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Axis Inhibition Protein 2 (*AXIN2*) Polymorphisms and Tooth Agenesis

N Callahan[1,2], **A Modesto**[3], **R Meira**[5], **F Seymen**[6], **A Patir**[6], and **AR Vieira**[1,2,3,4,7]

1 *Center for Craniofacial and Dental Genetics, University of Pittsburgh School of Dental Medicine*

2 *Departments of Oral Biology, University of Pittsburgh School of Dental Medicine*

3 *Pediatric Dentistry, University of Pittsburgh School of Dental Medicine*

4 *Department of Human Genetics, Graduate School of Public Health, Pittsburgh, PA, USA*

5 *Department of Pediatric Dentistry, Brazilian Lutheran University, Canoas, RS, Brazil*

6 *Department of Pedodontics, Istanbul University, Istanbul, Turkey*

Abstract

Tooth agenesis is a common congenital disorder that affects almost 20 percent of the world's population. A number of different genes have been shown to be associated with cases of tooth agenesis including *AXIN2*, *IRF6*, *FGFR1*, *MSX1*, *PAX9*, and *TGFA*. Of particular interest is *AXIN2*, which was linked to two families segregating oligodontia and colorectal cancer. We studied two collections of families affected with tooth agenesis and tested them for association with *AXIN2*. Significant association between tooth agenesis and *AXIN2* was found ($p = 0.02$) in cases with at least one missing incisor. Our work further supports a role of *AXIN2* in human tooth agenesis and for the first time suggests *AXIN2* is involved in sporadic forms of common incisor agenesis. Future studies should identify which specific tooth agenesis subphenotypes are consequence of *AXIN2* genetic variations. A subset of these cases could have an increased susceptibility for colon cancer or other types of tumors and this knowledge would have significant clinical implications.

Introduction

Dental abnormalities can provide some of the earliest warnings signs of some systemic disorders, such as bulimia (Bartlett, 2005) and consequences of antineoplastic treatments (Lopes et al., 2006). The dentist could be the first professional to notice these symptoms, which in turn can lead to early detection, faster treatment, and a higher survival rate. There is compelling evidence that we can soon add colorectal carcinomas to this list of diseases.

Tooth agenesis affects approximately twenty percent of the world's population. If third molars are excluded, still over five percent of the population is afflicted (Vastardis 2000). People with missing teeth have many problems with esthetics, phonetics, and mastication.

7Corresponding Author: Alexandre R. Vieira, Department of Oral Biology, 614 Salk Hall, University of Pittsburgh School of Dental Medicine, 3501 Terrace Street, Pittsburgh, PA, 15261, arv11@dental.pitt.edu.

Studies in a Finnish family with severe familial oligodontia who did not have mutations in *MSX1* (muscle segment homeobox 1) or *PAX9* (paired box 9), the two genes that when mutated can lead to oligodontia, disclosed the presence of a mutation in the axis inhibition protein 2 (*AXIN2*) gene. While studying the family's medical records, a history of familial adenomatous polyposis was discovered (Lammi et al., 2004). The possibility of the identification of a dental clinical marker for cancer brings additional interest to the studies of the genetic regulation of tooth agenesis.

The association with *AXIN2* and tooth agenesis was independently investigated in a relatively small case-control cohort from Poland and an association was found between tooth agenesis and markers in the gene (Mostowska et al 2006). On the other hand, germline *AXIN2* mutations were found to be rare in patients with multiple polyposis (Lejeune et al., 2006).

AXIN2 is a negative regulator of the Wnt signaling pathway. *Axin2* is expressed during mice odontogenesis in the dental mescenchyme, enamel knot, dental papilla mescenchyme, and in mesenchymal odontoblasts. There is also extensive evidence of the expression of *AXIN2* in colorectal tissues leading to carcinomas (Lammi et al. 2004).

The present study expands the investigations of *AXIN2* in tooth agenesis, and further supports a role of this gene in human tooth agenesis.

MATERIALS AND METHODS

Our study group consisted of 167 patients with tooth agenesis and their parents. The patients were from two different cohorts, 116 were from Rio de Janeiro, Brazil, which is an admixed population of Europeans (from Portugal) and Africans, with a very small percentage of Native South Americans. This population contains 71 sporadic cases and 45 familial cases. Seventy-four were females and 42 were males. The second cohort consisted of 51 trios from Turkey. Twenty six were females and 25 were males. All Turkish cases were of sporadic origin. Tooth agenesis was the sole disorder affecting these patients and second premolars were the teeth most commonly absent, followed by lateral incisors. None of the families reported history for clefts. Complete details about these two populations are presented elsewhere (Vieira et al., 2004; Vieira et al., 2008). The study was approved by the University of Pittsburgh Institutional Review Board (IRB), as well as the appropriate ethical committee at the Federal University of Rio de Janeiro and Istanbul University, and/or appropriate informed consent was obtained from human subjects. After informed consent was obtained, cheek swabs, saliva, or whole blood were collected from each individual. Clinical analysis, sample collection, and DNA extraction were performed using a consolidated protocol described elsewhere (Jezewski et al., 2003; Vieira et al., 2004; Vieira et al., 2008). Cases were analyzed not only as a single group, but also in three subgroups: cases with positive family history for tooth agenesis, cases with at least one missing incisor, and cases with at least one missing premolar (Table 1).

Three intragenic *AXIN2* markers were studied (rs7591, rs11867417, and rs2240308; Table 2). These markers were chosen based on the information on the gene structure and linkage disequilibrium blocks available at the International HapMap Project website (http://www.hapmap.org/). Genotypes were obtained using an ABI PRISM 7900 Sequence Detection System (Valencia, CA, USA) and TaqMan chemistry. Reagents and SNP genotyping assays were supplied by Applied Biosystems (Valencia, CA, USA). Pairwise calculations of linkage disequilibrium were computed with the Graphical Overview of Linkage Disequilibrium (GOLD) software for both the squared correlation coefficient (r2) and Lewontin's standardized disequilibrium coefficient (D′) (Abecasis and Cookson, 2000). Alleles at each marker were tested for association with tooth agenesis with the use of the Family Based Association Test (FBAT) software (Horvath et al., 2001;Horvath et al., 2004). The hbat

function of FBAT was used to calculate haplotype frequencies and associations between *AXIN2* haplotypes and tooth agenesis.

RESULTS

Table 3 provides the results for single marker analysis. Also, it presents the results for the subgroups of the cases with positive family history, cases with at least one missing incisor, and cases with at least one missing premolar. Table 4 provides a summary of the most significant haplotype results in the two populations. The pairwise linkage disequilibrium analysis confirms that the marker set we selected is for the most part in weak linkage disequilibrium (Table 5), which provides almost non-overlapping information about the gene.

The most significant association was between the *AXIN2* rs2240308 marker and cases with at least one missing incisor (p=0.037). This trend could also be seen in the haplotype analysis, in which most of the significant signals were for cases with at least one missing incisor.

Since the marker rs2240308 is a coding change in the gene (P50S: proline to serine at position 50), we used the software Polyphen (Sunyaev et al., 2000; Sunyaev et al., 2001; Ramensky et al., 2002) to predict the possible impact of the amino acid substitution on the structure and function of the AXIN2 protein. The change was predicted to be benign. We also used the ESE Finder 3.0 software to predict if the change would alter exonic splicing enhancers, which are thought to serve as binding sites for specific serine/arginine-rich proteins that help determine splicing (Cartegni et al., 2003; Smith et al., 2006). The A to G nucleotide change did not affect any predicted exonic splicing enhancer site.

DISCUSSION

Our work provides further evidence that *AXIN2* contributes to tooth agenesis. The marker rs2240308 (P50S) was significant by itself in Brazilian cases with at least one missing incisor (p=0.037), as well as was part of an associated haplotype in the combined Brazilian-Turkish dataset of cases with at least one missing incisor (p=0.021). The P50S change does not appear to be etiologic based on our *in silico* experiments. It is possible this variant is in linkage disequilibrium with the functional variant that contributes to tooth agenesis.

While concerned about multiple testing, we did not apply the strict Bonferroni correction as it would increase type II errors and a major focus of this study was to identify putative associations between *AXIN2* and tooth agenesis for further studies. For example, under the Bonferroni correction, we would have lowered the alpha to 0.0056 (0.05/9), and the possible association between *AXIN2* and cases with at least one missing incisor would have been ignored. Therefore we report here all results with p-values below 0.05. However, our data must be carefully interpreted since it is expected that some of the p-values below 0.05 can be due to chance.

The suggestive association with cases with at least one incisor missing raises interesting questions. The inactivating mutations described in *AXIN2* that lead to oligodontia and higher susceptibility to colon cancer affected incisor development in 11 out of 12 cases (Lammi et al., 2004). The study from Poland that suggested an association between *AXIN2* variation and tooth agenesis (Mostowska et al., 2006) was also enriched by cases missing the upper lateral incisors (36 cases in a total of 55). Interestingly, this report did not show an association between the *AXIN2* P50S change and tooth agenesis, and the significant results were found for another two *AXIN2* variants in strong linkage disequilibrium with each other, the silent mutation L688L and the intronic variant c.956+16A>G). According to the authors, these variants were not in linkage disequilibrium with P50S. In combination with our findings, these results may suggest that multiple *AXIN2* variants could contribute to tooth agenesis in humans. The L688L was

predicted to disrupt exonic splicing enhancer sequences, and although it is a synonymous change, this could contribute to the tooth agenesis phenotype. The c.956+16A>G was also suggested as possibly exerting a weak effect on splicing as it creates an additional donor-splicing site within the sequence of exon 2.

Finish, Polish, Turkish, and the studied Brazilian dataset, which is comprised by mainly Portuguese descendents, can be assumed to be mainly European subgroups. The current evidence regarding *AXIN2* and tooth agenesis suggests that this gene may play a role in congenitally missing teeth of groups of European ancestry.

Previously, we have suggested that different types of teeth are regulated by independent gene expression (Vieira, 2003). A number of Wnt genes are expressed in the developing teeth and changes in their expression may be one of the factors determining tooth agenesis (Zhang et al., 2005). There is evidence that genes related to the Wnt pathway are differentially expressed in molars versus incisors. Studies in mice targeting *Lef1* function, a transcription factor that can be activated by Wnt proteins, showed that transgene expression of human *LEF1* (lymphoid enhancer-binding factor 1) promoter can be seen where the incisor and molar teeth develop at E12.5. Transgene molar expression of the *LEF1* promoter during molar development was observed only in E12.5 embryos and disappeared in E13.5 embryos. Conversely, *LEF1* promoter expression during incisor tooth development persisted from E12.5 to 17.5 (Amen et al., 2007). Is it reasonable to hypothesize that whereas *AXIN2* loss-of-function affects both molar and incisor development (Lammi et al., 2004), hypomorphic *AXIN2* alleles would affect incisors more often due to the persistent expression of *LEF1* in these developing teeth, when compared to molars. In addition, the interactions between *LEF1* and *PITX2* (paired-like homeodomain transcription factor 2) could not only play important roles in dental development, but also in certain types of cancer (Amen et al., 2007).

In summary, we provide further evidence suggesting a role of *AXIN2* in tooth agenesis. Future studies should identify which specific tooth agenesis subphenotypes are consequence of *AXIN2* genetic variation. A subset of these cases could have an increased susceptibility for colon cancer or other types of tumors. It would be an impressive development if a patient could walk into a dental office with a congenitally missing tooth, something a medical doctor would most likely overlook, and just by taking a spit sample, the dentist was able to tell if the patient was at high risk for colorectal cancer.

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References

Abecasis GR, Cookson WO. GOLD – Graphical overview of linkage disequilibrium. Bioinformatics 2000;16:182–3. [PubMed: 10842743]

Amen M, Liu X, Vadlamudi U, Elizondo G, Diamond E, Engelhardt JF, Amendt BA. PITX2 and β-catenin interactions regulate lef-1 isoform expression. Mol Cel Biol 2007;27:7560–7573.

Bartlett DW. Three patient reports illustrating the use of dentin adhesives to cement crowns to severely worn teeth. Int J Prosthodont 2005;18:214–218. [PubMed: 15945308]

Cartagni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: a web resource to identify exonic splicing enhancers. Nucleic Acid Res 2003;31:3568–3571. [PubMed: 12824367]

Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype-phenotype associations. Eur J Hum Genet 2001;9:301–6. [PubMed: 11313775]

Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol 2004;26:61–9. [PubMed: 14691957]

Jezewski PA, Vieira AR, Nishimura C, Ludwig B, Johnson M, O'Brien SE, Daack-Hirsch S, Schultz RE, Weber A, Nepomucena B, Romitti PA, Christensen K, Orioli IM, Castilla EE, Machida J, Natsume N, Murray JC. Complete sequencing shows a role for *MSX1* in non-syndromic cleft lip and palate. J Med Genet 2003;40:399–407. [PubMed: 12807959]

Lammi L, Arte S, Somer M, Järvinen H, Lahermo P, Thesleff I, Pirinen S, Nieminen P. Mutations in *AXIN2* cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet 2004;74:1043–1050. [PubMed: 15042511]

Lejeune S, Guillemot F, Triboulet JP, Cattan S, Mouton C, Porchet N, Manouvrier S, Buisine MP. PAFNORD Group. Low frequency of *AXIN2* mutations and high frequency of MUTYH mutations in patients with multiple polyposis. Hum Mut 2006:931.

Lopes NN, Petrilli AS, Caran Em, França CM, Chilvarquer I, Lederman H. Dental abnormalities in children submitted to antineoplastic therapy. J Dent Child (Chic) 2006;73:210–213.

Mostowska A, Biedziak B, Jagodzinski PP. Axis inhibition protein 2 (*AXIN2*) polymorphisms may be a risk factor for selective tooth agenesis. J HumGenet 2006;51:262–266.

Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002;30:3894–3900. [PubMed: 12202775]

Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, krainer AR. An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. Hum Mol Genet 2006;15:2490–2508. [PubMed: 16825284]

Sunyaev S, Ramensky V, Bork P. Towards a structural basis of human non-synonymous single nucleotide polymorphisms. Trends Genet 2000;16:198–200. [PubMed: 10782110]

Sunyaev S, Ramensky V, Koch I, Lathe W III, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Hum Mol Genet 2001;10:591–597. [PubMed: 11230178]

Vastardis H. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. Am J Orthod Dentofacial Orthop 2000;117:650–656. [PubMed: 10842107]

Vieira AR. Oral clefts and syndromic forms of tooth agenesis as models for genetics of isolated tooth agenesis. J Dent Res 2003;82:162–165. [PubMed: 12598542]

Vieira AR, Meira R, Modesto A, Murray JC. *MSX1*, *PAX9*, and *TGFA* contribute to tooth agenesis in humans. J Dent Res 2004;83:723–727. [PubMed: 15329380]

Vieira AR, Seymen F, Patir A, Menezes R. Evidence of linkage disequilibrium between polymorphisms at the *IRF6* locus and isolate tooth agenesis, in a Turkish population. Arch Oral Biol 2008;53:780–784. [PubMed: 18452891]

Zhang YD, Chen Z, Song YQ, Liu C, Chen YP. Making a tooth: growth factors, transcription factors, and stem cells. Cell Res 2005;15:301–316. [PubMed: 15916718]

Table 1

Demographic data for both cohorts.

Family Characteristic	Brazilian N (%)	Turkish N (%)
Gender distribution		
Males	42 (36)	24 (47)
Females	74 (64)	27 (53)
Number of teeth missing		
1	44 (38)	7 (14)
2	46 (40)	17 (33)
3 or more	26 (22)	27 (53)
Type of teeth more often missing (total teeth missing)	274	207
Second premolar	104 (37.9)	75 (36.2)
Lateral incisor	82 (29.9)	54 (26)
First premolar	26 (9.7)	8 (3.8)
Second molar	24 (8.7)	11 (5.3)
Central incisor	16 (5.8)	32 (15.4)
First molar	11 (4)	9 (4.3)
Canines	11 (4)	18 (8.6)
Associated dental anomalies	16 (13.8)	-
Number of cases missing incisors	55 (47.4)	29 (56.8)
Number of cases missing premolars	63 (54.3)	44 (86.2)
Number of cases missing molars	14 (12)	8 (15.6)
Number of cases missing canines	7 (6)	10 (19.6)
Positive family history	41 (35.3)	-

Table 2

AXIN2 SNPs studied.

Variants	Alleles	Average Heterozygosity ± Standard Error	Location in *AXIN2*	mRNA Position
rs7591	A	0.466 ± 0.126	3′UTR	3858
	T			
rs11867417	C	0.486 ± 0.081	Intron 7	-
	T			
rs2240308	A	0.417 ± 0.186	Exon 1 (P50S)	462
	G			

Table 3

FBAT Results for combined cohorts of Brazilian and Turkish families.

Variants	Overtransmitted Allele	Brazil		Turkey		Combined	
All Trios		**n**	**p**	**n**	**p**	**n**	**p**
rs7591	-	56	0.742	30	0.286	86	0.373
rs11867417	-	56	0.359	34	0.199	90	0.128
rs2240308	-	56	0.154	32	0.334	88	0.086
Trios with Probands Missing at Least One Incisor		n	p	n	p	n	p
rs7591	-	26	0.612	17	0.22	43	0.241
rs11867417	-	26	0.086	21	0.857	47	0.172
rs2240308	A	28	**0.037**	17	0.587	45	0.055
Trios with Probands Missing at Least One Premolar		n	p	n	p	n	p
rs7591	-	35	0.500	25	1.000	60	0.600
rs11867417	-	37	0.674	28	0.262	65	0.292
rs2240308	-	32	0.886	28	0.374	60	0.471
Trios with Positive Family History		n	p	n	p	n	p
rs7591	-	19	0.317	-	-	-	-
rs11867417	-	19	0.852	-	-	-	-
rs2240308	-	19	0.480	-	-	-	-

Notes: n = number of informative families; p = p-value; bold indicates p-values lower than 0.05.

Table 4

Summary results of haplotypes analysis for combined cohorts of Brazilian and Turkish families.

Haplotype	Allele	Brazil		Turkey		Combined	
All Trios		**n**	**p**	**n**	**p**	**n**	**p**
rs7591 rs11867417	A-T-						
rs2240308	A	14	0.077	7	0.959	21	0.156
rs7591 rs11867417	T-C	47	0.162	26	0.231	73	0.069
Trios with Probands Missing at Least One Incisor		n	p	n	p	n	p
rs7591 rs11867417	A-T-						
rs2240308	G	15	**0.037**	14	0.131	29	0.096
	T-C-						
	A	17	0.099	13	0.956	30	**0.021**
	T-T-						
	A	3	0.083	2	0.784	5	**0.046**
rs7591 rs11867417	T-C	20	**0.027**	15	0.235	35	0.057
	A-C	15	0.654	10	0.091	25	0.519
rs11867417 rs2240308	C-A	23	**0.048**	16	0.594	39	0.065
Trios with Probands Missing at Least One Premolar		n	p	n	p	n	p
rs7591 rs11867417	T-T-						
rs2240308	G	3	0.083	-	-	3	0.083
	T-C-						
	G	18	**0.041**	17	0.33	35	0.465
rs7591 rs11867417	A-C	18	0.088	14	0.467	32	0.427
rs11867417 rs2240308	C-G	25	**0.041**	20	0.434	45	0.271
Trios with Positive Family History		n	p	n	p	n	p

Haplotype	Allele	Brazil		Turkey		Combined	
All Trios		**n**	**p**	**n**	**p**	**n**	**p**
rs7591 rs11867417 rs2240308	T-C-G	9	0.052	-	-	-	-

Notes: n = number of informative families; p = p-value; bold indicates p-values lower than 0.05.

Table 5

Linkage Disequilibrium analysis of the *AXIN2* markers studied.

Marker	rs7591	rs11867417	rs2240308
rs7591	-----	0.384	0.106
rs11867417	0.679	-----	0.143
rs2240308	0.377	0.488	-----

[a] r^2 is above the diagonal; D′ is below the diagonal.