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Association of *AXIN2* gene polymorphisms with nonsyndromic oligodontia in Turkish families

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Abstract

Tooth agenesis is the most common developmental abnormality of the human dentition characterized by the congenital absence of one or more permanent teeth. Oligodontia is the term used to describe severe tooth agenesis, where six or more permanent teeth are missing. The WNT gene pathway regulates multiple developmental processes during craniofacial and tooth development, and variations in WNT pathway genes have been reported in individuals with tooth agenesis. In this study, we investigated the association of 37 SNPs in/nearby 12 WNT pathway genes (*WNT3, WNT3A, WNT5A, WNT8A, WNT9B, WNT10A, WNT11, AXIN1, AXIN2, APC, LRP5, LRP6*) with oligodontia in 22 multiplex families. Genotypes were generated using Taqman chemistry in a real-time polymerase chain reaction assay. Family-based association tests were performed using FBAT. Pairwise-haplotype analysis was also performed. Bonferroni correction was used to adjust for multiple testing and P-values ≤ 0.001 were considered statistically significant. We found nominal association for *AXIN2* rs7591, located in the 3' UTR, with oligodontia (P=0.04). *In silico* analysis of SNP function predicted a binding site for miR-205 with potential impact on *AXIN2* expression. Although modest, these results continue to support a role for *AXIN2* in the etiology of familial tooth agenesis.

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Introduction

Tooth agenesis is the most common craniofacial congenital malformation in humans [1]. Up to 20% of the general population has agenesis of at least one third molar. Agenesis of other permanent teeth, excluding third molars, ranges from ~1.6 to 9%, depending on the population studied, and in 70-80% of these cases one or two teeth are missing [2,3]. Tooth agenesis can be identified as hypodontia (up to 5 teeth missing, excluding third molars), or oligodontia (lack of more than 6 teeth missing, excluding third molars) [1], in sporadic cases or segregating in families. In most of the familial cases, inheritance is autosomal dominant, however, autosomal recessive and X-linked inheritance have also been described [4].

Table 1. Details of study families.

Family No.	Individual No.	Phenotype	Relationship	No. of Missing Teeth	Type of Missing Teeth	Inheritance
1	1-1	Oligodontia	Proband	11	Incisors, premolars	Complex
	1-2	Unaffected	Mother	0		
	1-3	Unaffected	Father	0		
	1-4	Unaffected	Brother	0		
	1-5	Unaffected	Brother	0		
	1-6	Oligodontia	Aunt	10		
	1-7	Oligodontia	Uncle	7		
	1-8	Oligodontia	Uncle	7		
2	2-1	Oligodontia	Proband	28	Incisors, canines, premolars, molars	Complex
	2-2	Unaffected	Mother	0		
	2-3	Oligodontia	Father	8		
	2-4	Unaffected	Brother	0		
	2-5	Hypodontia	Sister	4		
	2-6	Hypodontia	Brother	4		
	2-7	Unaffected	Brother	0		
	2-8	Unaffected	Sister	0		
	2-9	Hypodontia	Brother	5		
	2-10	Oligodontia	Uncle	7		
	2-11	Hypodontia	Aunt	5		
	2-12	Oligodontia	Cousin	21		
	2-13	Oligodontia	Cousin	23		
	2-14	Oligodontia	Cousin	19		
	2-15	Oligodontia	Cousin	Unk		
3	3-1	Oligodontia	Proband	20	Incisors, premolars, molars	AR
	3-2	Unaffected	Mother	0		
	3-3	Oligodontia	Father	Unk		
	3-4	Hypodontia	Sister	Unk		
	3-5	Unaffected	Sister	0		
	3-6	Hypodontia	Brother	Unk		
	3-7	Oligodontia	Sister	17		
	3-8	Hypodontia	Cousin	Unk		
4	4-1	Oligodontia	Proband	8	Lower incisors, molars	AD
	4-2	Unaffected	Mother	0		
	4-3	Unaffected	Father	0		
	4-4	Hypodontia	Brother	4		
	4-5	Hypodontia	Aunt	Unk		
	4-6	Unaffected	Cousin	0		
	4-7	Hypodontia	Grandmother	Unk		

Unk, unknown missing tooth types
Inheritance patterns (suspected): AD, autosomal domimant; AR, autosomal recessive.

The etiology of tooth agenesis is complex and poorly understood [4]. Studies in mice have allowed the identification of genes directly or indirectly involved in the regulation of tooth development, and have been fundamental to the understanding of the basic genetic principles of tooth development and its defects [5]. Nevertheless, very few human mutations have been described in several genes known to arrest tooth development in mice. This may reflect basic differences in agenesis mechanisms because of species-specific characteristics, such as tooth type (only incisors and molars in mice) and number of dentitions (one dentition in mice vs. two dentitions - deciduous and permanent - in humans) [6]. Mutations in *PAX9* (Paired Box 9), *MSX1* (Msh Homeobox 1), and *EDA* (Ectodysplasin A), have been shown to cause arrest of tooth development in mice and humans, and these genes have been extensively studied [1-10].

Table 1 (Continued). Details of study families.

Family No.	Individual No.	Phenotype	Relationship	No. of Missing Teeth	Type of Missing Teeth	Inheritance
5	5-1	Oligodontia	Proband	13	Incisors, premolars	AR
	5-2	Unaffected	Mother	0		
	5-3	Unaffected	Father	0		
	5-4	Unaffected	Sister	0		
6	6-1	Oligodontia	Proband	23	Incisors, premolars, molars	Complex
	6-2	Hypodontia	Mother	2	Upper lateral incisors	
	6-3	Unaffected	Father	0		
	6-4	Unaffected	Brother	0		
7	7-1	Oligodontia	Proband	17	Incisors, premolars, molars	AR
	7-2	Unaffected	Mother	0		
	7-3	Unaffected	Father	0		
	7-4	Unaffected	Sister	0		
8	8-1	Oligodontia	Proband	9	Incisors, premolars	AD
	8-2	Unaffected	Mother	0		
	8-3	Hypodontia	Father	2	Upper lateral incisors	
9	9-1	Oligodontia	Proband	15	Incisors, premolars, molars	Complex
	9-2	Unaffected	Mother	0		
	9-3	Hypodontia	Father	Unk		
	9-4	Hypodontia	Brother	Unk		
	9-5	Hypodontia	Brother	Unk		
	9-6	Unaffected	Sister	0		
	9-7	Unaffected	Brother	0		
	9-8	Unaffected	Sister	0		
10	10-1	Oligodontia	Proband	9	Incisors, premolars, molars	X-linked
	10-2	Unaffected	Mother	0		
	10-3	Hypodontia	Father	3		
	10-4	Hypodontia	Sister	4		
	10-5	Unaffected	Brother	0		
11	11-1	Oligodontia	Proband	8	Incisors, canines, premolars	AR
	11-2	Unaffected	Mother	0		
	11-3	Unaffected	Father	0		
	11-4	Unaffected	Sister	0		
12	12-1	Oligodontia	Proband	12	Incisors and premolars	AD
	12-2	Oligodontia	Mother	Unk		
	12-3	Unaffected	Father	0		
	12-4	Unaffected	Brother	0		
	12-5	Oligodontia	Uncle	12		
13	13-1	Oligodontia	Proband	12	Incisors, canines, molars	AR
	13-2	Unaffected	Mother	0		
	13-3	Oligodontia	Father	7		
	13-4	Oligodontia	Brother	8		

Unk, unknown missing tooth types
Inheritance patterns (suspected): AD, autosomal domimant; AR, autosomal recessive.

The WNT gene pathway regulates multiple developmental processes during craniofacial and tooth development [11-12]. Previous evidence showing the expression of several Wnt genes during mouse tooth development strongly implicated this gene family in the etiology of tooth agenesis [11-14]. In recent years, mutations in WNT pathway genes, namely *AXIN2* (Axis Inhibition Protein 2), *WNT10A* (Wingless-Type MMTV Integration Site Family, Member 10A), *LRP6* (low-density lipoprotein receptor-related protein 6), and recently *WNT10B* (Wingless-Type MMTV Integration Site Family, Member 10B), have also been shown to cause tooth agenesis in humans [8, 10, 15-18]. Additional studies have also shown the association of common single nucleotide polymorphisms in a few WNT pathway genes with the milder form of tooth agenesis, hypodontia [19-22]. However, the association of WNT pathway gene polymorphisms in oligodontia pheno-

Table 1 (Continued). Details of study families.

Family No.	Individual No.	Phenotype	Relationship	No. of Missing Teeth	Type of Missing Teeth	Inheritance
14	14-1	Oligodontia	Proband	12	Incisors and premolars	Complex
	14-2	Hypodontia	Mother	4		
	14-3	Unaffected	Father	0		
	14-4	Hypodontia	Brother	4		
	14-5	Hypodontia	Cousin	2	Upper lateral incisors	
	14-6	Unaffected	Uncle	0		
	14-7	Hypodontia	Uncle's wife	2	Upper lateral incisors	
15	15-1	Oligodontia	Proband	7	Premolars, molars	Complex
	15-2	Unaffected	Mother	0		
	15-3	Hypodontia	Father	2	Upper lateral incisors	
	15-4	Unaffected	Sister	0		
	15-5	Hypodontia	Uncle	2	Upper lateral incisors	
	15-6	Hypodontia	Uncle	1	Upper lateral incisor	
	15-7	Hypodontia	Uncle	1	Upper lateral incisor	
16	16-1	Oligodontia	Proband	10	Incisors, canines, premolars, molars	AD
	16-2	Unaffected	Mother	0		
	16-3	Unaffected	Father	0		
	16-4	Unaffected	Brother	0		
17	17-1	Oligodontia	Proband	6	Upper lateral incisors, premolars	AR
	17-2	Unaffected	Mother	0		
	17-3	Hypodontia	Father	2	Upper lateral incisors	
	17-4	Unaffected	Brother	0		
	17-5	Hypodontia	Sister	4	Premolars	
18	18-1	Oligodontia	Proband	18	Incisors, premolars, molars	AD
	18-2	Unaffected	Mother	0		
	18-3	Unaffected	Father	0		
	18-4	Oligodontia	Sister	28	Incisors, canines, premolars, molars	
19	19-1	Oligodontia	Proband	10	Incisors and premolars	Complex
	19-2	Hypodontia	Mother	2	Upper lateral incisors	
	19-3	Unaffected	Father	0		
20	20-1	Oligodontia	Proband	13		AD
	20-2	Unaffected	Mother	0		
21	21-1	Oligodontia	Proband	28	Incisors, canines, premolars, molars	Complex
	21-2	Unaffected	Mother	0		
	21-3	Unaffected	Father	0		
22	22-1	Oligodontia	Proband	16	Incisors, canines, premolars, molars	Complex
	22-2	Hypodontia	Mother	2	Upper lateral incisors	
	22-3	Unaffected	Father	0		

Unk, unknown missing tooth types
Inheritance patterns (suspected): AD, autosomal domimant; AR, autosomal recessive.

types is still unclear. Therefore, in this study, we investigated the association of single nucleotide polymorphisms in 12 WNT pathway genes with oligodontia in multiplex families from Turkey.

Material and methods

Sample Population

This study was approved by the Istanbul University Institutional Ethical Review Board and the Committee for Protection of Human Subjects at the University of Texas Health Science Center at Houston. Clinical and demographic information and DNA samples from peripheral blood were obtained from the CRANI-RARE2 Project, an European Union-funded collaborative ERA-net Project on craniofacial malformations run at the Istanbul University, Istanbul Medical Faculty, Medical Genetics Department. All registry participants had signed an informed consent form agreeing to participate in genetic studies and pro-

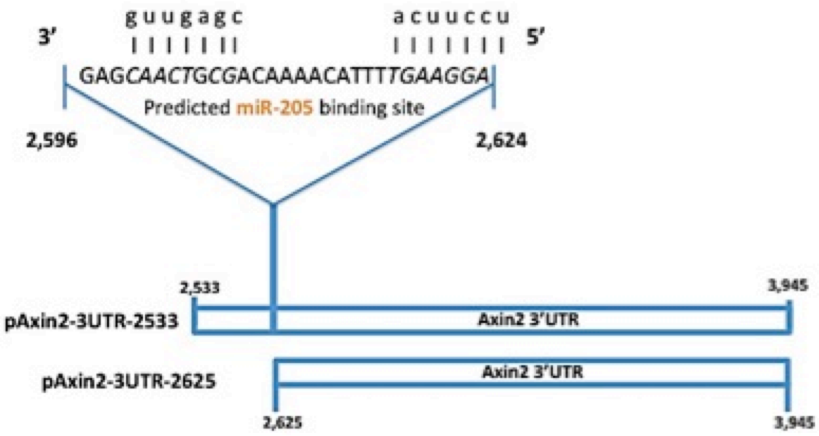

Figure 1. *AXIN2* rs7591 is predicted to bind to miR-205-5p.

vided a blood samples as a source of genomic DNA.

Probands were selected from the registry based on their radiographic records showing congenital tooth agenesis and were invited to participate. Individuals were considered to have oligodontia when six or more permanent teeth were missing in the oral cavity, excluding third molars. Families were ascertained through probands, and additional relatives were recruited. All probands and available family members were further examined clinically to confirm the tooth agenesis status and exclude syndromic cases. In a few cases, history of tooth agenesis was available by self-report from family members, or from statements by their dental provider. Our sample population consisted of 22 multiplex oligodontia families (117 total individuals, 67 affected, 50 unaffected), in which tooth agenesis segregated in both autosomal dominant and autosomal recessive forms, and an average of 2-18 teeth were missing in each affected individual. Details of studied families are presented in Table 1.

Selection of Candidate Genes and Single Nucleotide Polymorphisms

We selected 37 single nucleotide polymorphisms (SNPs) in/nearby *APC, AXIN1, AXIN2, LRP5, LRP6, WNT3, WNT3A, WNT5A, WNT8A, WNT9B, WNT10A,* and *WNT11* genes for genotyping in our families. SNPs were selected based on their locations within the genes, on their likelihood to have functional consequences (i.e., located in the promoters, exons, or near exon/intron boundaries), or if considered tag-SNPs for the linkage disequilibrium blocks surrounding the respective genes [23]. We used information available at the NCBI dbSNP (http://www.ncbi.nlm.gov/SNP/) and HapMap Project (http://www.hapmap.org) databases to select polymorphisms. Details of studied genes and polymorphisms are presented in Table 2.

Genotyping

Genomic DNA was extracted from blood using established protocols. Genotypes were generated using Taqman chemistry [24]. Reactions were carried out in 5-µL volumes in a ViiA7 Sequence Detection System (Applied Biosystems, Foster City, CA). Assays and reagents were supplied by Applied Biosystems. The results were analyzed using EDS software (Applied Biosystems). In order to ensure quality control of genotyping reactions, we included a non-template control (water instead of DNA) as negative control and a DNA sample of known genotype as positive control in each reaction.

Association analyses

Family-based association tests were performed using FBAT software version 1.06 [25]. We used Bonferroni correction to adjust for multiple testing (0.05/37) and P-values ≤ 0.001 were considered significant.

In silico prediction of SNP function

We performed *in silico* analysis of SNP function to predict the effects of the associated *AXIN2* rs7591 SNPs function using MiRBase software [26].

Results

Association analyses

We found evidence of altered allelic transmission for *AXIN2* rs7591, in the gene 3' UTR, with oligodontia (P=0.04).

***In silico* prediction of SNP function**

In silico analysis of the 3' UTR SNP in *AXIN2* rs7591 predicted a binding site for the microRNA miR-205-5p with potential effects on gene expression (Figure 1).

Discussion

In this study, we investigated the association of 12 WNT pathway genes (*APC, AXIN1, AXIN2, LRP5, LRP6, WNT3, WNT3A, WNT5A, WNT8A, WNT9B, WNT10A,* and *WNT11)* with nonsyndromic oligodontia in 22 well-characterized Turkish Caucasian multiplex families. Although modest, our results suggest a positive association between *AXIN2* and oligodontia, and corroborate the results of previous studies [19-21]. To our knowledge, this is the most comprehensive analysis of the association of WNT/β-catenin pathway genes with tooth agenesis, particularly oligodontia.

Over the years, many signaling pathways have shown to be involved in the organogenesis and embryogenesis of teeth [3, 5, 7, 12, 27]. Individuals with oligodontia constitute approximately 1% of all individuals with hypodontia, and both conditions can be found in the same family, indicating variable expression of shared genetic factors [2, 7]. The importance of the WNT/β-catenin signaling pathway during tooth development has been reported by several authors [11-14]. Many studies showed that Wnt pathway plays a critical role in tooth morphogenesis and several Wnt genes are expressed in craniofacial and dental tissues [11-14]. Wnt pathway activation has roles at the lamina-early bud stage and also important for molar cusps development [13]. During tooth development, AXIN2 is expressed in the dental mesenchyme, the odontoblasts and the enamel knot, and it is needed as a negative regulator of WNT-signaling at specific stages [12, 13].

Additional common variants in *AXIN2* have also been associated with increased susceptibility to hypodontia in Eastern Europeans [19]. However, the SNP associated in the present study, *rs7591,* located in the 3' UTR, has not been previously reported in association with tooth agenesis and warrants additional confirmatory studies. Previously, this same SNP was reported in association with oral clefts in families with increased susceptibility to colon cancer [28, 29]. Interestingly, *in silico* analyses predicted that this SNP harbors a binding site for the miR-205-5p, with a potential regulatory role in gene expression. Recent evidence has shown that a number of cellular functions, including development, differentiation, growth, metabolism, anabolism, and carcinogenesis can be affected by miRNA functions [30]. Although the role of miR-205-5p in craniofacial development is yet unknown, it has been suggested to play a role in cancer development and Parkinson's disease [31]. Further, the level of miR-205-5p expression was found to be down-regulated in various cancer cells, including breast, oral, prostate cancer cells, and melanoma [32]. Additional studies on miR-205-5p and its effect on the regulation of *AXIN2* might elucidate the role of these molecules in tooth agenesis.

In addition to a critical role in embryonic development, the WNT/β-catenin signaling pathway is also associated with tumorigenesis events [9]. Mutations in *AXIN2* were found segregating with autosomal dominant tooth agenesis and colorectal cancer in a large multiplex family, suggesting that a same gene may be involved in congenital anomalies and cancer later in life [15]. *AXIN2* mutations were also detected segregating in autosomal dominant pattern with oligodontia and other findings including colonic polyposis, gastric polyps, a mild ectodermal dysplasia phenotype with sparse hair and eyebrows, and early onset colorectal and breast cancers [33]. The *AXIN2* gene encodes the axis inhibition protein 2 that regulates the stability of beta-catenin and early organ differentiation and development and plays a key role in many basic cell functions, like cell homeostasis [9]. Since the report by Lammi et al. [15], numerous human genetic studies have focused on identifying variants in *AXIN2* in association with tooth agenesis or other birth defects such as cleft lip/palate [8-10, 19-21, 28], due to the previously suggested hypothesis that cancer-related genes may have a role in tooth agenesis. Notwithstanding, despite the positive associations reported, additional studies are needed to determine if potential correlations exist between *AXIN2*, birth defects and cancer.

Recently, *WNT10A* has been suggested as a major candidate gene for tooth agenesis, and oligodontia in particular, and rare variants in this gene have been found in individuals with tooth agenesis from multiple populations [33]. Interestingly, in the present study, we did not identify any association between common variants in *WNT10A* and oligodontia in our Turkish families.

In summary, although modest, our results continue to support a role for *AXIN2* and the WNT/β-catenin signaling pathway in human tooth agenesis. Discrepancies between the present and previous studies may be due to heterogeneity of the condition across distinct populations, and/or the common variant-common disease approach used in this association study, when rare variants in relevant genes could be the cause of the phenotype. Future studies should focus on the identification of potentially functional variants in *AXIN2* and additional WNT pathway genes to further establish a biological role of this pathway in tooth agenesis phenotypes.

Table 2. Details of SNPs genotyped in this study and association results.

SNP	Locus	Gene	Function	Alleles*	MAF	Informative families**	P-value***
rs861674	Chr.5: 112064475	*APC*	Intron	A/T	0.454	17	0.67
rs2431238	Chr.5: 112124369	*APC*	Intron	C/T	0.343	14	1
rs454886	Chr.5: 112146117	*APC*	Intron	C/T	0.348	18	0.65
rs351771	Chr.5: 112164561	*APC*	Synonymous	C/T	0.009	1	----
rs448475	Chr.5: 112181379	*APC*	3' UTR	C/G	0.482	18	0.67
rs2301522	Chr.16: 359953	*AXIN1*	Intron	A/G	0.4	16	0.5
rs7591	Chr.17: 63525082	*AXIN2*	3' UTR	A/T	0.143	11	*0.04*
rs7224837	Chr.17: 63528123	*AXIN2*	Intron	A/G	0.491	23	0.91
rs11867417	Chr.17: 63537898	*AXIN2*	Intron	C/T	0.201	13	0.84
rs3923086	Chr.17: 63549488	*AXIN2*	Intron	G/T	0.438	18	0.43
rs2240307	Chr.17: 63554307	*AXIN2*	Intron	A/G	0.179	7	0.28
rs740026	Chr.17: 63561681	*AXIN2*	Intergenic	A/G	0.232	14	0.86
rs634008	Chr.11: 68094741	*LRP5*	Intron	C/T	0.402	20	0.46
rs667126	Chr.11: 68177728	*LRP5*	Intron	C/T	0.267	16	0.29
rs312788	Chr.11: 68122295	*LRP5*	Intron	G/T	0.384	20	0.57
rs312014	Chr.11: 68084962	*LRP5*	Intron	C/G	0.438	17	0.77
rs10743980	Chr.12: 12412795	*LRP6*	Intron	C/T	0.438	17	0.45
rs4477532	Chr.12: 12279361	*LRP6*	Intron	A/G	0.037	4	----
rs7294695	Chr.12: 12323618	*LRP6*	Intron	C/G	0.393	20	0.65
rs121908120	Chr.2: 218890289	*WNT10A*	Missense	A/T	1	0	----
rs3806557	Chr.2: 218879152	*WNT10A*	Missense	A/G	0.009	1	----
rs199980023	Chr.2: 218882196	*WNT10A*	Missense	C/T	0.5	22	0.81
rs116998555	Chr.2: 218890118	*WNT10A*	Missense	C/T	0.009	1	----
rs4574113	Chr.2: 219762662	*WNT10A*	Intron	A/T	0.194	11	1
rs10177996	Chr.2: 219746561	*WNT10A*	Intron	C/T	0.223	12	0.41
rs3806557	Chr.2: 219743874	*WNT10A*	Intron	G/A	0.221	12	0.33
rs1533767	Chr.11: 75905800	*WNT11*	Intron	A/G	0.009	1	----
rs199498	Chr.17: 44865603	*WNT3*	Intergenic	C/T	1	0	----
rs111769	Chr.17: 44871987	*WNT3*	Intergenic	C/T	0.356	19	0.31
rs9890413	Chr.17: 44901449	*WNT3*	Intergenic	A/G	0.356	14	0.6
rs708111	Chr.1: 228191365	*WNT3A*	Intergenic	C/T	0.143	13	0.5
rs3094912	Chr.1: 228209815	*WNT3A*	Intron	A/T	0.38	18	0.24
rs752107	Chr.1: 228247351	*WNT3A*	3' UTR	C/T	0.256	13	0.23
rs1745420	Chr.1: 228251732	*WNT3A*	Intergenic	C/G	0.393	22	0.15
rs566926	Chr.3: 55520778	*WNT5A*	Intron	A/C	0.348	18	0.41
rs2040862	Chr.5: 137419989	*WNT8A*	Intron	C/T	0.446	23	0.9
rs2165846	Chr.17: 44941366	*WNT9B*	Intron	A/G	0.5	18	0.64

* Ancestral allele listed first, NCBI dbSNP build 147
** SNPs with less than 5 informative families were excluded from further analysis
***FBAT, P<0.001 denotes statistical significance. P-values <0.05 are shown in italic font.

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