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Understanding the implications of the PAX9 Gene in tooth development

ABSTRACT

Aim Tooth agenesis is characterised by the congenital absence of one or more teeth. The Pax9 gene has been associated with nonsyndromic forms.

Materials and methods To investigate the molecular mechanisms, we evaluated specific haplotypes frequency in exon 3 of the Pax9 gene in 26 patients and 21 controls, using an Italian population.

Results Presence of His239His and the Ala240Pro were confirmed in exon 3 of the Pax9 gene. A frequency of 20.2% of the T allele at position 717 and a C frequency of 33% of Ala240Pro polymorphism, that reached 40.5% in the control group, were observed. The 39 C/C-240 C/C or G/Chaplotype which we defined Pax9hapl a had a proportion of 61.9% in control individuals. The frequency of Pax9hapl a tested in the patients was different from controls, being 81.3% in normalcy and 18.8% in oligodontia ($p < 0.05$).

Conclusion Our observations suggest that Pax9hapl a may have a protective effect against sporadic oligodontia.

Keywords: Tooth agenesis; Hypodontia; PAX9 Gene.

Introduction

Growth factors, transcription factors, signal receptors and other soluble morphogens are involved in the development of dentition, thus it is not surprising that such a complex process may be influenced by various disturbances, resulting in tooth agenesis.

Tooth agenesis is a common human anomaly that affects approximately 20% of the population and is characterised by the congenital absence of one or more teeth, leading to masticatory dysfunction, speech

alterations, aesthetic problems and malocclusion. Although tooth agenesis is associated with numerous syndromes, several case reports describe nonsyndromic forms that are either sporadic or familiar in nature [Gorlin et al., 1990].

In 1953 Malavez [De Michelis et al., 1992] first elaborated a classification, universally used in clinical practice, distinguishing anodontia and oligodontia. Anodontia is the failure of development of a whole dentition and it may affect both deciduous and permanent dentitions (total anodontia or agenodontia) or of just of the permanent teeth (complete anodontia or ablastodontia). The absence of up to half of the number of teeth is called oligodontia [Das et al., 2002] and it includes the oligodontia, when less than 10 teeth of the deciduous series are developed, and the oligoblastodontia, if 16 or less permanent teeth are developed. Finally the hypodontia is the agenesis of less than half of the dental formula, and either deciduous or permanent teeth may be affected. Among the hypodontia cases, agenesis of at least one tooth remains the most common anomaly of dental development [Vastardis, 2000].

Beside a numerical classification, agenesis is clinically identified depending of its location in anterior (at least one missing tooth among incisors and canines), middle (if bicuspid or first molars are affected) and posterior agenesis (if at least one of second and/or third molars are missing). Finally, an agenesis is called combined if the missing teeth are contemporarily located in different sites of the mouth. The most commonly agenetic teeth are the lateral upper incisors [Graber, 1978], followed by the third upper and lower molars and the second upper and lower bicuspid [Kokich and Kokich, 2006], and few authors underline that the most distal tooth of each morphologic dental class is the more often affected by numerical anomalies [De Michelis et al., 1992].

The reduction of teeth has been differently discussed in literature and various aetiopathogenetic explanations have been reported. Owen [De Michelis et al., 1992] developed the theory of agenesis as an evolution of the race, in which the reduction of teeth in humans may be interpreted as a regressive metamorphosis into mammalian dentition, originally fixed in 44 teeth. Following this hypothesis the reduction of teeth should proceed along with civilization [Talbot and Röse in De Michelis et al., 1992]. Endocrinological (hypopituitarism), general (nutritional deficit during pregnancy, central system disease, etc.) and local (jaws trauma) factors have also been described. Finally, there is a clinical evidence that hypodontia often occurs throughout different generations, in one or more members of the same family, thus indicating a possible hereditary factor influence in its occurrence.

Considering the complexity of the process leading to the development of dentition, involving several interactions between growth factors, transcription factors and signal receptors, it may be speculated that a defect in any of these proteins can lead to some kind of tooth abnormality. Hence, it is not surprising that tooth agenesis is a common genetic condition.

In the past few years several studies have focused their attention on the molecular basis of tooth development,

trying to underline the genetic causes behind this complex condition. To date, three genes have been associated with nonsyndromic forms of tooth agenesis: MSX1, Pax9 and TGFA [Kolenc-Fusè, 2004; Ogawa et al., 2006; Vieira et al., 2004] and a possible role for another gene (AXIN2) has been proposed [Mostowska et al., 2006].

An interesting feature of this dental disorder is that it is strongly influenced by ethnicity [Frazier-Bowers et al., 2003] so that tooth agenesis varies with each class of tooth and for ethnic groups [Rølling, 1980; Salama et al. 1994; Chai et al., 1999; Endo et al., 2006]. It is interesting to speculate that different genetic backgrounds in the form of specific haplotypes within or around those genes involved in dental development may have occurred independently at some time in the remote past in different populations and were then set and maintained throughout several generations due to some potential

beneficial effect. In fact, only recently, there have been few attempts to study human tooth agenesis through a genetic epidemiological approach in ethnically homogeneous populations [Pereira et al., 2006; Vieira et al., 2004].

We here propose to investigate the molecular mechanisms behind this complex dental disease, particularly by the evaluation of different frequencies of specific haplotypes in exon 3 of the Pax9 gene, in our study group as compared with the control group, using an Italian population.

Materials and methods

Subjects

This investigation is part of a large study on the implication of candidate gene mutations in tooth agenesis, started in 2006. We decided to perform the present study to explore the relationship between mutations in exon 3 of the Pax9 gene and oligodontia. In November 2007, 26 patients with tooth agenesis between 10 and 69 years of age, characterised by nonsyndromic forms of oligodontia of different severity and referred to the specialised center of the Department of Dentistry of the AFaR-Fatebenefratelli Hospital in Rome (Italy), were recruited. Different members of the same family were included (i.e. grandparents, parents and children) when all of them presented familial agenesis, thus explaining such a high gap of age of the sample. This set of patients was compared with a sex- and age-matched group of 21 subjects, referred to the same Department because of third molars germectomy or extraction, and whose normal dentition was assessed through clinical visit and radiological evaluation on panoramic x-ray. The patients sample comprised 12 sporadic cases and 14 familial cases. For these patients all the clinical, demographic, anamnestic and radiological data were collected in an appropriate database and are summarised in Table 1. All the individuals in this study were of Italian origin and by all of them appropriate informed consent was obtained.

DNA sequencing

DNA was extracted from all the study population from peripheral blood samples according to standard procedures. Exon 3 of the Pax9 gene was PCR-amplified using primer pairs and conditions previously described [Pereira et al., 2006]. PCR fragments were purified using Nucleospin columns (M-Medical) according to manufacturer's instructions. PCR fragments were then

Patient	Age	Sex	Olig.	Tooth agenesis	Polym. 239	Polym. 240
1532	29	F	sporadic	1.4; 2.4	C/T	G/G
1364	19	F	familial	1.2; 2.2	C/C	G/G
1621	26	F	sporadic	1.2; 1.4; 1.5; 1.8; 2.2; 2.3; 2.4; 2.5; 2.8; 3.4; 3.5; 3.8; 4.4; 4.5; 4.8	C/T	G/G
1615	69	F	familial	1.3; 1.8; 2.2; 2.8; 3.8; 4.8	C/T	G/G
1366	15	F	sporadic	1.5; 2.5; 3.5	C/C	G/G
1178	15	M	sporadic	1.2; 1.7; 1.8; 2.2; 2.7; 2.8; 3.1; 3.6; 3.7; 3.8; 4.1; 4.6; 4.7; 4.8	C/T	G/G
1316	25	F	sporadic	1.2; 2.2; 3.5; 4.5	C/T	G/C
1554	14	F	sporadic	3.4; 3.5; 4.5; 4.6	C/C	G/C
1319	24	F	familial	1.3	C/C	G/C
1520	10	F	sporadic	1.2	C/C	G/C
1308	17	M	familial	1.2; 1.3; 1.8; 2.2; 2.3; 2.8; 3.8; 4.8	C/C	C/C
1521	12	F	sporadic	3.5	C/T	G/C
1352	36	M	familial	1.2; 1.3; 1.4; 1.5; 1.8; 2.2; 2.3; 2.4; 2.5; 2.8; 3.4; 3.5; 3.8; 4.4; 4.5; 4.8	C/C	C/C
1551	54	F	familial	1.2; 2.2	C/C	G/C
1322	17	F	familial	1.2; 2.2	C/T	G/G
1614	25	F	familial	1.8; 2.8; 3.1; 3.8; 4.1	C/C	G/C
1505	26	M	familial	1.2; 2.2; 1.5; 2.5; 3.5; 4.5	C/T	G/G
1612	32	F	familial	1.5; 1.8; 2.4; 2.5; 2.8; 3.5; 3.8; 4.5; 4.8	C/T	G/G
1613	45	F	familial	1.5; 2.5	C/T	G/G
1305	27	F	familial	2.5; 3.8; 4.8	C/C	G/C
1552	20	F	sporadic	1.2; 1.8; 2.2; 2.8; 3.1; 3.8; 4.1; 4.8	C/C	G/G
1597	12	M	sporadic	1.8; 2.5; 2.8; 3.8; 4.5; 4.8	C/C	G/G
1303	26	M	sporadic	3.5	C/C	G/C
1580	12	F	familial	1.2; 2.2; 3.2	C/C	G/G
1622	34	M	familial	1.2; 1.8; 2.2; 2.8; 3.2; 3.8; 4.2; 4.8	C/T	G/C
1309	16	F	sporadic	2.2	C/T	G/G

TABLE 1 - Clinical characteristics and genotype of hypodontia cases; agenetic teeth are expressed using FDI dental numerical system.

Genotype n, %	C/C		C/T		T allele frequency %			
	His/His	%	His/His	%	T/T	His/His	%	
individuals	47	29	61.7%	17	36.2%	1	2.1%	20.2%
Controls	21	15	71.4%	5	23.8%	1	4.8%	16.7%
Patients	26	14	53.8%	12	46.2%	0	0.0%	23.1%

TABLE 2 - Allele and genotype distribution of the His239His polymorphism.

Genotype n, %	G/G		G/C		C allele frequency %		
	Ala/Ala	%	Ala/Pro	%	C/C	Pro/Pro	%
individuals	47	44.7%	21	44.7%	5	10.6%	33.0%
Controls	21	33.3%	11	52.4%	3	14.3%	40.5%
Patients	26	53.8%	10	38.5%	2	7.7%	26.9%

TABLE 3 - Allele and genotype distribution of the Ala240Pro polymorphism.

sequenced on both strands on an ABI310 Genetic Analyzer (Applied Biosystems) using the BigDye terminator chemistry (Applied Biosystems).

Results

For each of the 26 patients and the 21 controls of the present study, exon 3 of the Pax9 gene was directly sequenced. Rather than new mutations, the analysis revealed the presence of 2 relatively common polymorphisms previously described [Nieminen et al., 2001; Pereira et al. 2006], namely the His239His (a C to T transition at position 717), and the Ala240Pro (a G to C transversion at position 718). In our study sample it was observed a frequency of 20.2% of the T allele at position 717, with a slightly higher frequency for the patients group, which was 23.1% (Table 2). The Ala240Pro polymorphism showed an even higher frequency of the C allele with 33.0% (Table 3) considering the entire group, and reaching 40.5% in the control group.

Table 4 shows the genotype observed at position 239 and 240 in the study group and the relative asset of tooth agenesis for each patient.

The C/C genotype at position 718 (homozygosity for the less frequent allele) was found in 2 (7.7%) affected individuals and in 3 (14.3%) control individuals.

We then analysed our data taking into account the frequencies of the haplotype derived by the combination of the most frequent single polymorphisms in the healthy

controls. When we considered the frequency of the 39 C/C-240 C/C or G/C haplotype (i.e. wild type at position 239 and homozygous recessive or heterozygous at position 240), which we defined Pax9hapl a- in our control panel, we found a proportion of 61.9% of individuals with this haplotype (Table 1). Frequencies of Pax9hapl a was 59.1% in our healthy controls and 40.9% in the study group. This difference was even more striking when the frequency of Pax9hapl a was tested comparing only controls with sporadic cases, being 81.3% in normalcy and 18.8% in oligodontia (χ^2 test $p < 0.05$) (Fig. 1).

Discussion

Pax9 gene codes for a paired domain-containing transcription factor that plays an essential role in the development of mammal dentition. It has been associated with selective tooth agenesis in humans and mice, which mainly involves the posterior teeth.

In the present study we identified 2 relatively common polymorphisms previously described [Nieminen et al., 2001; Pereira et al., 2006], namely the His239His (a C to T transition at position 717), and the Ala240Pro (a G to C transversion at position 718). The genotype and allele distribution of the two polymorphisms were only recently described by Pereira and collaborators [2006]. In their study they investigated three ethnically distinct populations and found different distributions of the 2 alleles. Interestingly, the European population, comprising 15 individuals, showed the highest frequency for both sequence alterations. These results are largely confirmed by our study, where we observed a frequency of 20.2% of the T allele at position 717 in all individuals tested (both patients and controls), with a slightly higher frequency for the patients group (23.1%).

We also observed that the C/C genotype at position 718 (homozygosity for the less frequent allele) was found in 2 affected individuals and in 3 control individuals. In their paper, Pereira et al. [2006] suggested that individuals carrying this polymorphism in a homozygous state are associated with absence of all 4 third molars. Our results demonstrate that this is true only for people affected by tooth agenesis, where indeed, in addition to the absence of other teeth, all 4 third molars were missing in both patients with the C/C genotype. In contrast, the 3 healthy individuals displaying this genotype had all 4 third molars. This may suggest the possibility that homozygosity for the less frequent allele at this position may not be tolerated in the presence of the other polymorphism at position 717, not even in a heterozygous state.

In this report we defined a different distribution of the frequency of the 39 C/C-240 C/C or G/C haplotype (i.e. wild type at position 239 and homozygous recessive or heterozygous at position 240) which we named Pax9hapl a- in exon 3 of the Pax 9 gene, between normal individuals and cases affected by nonsyndromic sporadic oligodontia. Our observations suggest that Pax9hapl a- may have a protective effect against sporadic oligodontia, being in 81.3% in normalcy and 18.8% in oligodontia. A study evaluating the reliability of Pax9hapl a- in discriminating oligodontia cases from normal

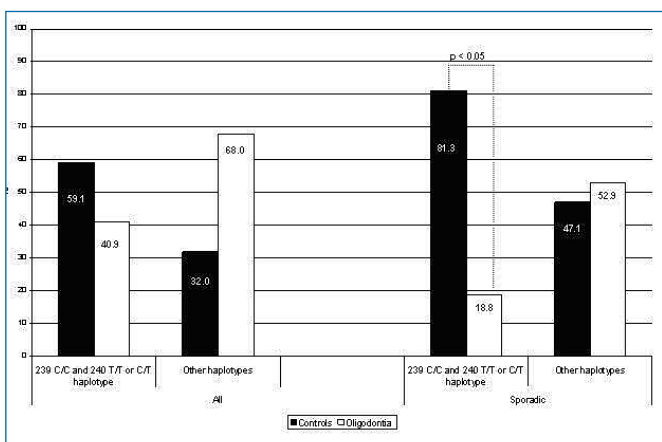


FIG. 1 - Frequency of Pax9hapl a tested comparing controls with sporadic cases.

individuals is in progress.

The fact that one of the constituent of the Pax9hapl a - the C→T transition at position 717 - results in a silent change at residue 239 (CAC→CAT, His239His), suggests that Pax9hapl a might affect mRNA folding, which in turn might affect mRNA translation rate and subsequent protein folding [Komar, 2007]. The idea that differences in the rate of Pax9 expression might have a direct impact on mammalian dental patterning has been already reported as the concluding remark of a study on Pax9 mouse mutants. The authors [Kist et al., 2005] described a novel, hypomorphic Pax9 mutant allele producing decreased levels of Pax9 wild-type mRNA and showed that this caused oligodontia in mice.

Even though our observations are found on correlative studies and certainly require confirmation in a larger cohort including normal individuals and oligodontia cases, they represent an attempt to discuss reasons to shift the attention from single polymorphisms to Pax9 exon 3 haplotypes implication in developmental abnormalities of dental patterning.

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