highlights from these results are presented below. No evidence of genomic inflation was observed in any of our independent GWASs or the meta-analysis (lambdas range between 0.99 and 1.02). Manhattan and quantile-quantile (Q-Q) plots of GWAS results for enamel hypoplasia for each cohort and meta-analysis are shown in Figure 1. Association results for the lead SNPs at associated loci with enamel hypoplasia are shown in Table 2. Furthermore, lead SNPs that showed association with enamel hypoplasia were further visualized using LocusZoom [30] plots in order to assess whether genes in such loci may have a possible role in tooth development and dental/oral health (Figure 2).

POFC GWAS: Several signals were near genes with potentially relevant biological roles to tooth development and enamel hypoplasia. One of the top hits involved SNP rs60248638 ($P = 5.87\times10^{-8}$, Figure 2A) which is intronic to *ULK4* (Unc-51-like kinase 4). *ULK4* has been shown to have an essential role in regulating the wnt signaling pathway. This is specifically

relevant as the wnt signaling pathway is a fundamental regulator of tooth development [31]. In addition, the wnt signaling pathway is crucial during brain development. Indeed, *Ulk4* mutant mice appear to have severe reduction of cortex and neural stem cells, which indicates that embryonic neurogenesis is affected [32, 33]. Furthermore, a study that investigated, among other things, a rare deletion of 143,487 bp at Chr3:41611009–41754496 which encodes exons 25–30 of *ULK4* in six patients found that these patients manifested heterogeneous clinical symptoms, including behavioral problems, developmental delay, learning difficulties, language delay, speech delay, and enamel hypoplasia [32]. These manifestations support the previous evidence that *ULK4* has a role in brain development and/or function and could possibly hint at a possible role of *ULK4* during odontogenesis (tooth development). Rs60248638 is also located approximately 400kb downstream of *CCK*, which encodes a member of the gastrin/cholecystokinin family of proteins and has a role as a hormone and neuropeptide for multiple functions including the release of digestive enzymes from the pancreas and as an autocrine growth factor [34]. A previous genome-wide screening study evaluated enamel organ (EO) cells from the secretory and maturation stage demonstrated that *CCK* was highly up-regulated during enamel maturation [35]. Based on this supporting biological evidence, *ULK4* and *CCK* may influence tooth development and/or enamel hypoplasia. However, their exact roles need to be investigated more to confirm their association with enamel hypoplasia.

The SNP rs12043922 ($P = 7.45\times10^{-8}$, Figure 2B) is intronic to *NUP210L* and the function of this gene is still unknown. This SNP is also located approximately 400kb downstream of *ADAR*, which produces RNA-specific adenosine deaminase 1 (*ADAR1*) protein. The expression of Adar1 is very strong in mice dental papilla, ameloblasts, and odontoblasts during the process of tooth development [36]. In a previous human study, a novel mutation in *ADAR1* led to dental anomalies, specifically dens evaginatus and dens invaginatus [36]. The precise function of *ADAR1* in tooth development is not yet clear, however the previous study suggested that it might influence enamel hypoplasia and tooth development.

A suggestive association signal was detected near rs2414459 ($P = 9.58\times10^{-8}$, Figure 2C), which is located approximately 400kb upstream of *PYGO1*. Mice lacking both *Pygo1* and *Pygo2* in epithelial cells were able to develop teeth, yet the enamel has been structurally disorganized, bright white and had reduced iron content compared to control mice. These characteristics are very similar to the ones humans develop with amelogenesis imperfecta (AI) [37]. This SNP is in high LD with rs77220396 ($r^2=0.86$), and the C allele of rs77220396 could change the affinity of Sox_2 (score: 13–13.2). *Sox2* has been found to be expressed during tooth development in mice at different stages [38, 39]. While it is still unclear how *PYGO1* and *SOX2* may influence the risk of developing enamel hypoplasia, there is biological evidence that both genes may impact tooth development and/or enamel hypoplasia.

The second top association signal with enamel hypoplasia was detected near rs9616163 ($P = 3.22\times10^{-7}$, Figure 2D), which is intronic to *TBC1D22A,* however the precise biological role of this gene is unknown. This SNP is in high LD with several SNPs that show enhancer chromatin marks in many different cell types, including osteoblasts (i.e., bone forming cells), which can be found in periodontal ligament and alveolar bone in the oral

cavity. Rs9616163 is located 400kp upstream of *CELSR1*. The protein encoded by *CELSR1* belongs to the flamingo subfamily, which has a role during early embryogenesis. *Celsr1* expression was analyzed alongside other genes including *Fzd3*, *Fzd6*, *Vangl2*, *Dvl2* and *Dvl3* using mouse molar teeth from the bed to bell stage. Overall, *Celsr1* was expressed in different tooth cells, including odontoblast and ameloblast, and during different tooth development stages. In addition, *Celsr1* was found to be highly expressed in the enamel knot during the cap stage [40]. Further investigations are needed to clarify how *CELSR1* may influence tooth development and/or enamel hypoplasia.

COHRA1 GWAS: The genome-wide significant loci in COHRA1 did not include genes with clear biological evidence for tooth development, enamel, oral health, and/or craniofacial development. For example, the top significant association signal was detected near rs245664 ($P = 3.92\times10^{-8}$) and this SNP is located 500kb downstream to Neurexophilin (*NXPH1),* which promotes adhesion between dendrites and axons has been linked to neuroticism [41]. Both of the following most strongly associated variants (rs62522905 and rs6954583) are within 200kb from genes (*KHDRBS3, LINC01005),* and both of these genes' biological functions are still unknown.

A suggestive variant identified was rs3888090 ($P = 2.37\times10^{-7}$, Figure 2E), intronic to *NLRP12* which is an atypical intracellular sensor of the NLR family and plays a role in negatively regulating several inflammatory conditions and osteoclastogenesis. A recent study that investigated the role of *NLRP2* in the immune response and bone loss initiated by a bacterial infection in the oral cavity, more specifically during apical periodontitis, found that *NLRP12* could help in reducing alveolar bone loss and dampening inflammatory response and osteoclastogenesis through the negative regulation of the NF-κB pathway[42]. Interestingly, this SNP shows enhancer chromatin marks in osteoblasts, which can be found in periodontal ligament and alveolar bone in the oral cavity, and that could indicate that variation in *NLRP12* can be associated with periodontitis. It is unclear, though, how variation within this genomic locus could influence enamel hypoplasia and/or tooth development.

COHRA2 GWAS: The strongest association signal among the four cohorts was detected in this cohort at rs58089913 ($P = 1.57\times10^{-9}$, Figure 2F). This SNP is intronic to *ANXA3*(Annexin A3), which has a regulatory role in several biological process, such as containing cell proliferation, angiogenesis, and cancer progression [43]. This SNP is also located 200kb upstream of *BMP2K*. The encoded protein of *BMP2K* plays a presumed role in the regulation of the mitigation of osteoblast differentiation, which makes the *BMP2K* associated with the development of the bone and the cartilage [44, 45]. In fact, *BMP2K* is known as a *BMP2*-inducible gene [46]. *BMP2* has a well-established role in early embryogenesis, skeletal development, and tooth development [47–50]. Motif analysis showed that the A allele of rs58089913 could change the affinity of FOX (score: 11.5–11.9). *FOX*, specifically FOX-F, has been found to have a role in tooth formation [51]. Further investigations are needed to confirm the role that *BMP2K* might have in enamel hypoplasia.

Similar to the COHRA1 cohort, some of top associated SNPs (rs11232439 and rs57555457) were intronic to genes (*LOC100506433, MIR4300*) with unknown biological functions.

A suggestive variant, rs62575356 ($P = 2.7\times10^{-7}$, Figure 2G), is intronic to long intergenic non-protein coding RNA 474 (*LOC101928775).* This SNP is also located approximately 400kb upstream of *PAPPA*, which has a role in bone formation, inflammation, and wound healing. In transcriptomic analysis of developing murine teeth, *Pappa* were found to be expressed in incisor teeth. In addition, *Pappa* was found to be in one of the networks genes that was centered in NFκB pathway, which is one of the signaling pathway that regulate odontogenesis [52]. This SNP shows enhancer chromatin marks in many different cell types, including osteoblasts, which can be found in alveolar bone in the oral cavity, and this confirms the role that *PAPPA* plays during tooth development. However, it is still unclear how *PAPPA* could influence enamel hypoplasia, which suggests the need for more investigations.

Another suggestive variant, rs754479 ($P = 8.0\times10^{-7}$, Figure 2H), is located 50kb upstream of *GSC*. The encoded protein of *GSC* plays a role as a transcription factor. Deletion of *Gsc* in mice leads to many craniofacial defects. *Gsc* is detected in mice in the osteogenic mesenchyme of the mandible, which indicates its role in craniofacial development [53, 54].

Dental SCORE GWAS: No SNPs reached genome-wide significance, however there were several regions that showed suggestive evidence.

The top 4 lead SNPs were near genes with no biological relevance to tooth development, enamel, and/or craniofacial development. For example, rs2142491 ($P = 6.18\times10^{-8}$), is located approximately 300kb from *USP25*, and this gene has been linked to Down Syndrome [55]. Rs28504363 ($P = 1.94\times10^{-7}$) is approximately 100kb downstream of *ATOHA1*, which has a role in neurogenesis [56]. Rs36010081 is approximately 50kb upstream of *PPP2R5A* ($P = 2.67\times10^{-7}$), and the protein encoded by this gene has been found to be expressed in skeletal muscles [57]. Rs7780210 ($P = 3.06\times10^{-7}$) is intronic to *CLEC2L*. The biological function of this gene is still not fully understood. Further investigations can help in establishing if variation within these genomic regions may influence tooth development and/or enamel hypoplasia.

One of the suggestive variants in Dental SCORE was rs3010205 ($P = 7.22\times10^{-7}$, Figure 2I), which is located approximately 100kb from *UBIAD1*, which has a role in cholesterol metabolism [58]. Rs3010205 is also located approximately 200kb upstream of *MTOR*. The encoded protein of *MTOR* (Mammalian target of rapamycin, mTOR), is a conserved protein kinase and required for multiple developmental processes, including differentiation, apoptosis, metabolism, and development of neural cranial crest cells (NCCs), which makes *mTOR* essential for craniofacial development. Further, when disrupted, mTOR can cause defective facial growth, including defects or malformation in teeth, tongue and palate [59].

GWAS Meta-Analysis Results: Meta-analysis was conducted to combine the GWAS results obtained from the four cohorts, see Table 3 for the lead SNPs results. The heterogeneity statistics results for the meta-analysis are shown in supplementary materials, Table S3. In addition, the lead SNPs from each individual cohort were looked up in the meta-GWAS results and in the other cohorts (Table S4 in the supplementary materials).

None of the variants in the meta-analysis reached the genome-wide significance ($P < 5 \times 10^{-8}$), however there were several that showed suggestive significance ($5 \times 10^{-8} < P < 5 \times 10^{-6}$).

The strongest suggestive signal was near rs1359694 ($P = 5.86 \times 10^{-7}$, Figure 2J), which is intronic to *PTPRD*, and this gene encodes a member of the PTP (protein tyrosine phosphatase) family and has a role as a signaling molecule regulating different cellular processes such as differentiation, cell growth, and the mitotic cycle. *PTPRD* has been previously reported to be associated with smooth and pit-and-fissure surface caries in the primary dentition in children [60].

Several association signals were detected in the meta-analysis near genes that did not have clear biological relevance to tooth development nor enamel hypoplasia. For example, rs12830414 ($P = 6.07 \times 10^{-7}$), is located approximately 400kb from Neuron Navigator 3 (*NAV3*), which is highly expressed in the brain and the neuron system [61]; rs682846 ($P = 6.36 \times 10^{-7}$) is located within myosin heavy chain 14 (*MYH14*) which has been linked to deafness [62]; rs56282801 ($P = 1.00 \times 10^{-6}$) is located within *DHX37* which has been linked to neurodevelopmental disorders [63]; and rs4649222 ($P = 1.00 \times 10^{-6}$) is intronic to *KIAA1804,* also known as *MLK4*, which can regulate the activation of transcription factor NF-κB [64].

One of the suggestive association signals in the meta-analysis was detected at rs2840075 ($P = 2.20 \times 10^{-6}$, Figure 2K), which is located approximately 100kb upstream of *SLC4A4,* and this gene encodes sodium bicarbonate cotransporter (NBC), which has three main variants, the N-terminally spliced (NBCe1-A and NBCe1-B) and the C-terminally spliced (NBCe1-C). The NBCe1 is essential in regulating the pH of enamel matrix during tooth development, and more than one study, in humans and mice, has found that mutation in *SLC4A4* can cause an abnormal enamel phenotype [65–67]. In addition, this SNP is a strong eQTL for *SLC4A4.* The lead SNP (rs2840075) and other SNPs (rs114654867, rs11729023) in high LD ($r^2 \geq 0.8$) at this locus show promoter and enhancer chromatin in many different cell types including osteoblasts. Motif analysis showed that the A allele of rs2840075 could change the affinity of Myc_disc5 (score: 10.2–13.4). The MYC family, which Myc_disc5 belongs to, contains three members (C- MYC, N-MYC, and L-MYC). It has been reported that C-MYC specifically was found to be involved in tooth development [68]. All of this evidence could indicate that variation within this genomic region can influence tooth development and/or enamel hypoplasia.

Another suggestive variant from the meta-analysis results is rs62196465 ($P = 2.6 \times 10^{-6}$, Figure 2M), which is intronic to *MACROD2*. *In situ* hybridization of mice showed that *Macrod2* was significantly expressed in different tissues and organs, including teeth, during mouse embryonic development [69]. *MACROD2* needs to be investigated in both animal and human studies to understand the exact role it might have during tooth development.

Functional Annotation of Meta-GWAS Results: Functional Mapping and Annotation (FUMA) of the meta-GWAS (Table 3) was used to obtain more information regarding the different biological aspects of the observed associations. FUMA meta-GWAS mapped a total

of 20 genes to the 18 risk loci identified (Figure 1 in the supplementary materials). These genes' expression was examined across different human tissues from different databases. FUMA results of the meta-GWAS risk loci ($P < 5.0 \times 10^{-6}$) are shown in supplementary materials, Figure 1. In addition, functional mapping linked lead SNPs and nearby SNPs in tight LD with the GWAS catalog (https://www.ebi.ac.uk/gwas/). All of the results of the GWAS catalog-based annotation are presented in supplementary materials, Table S5.

The gene-based test based on the meta-GWAS results of all cohorts was computed by MAGMA (Multi-marker Analysis of GenoMic Annotation) using the default settings implemented in FUMA. Lead SNPs from the meta-GWAS analysis were mapped to 19182 protein coding genes. The genome-wide significance level was calculated based on the number of tested genes and it was set at P of $0.05/19182 = 2.61 \times 10^{-6}$. None of the genes reached the genome-wide significance level. A manhattan plot of the gene-based test are shown in supplementary materials, Figure 2.

Gene set enrichment analyses were conducted with GENE2FUNC implemented in FUMA. The Bonferroni-corrected threshold for significant gene set enrichment was set at $P \leq 0.05$. A gene-set at chr17q23 was overexpressed ($P = 2.46 \times 10^{-7}$) in the Positional gene sets (MSigDB C1), which is a set of genes that can help in identifying effects based on chromosomal changes (e.g. deletions, or amplifications). Full results of the gene set enrichment analyses are shown in supplementary material, Table S6.

DISCUSSION

Enamel hypoplasia is a multifactorial condition believed to be caused by the interaction between environmental and genetic factors. Some epidemiological studies show that the prevalence of enamel hypoplasia differs depending on several factors, including which teeth are considered, examination method and the population investigated. The reported prevalence of enamel hypoplasia in the current study was 7.1% combining across cohorts, falling within the reported range of enamel hypoplasia prevalence in other published studies (0.8% to 17% for the permanent dentition) [9–12]. However, there was considerable variation in prevalence across our 4 study cohorts, which may be due to factors differing across the cohorts such as population diversity, environmental and/or socioeconomic factors, and enamel hypoplasia assessment methods.

In our current study we found that the most affected teeth by enamel hypoplasia in POFC were the maxillary left central incisors (7.5%), followed by maxillary right central incisors (7.33%) and upper right lateral incisors (7.33%). In previous studies [10, 12], maxillary incisors were found to be the most affected teeth by enamel hypoplasia. In COHRA1, the most affected teeth with enamel hypoplasia were the maxillary right centeral incisors (7.84%) followed by the maxillary right first molars (6.86%) and maxillary left lateral incisors (5.88%). In COHRA2, the most affected teeth by enamel hypoplasia were maxillary right first molar (6.60%), followed by maxillary left first molar (6.13%), and maxillary left lateral incisor (5.18%). Finally, the most affected teeth with enamel hypoplasia in dental SCORE were the maxillary right first molar (11.34%) followed by maxillary right second molar (10.31%), and maxillary left lateral incisors (6.18%). These results are shown in Table

1. Previous studies found incisors, especially in the maxilla, and molars are the most affected teeth with enamel hypoplasia [10, 12], and our findings were similar to what has been found in those studies.

The cohorts included in this study had somewhat different protocols for collecting and measuring enamel hypoplasia from the participants. Thus, to harmonize analyses across the different cohorts, we used a binary definition of enamel hypoplasia, i.e. approximating a case-control study design that can provide a threshold level of an underlying continuum of enamel hypoplasia risk. Similar binary traits were also successfully used in the past in studying other complex oral traits, such as dental caries [70]. Note that recording enamel hypoplasia as a binary trait did not contain information on its extent or severity, which may have reduced the statistical power of our analysis. Thus, ideally future studies should capture the full extent of the trait. However, it is important to emphasize that although we had varying protocols to collect enamel hypoplasia in our study, variability in phenotype definition should not lead to biased genetic results, although it may have reduced statistical power [71].

To the best of our knowledge, this study represents the first large-scale GWAS performed for enamel hypoplasia. The main aim of this study was to identify genetic loci that are associated with enamel hypoplasia. We were able to identify and nominate several variants near genes with reasonable biological roles in tooth and/or enamel development.

The only previously published GWAS in the literature that focused on enamel defects was on molar-incisor hypomineralization (MIH) in children from Munich [72]. MIH differs from the enamel hypoplasia trait of the current study, but it can be hard to distinguish teeth that are affected with enamel hypoplasia from teeth affected with MIH. Enamel hypoplasia is characterized by reduced thickness of enamel, while MIH affects the translucency of enamel [73]. The top association signal with MIH (rs13058467, $P = 3.72\times10^{-7}$) was near *SCUBE1*, which plays a role in the development of the tooth and in craniofacial development [74, 72]. In our meta-analysis the p-value for association with enamel hypoplasia for rs13058467 was 0.328. We also looked in the region around that lead SNP (+ 500 kb), but no other SNPs in that region were significantly associated in our meta-analysis results.

The current study identified several genome-wide significant and suggestive association signals, near genes that have plausible roles, directly or indirectly, in tooth development and enamel, and thus could possibly contribute to increasing the risk of developing enamel hypoplasia. These include but are not limited to: *BMP2K* which is involved in tooth development [46]; *MTOR*, which has been implicated in craniofacial development and the differentiation of odontoblasts [75]; *MACROD2*, which has been reported to be expressed in teeth during embryonic development in mice [69]; *SLC4A4*, which has strong biological evidence for the role it plays in influencing enamel hypoplasia; and *PTPRD,* which has been reported to be associated with smooth and pit-and-fissure surface caries in the primary dentition in children [60].

The fact that *PTPRD* has also been found to be associated with dental caries indicates that there may be an etiologic relationship between dental caries and enamel hypoplasia [60].

Indeed, enamel hypoplasia can create an enhanced environment for adherence and retention of cariogenic bacteria for a long period of time, which can increase the risk of developing dental caries [8] and can be considered as a potential confounder for dental caries. In our different cohorts, we had an average mean score of 6.88 of dental caries assessed by counts of decayed and filled teeth due to caries (DFT) index, which is considered to be high. The highest DFT mean score was seen in Dental SCORE (12.07), which was not surprising given that most participants in Dental SCORE were older, on average, than the other cohorts' participants. We used the DFT index instead of DMFT (counts of decayed, missing, and filled teeth due to caries) because we had incomplete information regarding the underlying cause of missing teeth, i.e. whether teeth were missing due to caries or for other reasons, such as injury.

The fact that some of the prioritized loci are related to pathways that regulate the tooth development process is consistent with the current understanding of the etiology of dental anomalies, which include enamel hypoplasia. For example, one of the lead SNPs in the POFC GWAS was near *ULK4* which has an important role in regulating the wnt signaling pathway. Thus mutations in wnt signaling components could lead to developing dental anomalies, such as tooth agenesis [31]. In addition, enamel hypoplasia was one of the manifestations that some patients reported as a result of a rare deletion of *ULK4* [32]. This evidence suggests that *ULK4* has a possible role during odontogenesis (tooth development) and/or enamel hypoplasia.

Under the hypothesis that enamel development genes could influence enamel hypoplasia, we also reviewed the p-values of 23 SNPs in the meta-analysis results that have been previously examined and reported [76–78] in and near some of the enamel matrix candidate genes (*ENAM, AMELX, AMBN, TFIP11, TUFT1*). In addition, we also investigated genes that were recently reviewed [79] to be involved in hereditary enamel defects in humans. Therefore, we investigated a total of 32 SNPs. A threshold of p-value of less than 0.001 was chosen to declare statistical significance, which corresponds to the Bonferroni correction for 32 SNPs. Interestingly, two SNPs showed statistical significance (p-value < 0.001); rs10518733 within *WDR72*, and rs11136305 within *FAM83H*. The full list of the results of these 32 variants is presented in Table 4.

Additional SNPs near the enamel matrix candidate genes (*ENAM, AMELX, AMBN, TFIP11, TUFT1*) emerged within the 500 kb regions flanking these genes in our meta-analysis results. A total of 8544 SNPs were in and near these enamel genes and considering that some of these SNPs are in linkage disequilibrium (LD), the Li and Ji method for determining the number of independent tests [80] was used leading to an adjusted threshold p-value of 1.79×10^{-4} to declare statistical significance. There was one SNP that reached statistical significance (rs2840075, $P = 2.20 \times 10^{-6}$), located approximately 500 kb downstream from *ENAM*. The encoded protein of *ENAM* (enamelin) is required for the proper development of teeth and enamel [4]. *AMBN*, another gene that has a role in the development of enamel, is located approximately 600 kb upstream of this SNP, though given the distance of this SNP from *AMBN* (600 kb) it is unclear if this SNP affects *AMBN* function or expression. The full list of the association results with the enamel matrix candidate genes is shown in Table S7.

One of the challenges in this study was the lack of replication of association signals across cohorts. This could be due to several possible sources of heterogeneity, such as different environmental factors, different phenotype assessment and study designs, different ethnic groups, and using different genotyping chips. In addition, the SNPs that are in linkage disequilibrium with the lead SNPs may differ from one ethnic group to another; formal assessment of the heterogeneity in genetic effects (I^2) showed little evidence for heterogeneity in the top associated loci, but with only four cohorts the power to detect heterogeneity may have been limited.

There are limitations of the current study. First, the study only identified the associations between genetic variants and enamel hypoplasia, not causation nor the biological effect of these variants and implicated genes on enamel hypoplasia. Second, the p-values from EMMAX are accurate but effect sizes are not informative because EMMAX is based on mixed models rather than a generalized mixed model. Finally, the study did not replicate most of the association signals across the different cohorts. These limitations are balanced by the fact that several of the association signals described and investigated in our study were near genes supported by a body of literature with involvement in oral and tooth biological processes.

The current study provides a wealth of new information on possible candidate genes and loci that might be involved in enamel hypoplasia and will require additional investigation in animal and human studies, replication and validation in larger studies. In addition, other suggestive signals near genes with no previously known biological roles in tooth development or enamel hypoplasia should also be further investigated.

This study is the first GWAS that aimed to identify genetic loci that associated with enamel hypoplasia and did nominate several for further investigation, including *ULK4*, *CCK*, *ADAR1, PYGO1, CELSR1, BMP2K, MTOR, PAPPA,* and *SLC4A4* because of their plausible biological roles in enamel development. Further studies are required to confirm the role these genes play in enamel development during odontogenesis using both human and mouse models. In addition, more research to replicate the analyses in independent cohorts is essentia

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DATA AVAILABILITY STATEMENT

The datasets analyzed in this paper are available in the dbGaP repository (Studies Accession: phs000774.v2.p1, phs001591.v1.p1, phs000095.v3.p1). See also Table S2. Authorized members and NIH Investigators are eligible to apply to access these datasets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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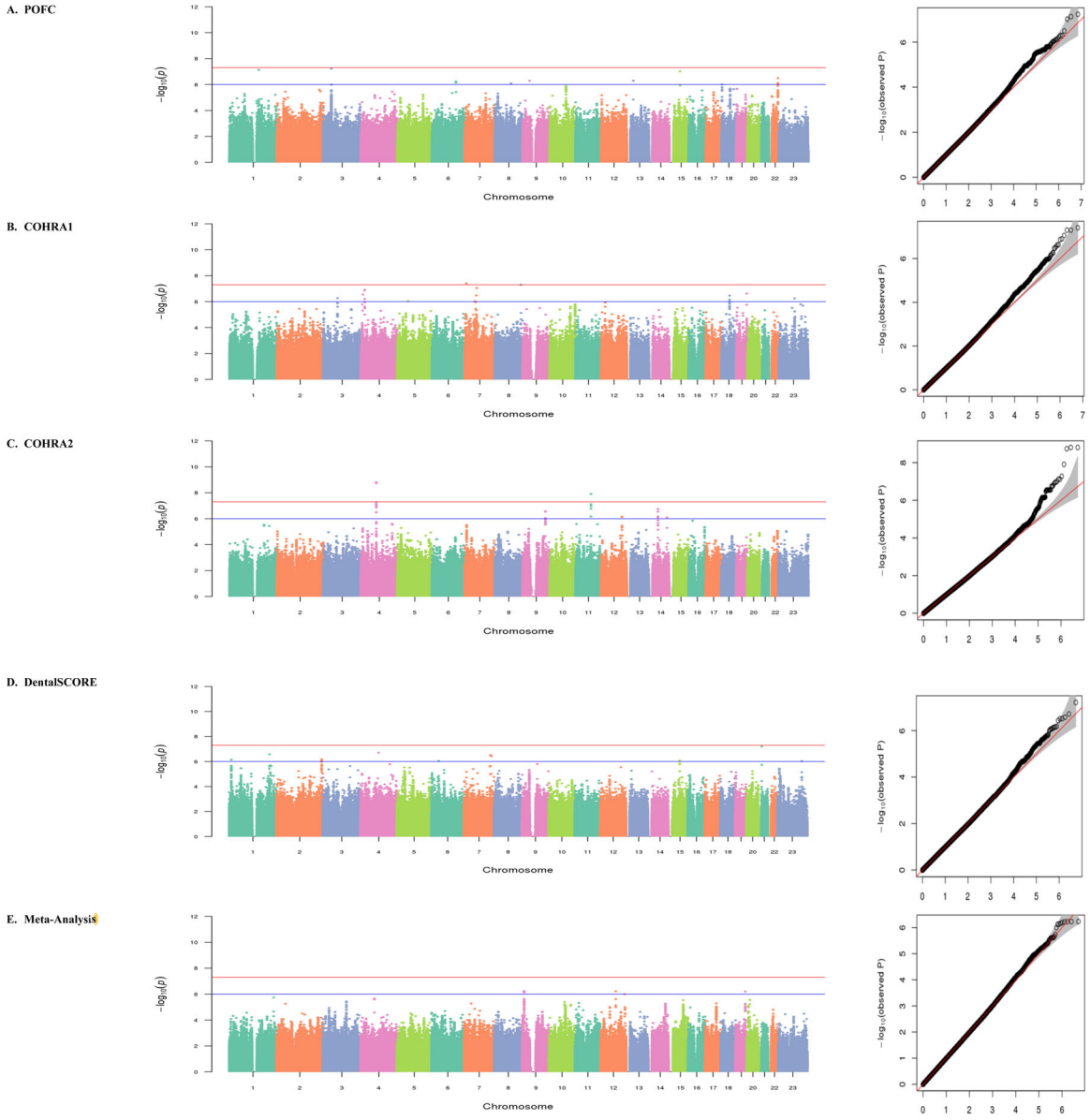
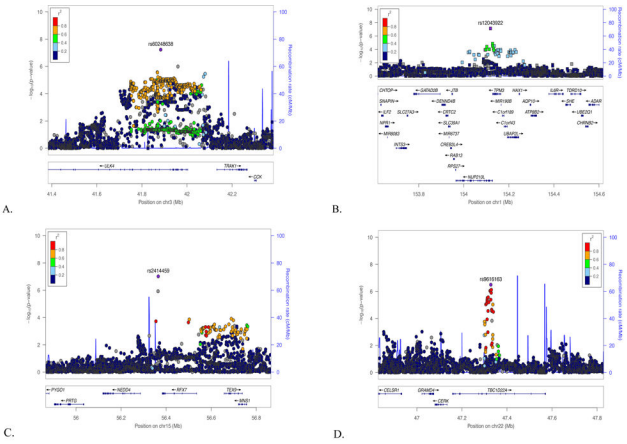


Fig. 1.

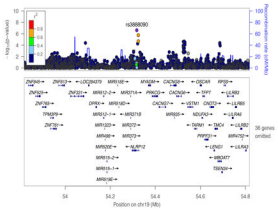
Manhattan plots and quantile-quantile (Q-Q) plots show GWAS results for the analyses. Red lines represent thresholds for genome-wide significance (p-value < 5×10^{-8}). Blue lines represent thresholds for suggestive significance (p-value < 5×10^{-6}).

POFC



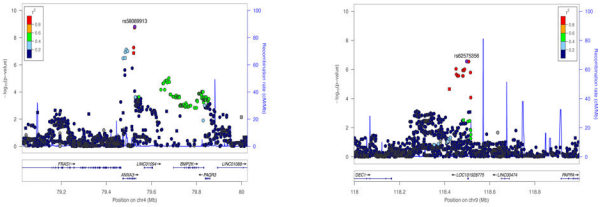
A. B. C. D.

COHRA1

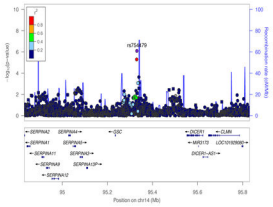


E.

COHRA2



F. G.



H.

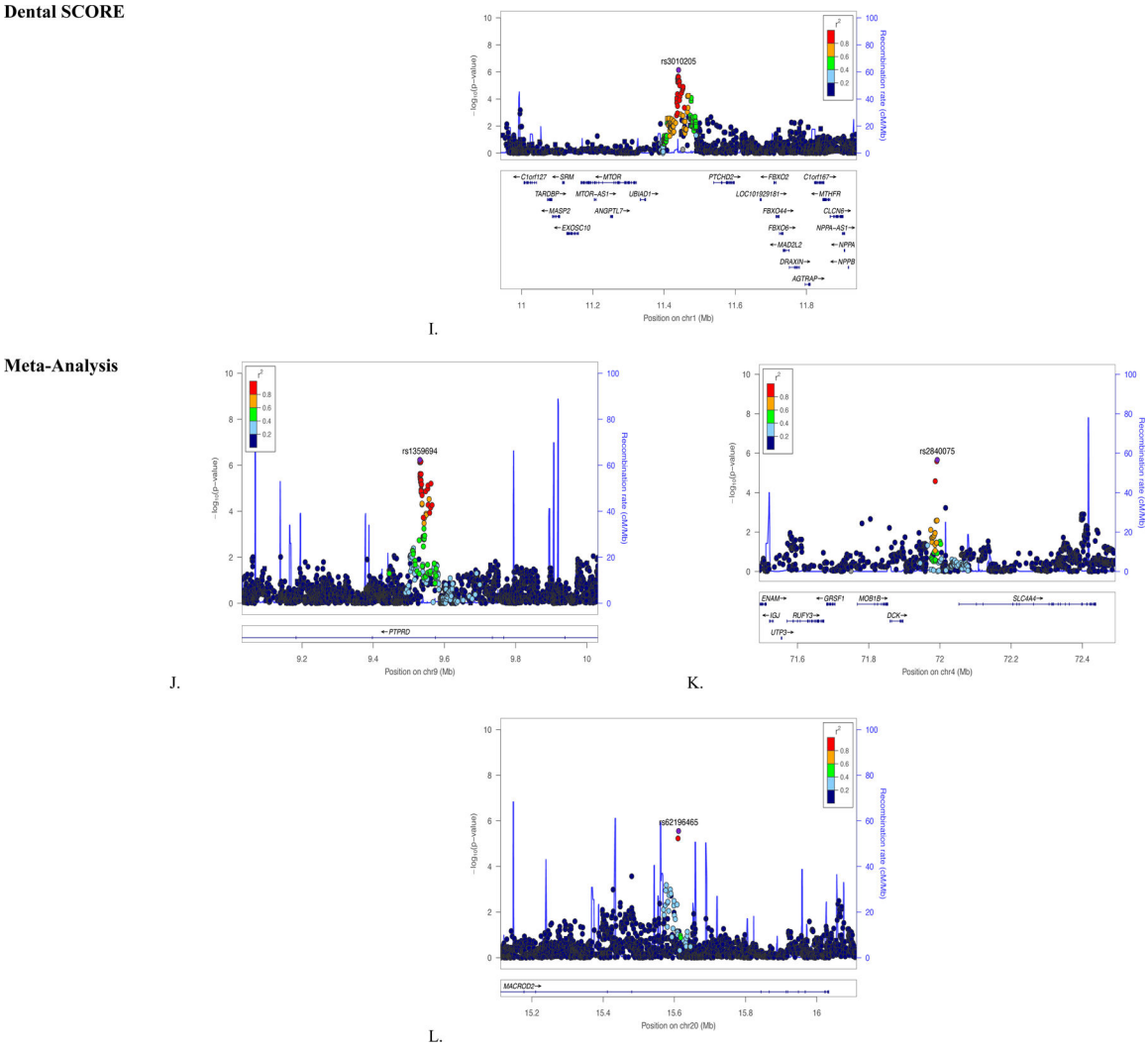


Fig. 2.

LocusZoom of regions of Interest. The genome build used for the recombination rate was based on 1000 Genomes November 2014 EUR data. All of the gene positions and directions of transcription are annotated on the plots.

Table 1.

Demographics of Each cohort included in the study

	Male, n (%)	Female, n (%)	Total, n (%)	Age *Mean* ± *SD*	Enamel Hypoplasia		Most Affected Teeth with Enamel Hypoplasia	DFT* *Mean* ± *SD*
					No	*Yes*		
POFC	1505 (42.1%)	2074 (57.9%)	3579 (50%)	31 ± 14.7	3297 (92.1%)	282 (7.9%)	Maxillary left central incisors (15.24%), maxillary right central incisors (14.89%), and maxillary right lateral incisors (14.89%).	4.36 ± 4.28
COHRA1	783 (42.6%)	1054 (57.4%)	1837 (25.7%)	25.5 ± 13.7	1789 (97.4%)	48 (2.6%)	Maxillary right centeral incisors (7.84%), maxillary right first molars (6.86), maxillary left lateral incisors (5.88%).	5.96 ± 5.11
COHRA2	32 (2.7%)	1163 (97.3%)	1195 (16.7%)	30.9 ± 7.9	1115 (93.3%)	80 (6.7%)	Maxillary right first molar (6.60%), maxillary left first molar (6.13%), and maxillary left lateral incisors (5.18%).	5.13 ± 6.93
Dental SCORE	179 (32.7%)	369 (67.3%)	548 (7.6%)	63.1 ± 7.5	451 (82.3%)	97 (17.7%)	Maxillary right first molar (11.34%), maxillary right second molar (10.31%), and maxillary left lateral incisors (6.18%).	12.07 ± 5.33
Total	2499 (34.9%)	4660 (65.1%)	7159	37.6 ± 10.95	6652 (92.9%)	507 (7.1%)		6.88 ± 5.41

*DFT counts of decayed and filled teeth due to caries

Table 2.

GWAS Results for Enamel Hypoplasia

rsID	Chr	BP	Beta	*P*	A1/A2*	Nearby Gene(s)[1]
			POFC			
rs60248638[2]	3	41883692	−0.0600436	5.87E-08	A/G	*ULK4, CCK*
rs12043922[2]	1	154118497	−0.0741821	7.45E-08	A/G	*NUP210L, ADAR*
rs2414459	15	56365357	−0.0610045	9.58E-08	C/T	*PYGO1*
rs9616163[2]	22	47328584	−0.0683888	3.22E-07	A/G	*TBC1D22A, CELSR1*
			COHRA1			
rs245664	7	9089477	−0.0643447	3.92E-08	C/G	*NXPH1*
rs62522905	8	136395352	−0.0504171	5.04E-08	A/G	*KHDRBS3*
rs6954583	7	63292425	0.05167179	8.91E-08	C/T	*LINC01005*
rs3888090[2]	19	54317914	−0.0647593	2.37E-07	A/G	*NLRP12*
			COHRA2			
rs58089913[2]	4	79526046	−0.1514529	1.57E-09	A/G	*ANXA3, BMP2K*
rs11232439	11	80743780	−0.1037051	1.24E-08	G/T	*LOC101928944*
rs57555457	14	48260934	−0.1046353	1.80E-07	C/T	*MIR548Y*
rs62575356	9	118497768	−0.1189798	2.77E-07	C/T	*LOC101928775, PAPPA*
rs754479	14	95331008	−0.1108255	8.01E-07	C/T	*GSC*
			Dental SCORE			
rs2142491	21	16916381	−0.2386754	6.18E-08	C/T	*USP25*
rs28504363[3]	4	94836997	−0.2762404	1.94E-07	A/G	*ATOH1*
rs36010081[2]	1	212423782	−0.2066991	2.67E-07	A/C	*PPP2R5A*
rs7780210	7	139231244	−0.1753172	3.06E-07	C/G	*CLEC2L*
rs3010205	1	11440957	0.1198029	7.22E-07	A/G	*UBIAD1, MTOR*

[1] Gene within the+500kb window

[2] These SNPs were intronic.

[3] This SNP is the only one that was genotyped, the rest of SNPs in the table are imputed

* A1 is the effect allele, A2 is the other allele

Table 3.

Meta-GWAS Results

						Results for the meta-GWAS SNPs in each individual cohort							
						POFC		**COHRA1**		**COHRA2**		**Dental SCORE**	
SNP*	**Chr**	**A1/A2[1]**	**Nearby Gene(s)[2]**	**Meta-*P*[3]**	**Direction**	***P***	**MAF**	***P***	**MAF**	***P***	**MAF**	***P***	**MAF**
rs1359694	9	A/C	*PTPRD*	5.86E-07	----	0.0245855	0.1223	0.000493	0.1809	0.220907	0.1825	0.002401	0.1825
rs12830414	12	C/T	*NAV3*	6.07E-07	++++	0.0561199	0.09428	0.006441	0.06867	0.208079	0.07289	4.41E-05	0.07289
rs682846	19	C/G	*MYH14*	6.36E-07	----	0.0304989	0.3097	0.003379	0.4438	0.023739	0.4283	0.009207	0.4283
rs56282801[4]	12	A/G	*DHX37*	1.00E-06	----	0.0004047	0.1984	0.001016	0.2045	0.143019	0.3195	0.135073	0.3195
rs4649222[4]	1	A/G	*KIAA1804*	1.83E-06	++++	0.0450894	0.2032	0.003782	0.2803	0.003620	0.2879	0.083122	0.2879
rs2840075	4	C/T	*SLC4A4, ENAM*	2.20E-06	----	7.86E-06	0.07779	0.098322	0.1058	0.053585	0.0984	0.156516	0.0984
rs62196465[4]	20	A/G	*MACROD2*	2.80E-06	----	0.0116859	0.05604	0.244040	0.05776	4.20E-05	0.05242	0.112376	0.05242
rs16968212[4]	15	C/T	*SCAPER*	2.92E-06	++++	0.2694265	0.06341	0.016560	0.07246	0.014049	0.07978	0.000683	0.07978
rs9846530[3]	3	C/G	*SEMA5B*	3.69E-06	----	0.1448401	0.2127	0.005046	0.2219	2.13E-05	0.2238	0.457281	0.2238
rs7921002	10	G/T	*ZMIZ1*	4.10E-06	----	0.0093433	0.3218	0.458316	0.178	0.002012	0.2056	0.005373	0.2056

[1] A1 is the effect allele, A2 is the other allele

[2] Gene within the+500kb window

[3] There is no statistically significant heterogeneity in the meta-analysis results across the cohorts, see supplementary materials, Table S3.

[4] These SNPs were intronic.

* All the SNPs presented in the table are imputed.

Table 4.

Previously Identified SNPs in Enamel Matrix Genes (*ENAM*, *TUFT1*, *AMBN*, *TFIP11*, and *AMELX*)

SNP	Gene	Chr	BP	A1/A2*	*P*	Type	dbSNP Functional Annotation
rs17733915	*AMBN*	4	71447093	T/C	0.1846	Genotyped	Upstream
rs4694075	*AMBN*	4	71466914	T/C	0.4764	Imputed	Intronic
rs17149026	*AMBN*	4	71440940	G/T	0.8275	Imputed	Upstream
rs2106416	*AMLEX*	23	11316742	T/C	0.4535	Imputed	Synonymous
rs946252	*AMLEX*	23	11313027	T/C	0.7673	Imputed	Intronic
rs1967376	*ENAM*	4	71501744	C/T	0.3458	Imputed	Intronic
rs12640848	*ENAM*	4	71506412	G/A	0.6669	Imputed	Intronic
rs2097470	*TFIP11*	22	26904965	T/C	0.1351	Imputed	Intronic
rs713900	*TFIP11*	22	26898242	A/G	0.214	Imputed	Intronic
rs134135	*TFIP11*	22	26898962	C/G	0.5798	Imputed	Intronic
rs5997096	*TFIP11*	22	26895957	C/T	0.6212	Imputed	Intronic
rs134136	*TFIP11*	22	26899474	T/C	0.6672	Imputed	Intronic
rs134134	*TFIP11*	22	26898891	T/C	0.7116	Imputed	Intronic
rs134145	*TFIP11*	22	26909750	A/G	0.7835	Genotyped	Intronic
rs6005060	*TFIP11*	22	26895736	A/T	0.9559	Imputed	Intronic
rs2337359	*TUFT1*	*1*	*151495796*	*C/T*	*0.004234*	Imputed	Upstream
rs10158855	*TUFT1*	1	151515654	G/T	0.07768	Imputed	Intronic
rs17640579	*TUFT1*	1	151521933	G/A	0.1621	Imputed	Intronic
rs3790506	*TUFT1*	1	151538366	A/G	0.198	Imputed	Intronic
rs16833391	*TUFT1*	1	151547253	T/C	0.52	Imputed	Intronic
rs1045298	*TUFT1*	1	151510825	T/C	0.5601	Imputed	3′ UTR
rs12749	*TUFT1*	1	151555741	A/G	0.8748	Imputed	3′ UTR
rs4970957	*TUFT1*	1	151517388	G/A	0.9746	Imputed	Intronic
rs10518733	*WDR72*	15	53940307	A/C	0.0005654	Genotyped	Intronic
rs11136305	*FAM83H*	8	144689914	C/T	0.001001	Imputed	Upstream
rs3745542	*KLK4*	9	51587643	C/T	0.002304	Genotyped	140bp 5’ of *KLK14*
rs8005614	*SLC24A4*	14	92971222	G/T	0.002784	Imputed	8.6kb 3’ of *SLC24A4*
rs2819865	*RUNX2*	6	45443176	A/G	0.003116	Imputed	Intronic
rs12804929	*MMP20*	11	10264250	G/A	0.003788	Imputed	Downstream
rs3803878	*DLX3*	17	48067581	G/C	0.00385	Imputed	3′ UTR
rs10044464	*ADAMTS2*	5	178692688	C/T	0.004017	Imputed	Intronic
rs9895891	*FAM20A*	17	66329586	G/A	0.004428	Imputed	Downstream

*A1 is the effect allele, A2 is the other allele