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Genetic Variation in *Ameloblastin* Is Associated with Caries in Asthmatic Children

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Abstract

Aim—Evidence suggests caries experience is higher in children with asthma. In this study, we compared caries experience in asthmatic and non-asthmatic children and defined whether variation in the distribution of caries experience differs between the two groups and is dependent of the presence of genetic variation in enamel formation genes.

Methods—Children with asthma were recruited at the Istanbul University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Allergy and Pulmonary Diseases, and non-affected children were recruited at the Istanbul University, Faculty of Dentistry, Department of Pedodontics. Cases (N=100) were defined as children between the ages of 6 and 12 years with asthma and controls (N=100) as children without asthma. Cases and controls were matched by sex and age. All study subjects received a complete dental exam, provided demographic and other caries and asthma risk factors data, and a saliva sample for DNA extraction. Caries experience was defined based on DMFT/dmft and DMFS/dmfs scores. Genotypes of 11 SNPs were selected in intronic regions of enamel development genes. PCR with TaqMan chemistry were used for genotyping all selected markers. Association between caries experience (caries free versus caries affected) depending on asthma status and SNPs was tested with PLINK by logistic regression, adjusting by risk, and other preventive measures. P-values below 0.0045 (0.05/11) were considered statistically significant.

Results—Logistic regression analysis showed an association between *AMBN* rs4694075 and caries experience (p=2.525e-007).

Conclusions—Our study provides, for the first time, evidence that ameloblastin is associated with caries in asthmatic children.

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Keywords

AMBN; Enamelin; Tuftelin; Amelogenin; Tuftelin Interacting Protein; Carious Lesion; White Spot Lesion

Introduction

Asthma is one of the most common chronic medical ailments in children and its frequency has steadily increased in the last two decades (Mannino et al., 1998; Steinbacher and Glick, 2001). A number of studies have investigated oral health in individuals with asthma, but the results are conflicting. Meta-analysis suggests that being affected by asthma doubles the risk of caries in both primary and permanent dentition (Alavaikko et al., 2011). Our data confirms the results of the meta-analysis by Alavaikko et al. (2011) that suggested an association between higher caries experience and asthma in adults (Anjomshoaa et al., 2009). Individuals with asthma appear to accumulate higher amounts of dental biofilm, as well as higher salivary levels of mutans streptococci (Botelho et al., 2011). $_2$ agonists cause decreased saliva secretion rate and patients taking these medications have increased levels of *lactobacilli* and mutans streptococci (Ryberg et al., 1987,1991). Although it is possible that medication intake increases susceptibility for caries, our data does not suggest that medications are associated with higher caries experience in asthmatics (Johnston and Vieira, 2012). Genes in the immune signaling pathway are differentially expressed in asthmatic individuals (Schmidt-Weber, 2006) and could underlie the association between asthma and high caries experience. One of these genes is *CD-14*, which is described as a classical example of gene-environment interactive factor in asthma (Simpson et al., 2006). Variation in *CD-14* has been also associated with resistance to abscess or fistula formation in children with four or more caries lesions (De Soet et al., 2008). Immune response regulators may be the common factors that underlie the association between asthma and caries.

Asthma is unlikely to be a single disease but rather a series of complex, overlapping individual diseases or phenotypes, each defined by its unique interaction between genetic and environmental factors. In adults, these conditions include syndromes characterized by allergen-exacerbated, non-allergic, and aspirin-exacerbated factors along with syndromes best distinguished by their pathologic findings (eosinophilic, neutrophilic, pauci-granulocytic), response to therapy (corticosteroid resistant), and natural history (remodeling prone) (Borish and Culp, 2008). Allergic sensitization can be detected by a positive skin test result to at least one common allergen in 93.5% of cases with severe asthma (Expert Panel Report 3, 2007). Non-allergic asthma has a more likely onset during adulthood, female predominance, and a higher degree of severity (Bell, 2004). This is interesting since our previous studies showed more women affected by asthma (Anjomshoaa et al., 2009).

Evidence suggests that children with asthma have two to three times more defects in the dental enamel in comparison to non-asthmatics (Ford et al., 2009; Jälevik et al., 2001; Suckling et al., 1987; Wogelius et al., 2010). Alterations in enamel are commonly thought to be consequence of local trauma or infection during the period of tooth development but evidence that children with permanent first molar alterations have alterations in the

permanent incisors 2.5 times more frequently than children without affected permanent first molars indicates a systemic, and not a local origin of these defects (Wogelius et al., 2008). Our hypothesis is that individuals with asthma may have higher caries experience due to genetic variation in enamel formation genes. We have previously shown that genetic variation in enamel formation genes is associated with higher caries experience (Deeley et al., 2008; Patir et al., 2008; Shimizu et al., 2012). To test this hypothesis, we designed a study that compared caries experience in asthmatic and non-asthmatic children and defined whether variation in the distribution of caries experience differs between the two groups and is dependent of the presence of genetic variation in enamel formation genes.

Method

Subjects

One hundred bronchial asthmatic children between 6 and 12 years of age being treated at the Istanbul University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Allergy and Pulmonary Diseases, and 100 non-asthmatic children between 6 and 12 years of age being treated at the Istanbul University, Faculty of Dentistry, Department of Pedodontics were included in this study. The protocol of this study was evaluated and approved by the Istanbul University ethics committee and the University of Pittsburgh Institutional Review Board. All subjects were recruited after the study was explained to parents of potential subjects in detail and written informed consent and appropriate assent were obtained from all parents and their children. Asthmatic cases were defined as children who were diagnosed with bronchial asthma while controls were defined as children who do not have any systemic diseases. Cases and controls were matched by sex and age (Table 1). Asthma diagnosis was defined by one of the authors (Z.T.) based on evaluation of patient history, physical examination and pulmonary function test results (spirometry).

Caries Assessments

All individuals received oral and dental examination performed by a single examiner (N.E.) and DMFT/dmft and DMFS/dmfs (decayed, missing due to caries, filled teeth and surfaces) scores (Table 1) were obtained according to the World Health Organization guidelines with some modifications. Examinations were done with the use of a flashlight and mouth mirror. Visible lesions in dentin, as well as visible active lesions in enamel (white spots) were scored as decayed. An explorer was gently used for assessing the smoothness of tooth surfaces. Gauze was used to dry and clean teeth prior to exam. Artificial light and a dental operatory were used for all evaluations of controls and a bed was used to lay down asthmatics subjects at medical offices. Exam calibrations were performed according to the following protocol: First, the calibrator (F.S.) presented to the examiner (N.E.) the criteria for caries detection, showing pictures of several situations to be observed in the exam (sound and decayed tooth surfaces, filled teeth with and without secondary lesions, and missing teeth due to caries or due to other reasons) and discussing each of these situations in a session that lasted one to two hours. Next, the calibrator and examiner examined 10 to 20 subjects and discussed each case. The intraexaminer agreement was assessed by a second clinical exam in 10% of the sample after 2 weeks, with a κ of 1.0.

Demographic Data and Biological Samples

Data recorded for all participants included age, sex, severity of asthma (mild or moderate), self-reported allergy, medication intake (beta2-adrenergic agonist, corticosteroid inhaler, leukotriene antagonist, combinations of any two medications, other medications), duration of medication intake, any other systemic disease, any asthmatic individual in the family (positive family history of asthma), asthmatic attack in the last year, snoring at night, sneezing, coughing, ventolin (albuterol) needed more than two times in a week, coughing during physical activities, mouth rinsing after using inhaler, tooth brushing frequency, education of parents, smoking status of parents or caregivers, mouth breathing, malocclusion (molar relationships of classes I, II, or III), DMFT/dmft and DMFS/dmfs scores, and plaque index (Silness and Löe, 1964). Table 1 summarizes the distribution of these variables in cases and controls. 2 mL of unstimulated saliva were obtained from each subject by spitting and samples were stored in Oragene DNA OG-500 Self-Collection kits (DNA Genotek Inc., Ontario, Canada). Subjects were informed to not eat, drink or chew gum for 30 minutes before sample collection. The accessory sponge kit was used for children who could not spit enough saliva. Until DNA extraction, samples were stored in the kits at room temperature (15-30°C). DNA was extracted according to the manufacturer's protocol.

Genotypes

Eleven single nucleotide polymorphisms (SNPs) were selected in intronic regions of enamel development genes (Table 2). Polymerase chain reactions with TaqMan SNP Genotyping Assays (Applied Biosystems) held in total 3.0 uL reaction with 2.0 ng/uL of DNA were used for genotyping all selected markers in a Tetrad PTC225 thermocycler (MJ Research, Waltham, MA, USA). Genotype detection and analysis were performed on the ABI 7900HT with ABI SDS software (Applied Biosystems, Foster City, CA, USA).

Statistical Analyses

Chi-square and Fisher's exact tests were used to compare frequencies of the above listed variables between asthmatic and non-asthmatic children. Student's *t* test was used to compare the age distribution between asthmatic and non-asthmatics. Hardy-Weinberg equilibrium was assessed in the genotyping data in both asthmatic and non-asthmatic groups by the chi-square test. Association between caries experience (caries free versus DMFT/dmft or DMFS/dmfs equals 1 or higher) depending on asthma status and SNPs was tested with PLINK (whole genome data analysis toolset – Purcell et al., 2007) by logistic regression, adjusting the analysis for the variables statistically significant different between cases and controls: mother's and father's education, tooth brushing habits, presence of visible plaque, current caries activity, and other preventive measures (details of each covariate in Table 1). P-values below 0.0045 (0.05/11) were considered statistically significant.

Results

Genotypes are in Hardy-Weinberg equilibrium (Supplemental Material). Logistic regression analysis (summary results in Table 3) showed an association between *AMBN* rs4694075 and caries experience (p=2.525e-007). Borderline results could be seen for *TUFT1* rs3790506 (p=0.03) and *TFIP1*1 rs134136 (p=0.03). The statistically significant result between

variation in *AMBN* and caries experience means that the less common allele T was more often than expected by chance in the group of asthmatics children in comparison to the control group, independent of mother's and father's education, tooth brushing habits, presence of visible plaque, current caries activity, and performing other preventive measures. PLINK allows for multiple covariates when testing the presence of disease trait SNP association. The regression coefficient represents the effect of the less frequent allele, meaning it increases risk/phenotype mean. Since it is reasonable to assume that the risk/preventive environmental factors used as covariates (mother's and father's education, tooth brushing habits, presence of visible plaque, current caries activity, and other preventive measures) in the model are independent from the individual's genotype, which is determined from birth but could have interaction effects on caries, adjusting for covariates in the analysis is justified.

Discussion

Our study provides, for the first time, evidence that genes involved in enamel formation are associated with caries in asthmatic children. We found evidence that caries is associated with *AMBN* in children diagnosed with asthma. AMBN is believed to control the elongation of enamel crystals and generally directs enamel mineralization during tooth development. Genome wide association studies have suggested approximately 30 SNPs to be associated with asthma (reviewed by Ramasamy et al., 2012). Among the genes marked by these SNPs, are the *SPRY1*/*ANKRD50* and *SLC7A11*/*PCDH18* genes in chromosome 4. These genes are located approximately 70 million base pairs from *AMBN*, which we found associated with caries in asthmatic children. Also *IL2RB* on chromosome 22 is approximately 11 million base pairs from *TFIP11*, which had a suggestive association result for caries in asthmatic children. But, aside from these two loci of enamel formation genes that are in the same chromosome of asthma associated SNPs, there is no coincidental findings between the results of genome-wide association analysis of asthma cases and the physical location of enamel formation genes.

The role of enamel formation genes in asthma is of interest because evidence suggests individuals diagnosed with asthma have a higher prevalence of enamel defects (Ford et al., 2009; Jälevik et al., 2001; Suckling et al., 1987; Wogelius et al., 2010). We have no reason to believe the increased prevalence of enamel defects is due by chance but the consequence of an underlying common biological mechanism. We have been investigating the possible contribution of genetic variation of enamel formation genes in caries (Deeley et al., 2008; Patir et al., 2008; Shimizu et al., 2012), a disease that leads to demineralization of the enamel. Our data also suggest that asthmatic individuals have more severe caries experience (Anjonshoaa et al., 2009), although recent evaluations of asthmatic children show that recently implemented asthma management and educational programs are reducing caries experience in these children (Lindemeyer et al., 2012). Children with asthma more often have enamel hypoplasia and these clinical defects are consequence of either primary ameloblast defects or defective interactions between odontoblasts and ameloblasts (Musale et al., 2010). Our hypothesis is that mechanisms that predispose to asthma, which should be in operation prior to the first asthma attack, interfere somehow with enamel formation, particularly in the presence of genetic variation altering the function of ameloblasts. Our

future studies will focus on understanding the molecular mechanism linking asthma to enamel defects, which in turn increase individual susceptibility to caries.

It is possible that the sensitization phase prior to the first asthma attack disrupts enamel development making this structure more susceptible to demineralization and caries lesion progression. Interfering with the sensitization phase is a promising venue of research. Evidence exists that human-induced pluripotent stem cells and mesenchymal stem cells may be used in the treatment of asthma (Sun et al., 2012) and these strategies may impact enamel development by avoiding that the inflammatory systemic reaction during sensitization interferes with dental development, allowing for the formation of an enamel structure less susceptible to the caries attack. Our hypothesis arises from the fact that is not uncommon to observe an enamel defect in a permanent tooth that was formed under a primary tooth that was affected by caries by the age of five (Broadbent et al., 2005).

Another possible mechanism influencing caries experience in asthmatics involves the disease treatment. Chronic corticosteroids use has adverse effects on bone mineral accretion. An aggravating factor in the pediatric population is the high prevalence of vitamin D insufficiency, which decreases bone mineral density. Increased use of inhaled and oral corticosteroids (i.e., two courses of oral corticosteroids per year) is associated with decreased bone mineral density in vitamin D insufficient boys 5 to 12 years of age (Tse et al., 2012). The chronic use of corticosteroids could be hypothesized to interfere in the process of mineralization of teeth as well, both during development, as well as impact the demineralization-remineralization balance and influence caries experience. Furthermore, individual levels of vitamin D could modulate this process. There is evidence that low vitamin D levels are associated with higher caries experience, but controlled clinical trials of supplemental vitamin D did not clearly reduced caries experience (Hujoel, 2013). In aggregate, these data suggest that the mechanism underlying vitamin D and asthma treatment effects on caries may be influenced by individual genetic variation and the identification of these variants will possibly allow us to identify the individuals that would benefit the most from personalized control of corticosteroid use or vitamin D supplementation in terms of reducing future caries experience.

Conclusion

Our study provides, for the first time, evidence that ameloblastin is associated with caries in asthmatic children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Age, sex, and caries experience of the studied subjects.

		Cases (n=100)	Controls (n=100)	*p-value*
		Mean±Standard Deviation (Median)	**Mean±Standard Deviation (Median)**	
Age		8.65±1.94	8.65±1.94	**p=1.0**
Plaque Index		1.35±0.70 (1.33)	1.19±0.77 (1.0)	**p=0.14**
DMFT		1.54±1.90 (1.0)	1.37±1.11 (1.0)	**p=0.58**
dmft		3.09±3.19 (2.0)	1.47±1.77 (1.0)	**p=0.001**
DMFS		1.87±2.60 (1.0)	1.78±1.79 (1.0)	**p=0.22**
dmfs		5.68±6.23 (4.0)	2.17±3.05 (1.0)	**p=0.001**
		n	**n**	
Sex	Female	50	50	p=1.0
	Male	50	50	
Brushing	Once a day	55	46	p=0.18
	Twice a day	21	34	
	Sometimes	23	18	
	Never	1	2	
Mother's education	High school	61	35	p=0.001
	University	10	43	
	Less than high school	29	22	
Father's education	High school	69	32	p=0.001
	University	18	46	
	Less than high school	13	22	
Visible plaque	Yes	61	39	p=0.002
	No	39	61	
Caries activity	None	45	70	p=0.003
	Active white spot lesion	18	6	
	Untreated cavity in dentin	27	18	
	Filling in the last 3 years	10	6	
Preventive factors	None	75	61	p=0.07
	Fluoridated toothpaste	19	33	
	Fissure sealant	6	6	
Any other systemic disease	Yes	12	0	p=0.001

		Cases (n=100)	Controls (n=100)	*p-value*
		Mean±Standard Deviation (Median)	**Mean±Standard Deviation (Median)**	
	No	88	100	
Smoking status	Mother	11	30	p=0.009
	Father	42	34	
	Both	26	17	
	None	21	19	
Mouth Breathing	Yes	64	12	p=0.001
	No	20	79	
	Sometimes	16	9	
Dental Malocclusion	No	72	89	p=0.003
	Class I	8	6	
	Class II div 1	16	3	
	Class II div 2	0	2	
	Class III	4	0	

DMFT/dmft=Decayed, Missing due to caries, Filled Teeth

DMFS/dmfs=Decayed, Missing due to caries, Filled Surfaces

Table 2

Enamel formation gene markers studied.

Gene	Locus	Marker public ID	Base pair change
Ameloblastin (AMBN)	4q21	rs34538475	G/T
		rs4694075	C/T
Amelogenin (AMELX)	Xp22.31-p22.1	rs17878486	C/T
		rs946252	C/T
Enamelin (ENAM)	4q13.3	rs3796704	A/G
		rs12640848	A/G
Tuftelin (TUFT1)	1q21	rs3790506	A/G
		rs233736	A/G
		rs4970957	A/G
Tuftelin interacting protein 11 (TFIP11)	22q12.1	rs134136	C/T
		rs5997096	C/T

Table 3

Logistic regression analysis of the association between genetic variation and caries experience, depending on asthma and adjusted by mother's and father's education, brushing habits, visible plaque, caries activity, and other preventive factors (implemented in PLINK).

Gene	Locus	SNP	Number of Informative Samples	p-value
Tuftelin	1q21.3	rs2337360	167	0.16
		rs4970957	195	0.5
		rs3790506	171	0.03
Ameloblastin	4q13.3	rs4694075	177	**2.525e-007**
		rs34538475	181	0.19
Enamelin	4q13.3	rs12640848	200	0.16
		rs3796704	191	0.54
Tuftelin interacting protein 11	22q12.1	rs5997096	192	0.61
		rs134136	187	0.03
Amelogenin	Xp22.2	rs946252	103	0.6
		rs17878486	116	0.55