ELECTRONIC LETTER

A unique form of hypodontia seen in Vietnamese patients: clinical and molecular analysis

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Tooth agenesis is a clinically heterogeneous disorder affecting various teeth at different rates, and is strongly influenced by ethnicity.^{1,2} Evidence confirms the role of genetic heterogeneity in this common dental anomaly, and alterations in two genes have been identified as causing this condition namely, *MSX1* and *PAX9*. Interestingly, our understanding of the genetic aetiology of human tooth agenesis is still largely based on the selective agenesis of predominantly posterior teeth. Specifically, mutations in the transcription factor *PAX9* were shown to be responsible for "molar oligodontia" in three independent families,³⁻⁷ and a large deletion of *PAX9* in one nuclear family led to the absence of all posterior teeth.⁸ Moreover, point mutations in the transcription factor *MSX1* have been identified in families affected with selective posterior tooth agenesis predominantly involving premolars and occasionally molars.⁹⁻¹²

Although recent studies of human tooth agenesis have greatly advanced our knowledge of the genetic basis of this disorder, very few studies have specifically considered the genetic aetiology of anterior tooth agenesis.¹³⁻¹⁵ In one study, a mutation in the *SHH* gene was identified in association with a solitary maxillary incisor¹⁴; however, this pattern of tooth agenesis presents as a clinical variation of the holoprosencephaly syndrome. Interestingly, in certain Asian populations (including Japanese, Chinese, and Malaysian people), the incidence of non-syndromic anterior tooth agenesis has been reported to be 2%,^{16,17} which contrasts with the 0.08%–0.23% occurrence rate in a white population.¹⁸ Despite the high prevalence of incisor hypodontia, there is little known about the genetic aetiology in this pattern of tooth agenesis.

Previous studies¹⁻⁷ have increased our current understanding of the biology of tooth development, specifically concerning the roles of *PAX9* and *MSX1*. Moreover, human genetic studies have provided interesting data that allow us to infer a genotype/phenotype correlation. Several affected people in previous human studies present with classical patterns of premolar or molar agenesis, but many also lack mandibular

incisors.^{3,4,6} Furthermore, expression studies in mice show that *Pax9* is present in early odontogenic mesenchyme where incisors and molars first develop. Taken together, these data suggest that *PAX9* may be associated with a temporal event, thus controlling the development of molars and mandibular incisors. This may begin to explain the presence of mandibular incisor agenesis that occurs with molar agenesis.

In the study presented here, we describe the clinical features, inheritance, and mutational analysis of familial mandibular incisor agenesis in people of Vietnamese descent located in the Houston area. Mutational analysis of *PAX9* and *MSX1* has excluded these genes as the cause of mandibular incisor agenesis in this population. Further, pedigree analyses suggest that mandibular incisor agenesis is inherited in an autosomal dominant manner with incomplete penetrance. Taken together, these studies strongly suggest that this pattern of tooth agenesis in this population is the result of mutations in a gene other than *PAX9* or *MSX1*.

MATERIALS AND METHODS

Patient recruitment and clinical diagnosis

Seven hundred sequential dental records from consenting and participating dental offices were reviewed, and screenings at health fairs were performed to identify affected people of Vietnamese descent. Affected people were also identified through referrals from dentists. Patients screened for this study ranged in age from 5 to 40 years, with roughly equal numbers of male and female patients (table 1). Pedigrees were extended laterally and vertically for six of 20 identified families, and are designated families 1 to 6 (fig 1A-F). The diagnosis of mandibular incisor hypodontia (fig 2A and B) for all patients was based on a clinical examination and either panoramic or periapical/bite wing dental radiographs. For this study, mandibular incisor agenesis includes those involving central or lateral incisors as it is difficult to distinguish definitively between these two teeth. Cephalometric analysis was performed to determine the presence of skeletal or dental malocclusion in those patients who were receiving orthodontic care. This study was approved by the University of Texas Health Science Center at Houston committee for the protection of human subjects. Consent to participate in this study (including release of dental records) was obtained from every adult participant, or a parental guardian in the case of minors.

Pedigree construction and power analysis

A pedigree analysis by inspection was performed for six families to determine the mode of inheritance of the hypodontia phenotype. This analysis suggested an autosomal dominant mode of inheritance with incomplete penetrance. Power

Key points

- Several families of Vietnamese descent were identified with mandibular incisor hypodontia that was transmitted as an autosomal dominant trait with incomplete penetrance.
- Mutational analysis was performed using the candidate gene approach to determine whether mutations in the coding regions of two genes known to cause tooth agenesis, namely *MSX1* and *PAX9*, are responsible for this unique pattern of incisor hypodontia.
- Direct sequencing of the coding regions of *PAX9* and *MSX1* excluded the involvement of these genes in mandibular incisor hypodontia.

*The first two authors contributed equally to this work.

Table 1 Twenty cases of lower incisor agenesis in Vietnamese people

Case	Age (years)	Sex	Missing permanent tooth	Dental findings	Skeletal classification	Presence of agenesis in family
1	11	F	Two mandibular incisors	Class III molars, class II cuspids, shovel shaped maxillary incisors	Skeletal class II	Yes: maternal cousin
2	15	M	One mandibular incisor	Full molar class III, class III cuspids, shovel shaped maxillary incisors	Skeletal class I	Yes: mother
3	8	F	One mandibular incisor	Class III molar relationship, cuspids not erupted yet, shovel shaped incisors	Skeletal class III	Yes: brother
4	23	F	Two mandibular incisors	Class III molar, class III cuspids, anterior open bite,	Skeletal class II	Yes: daughter, son
5	12	F	One mandibular incisor	Class III molar subdivision right, class III cuspids, shovel shaped incisors	Skeletal class I,	Yes, mother missing 1 lower incisor
6	16	F	One mandibular incisor	Class III molar, block out left maxillary cuspid, right peg lateral maxillary incisor	Skeletal class II	Yes: father
7	12	M	One mandibular incisor	Class III molar, minimal, shovel shaped incisors	No ceph	Yes: brother
8	14		One mandibular incisor	Class III molar, moderate, shovel shaped incisor	No ceph	Yes: brother
9	18	F	Two mandibular incisors	Class III molar, class III cuspids arch, shovel shaped incisor	No ceph	Yes: father
10	16	M	One mandibular incisor	Class I molar, class III cuspid, shovel shaped incisors	Skeletal class I	No, brother has congenitally 1 missing maxillary lateral incisor
11	18	F	Two mandibular incisors	Class III molar, class III cuspids, shovel shaped incisors	Skeletal class I	None from immediate family
12	18	M	Two mandibular incisors	Class III molar, class III cuspids, shovel shaped incisor	Skeletal class I,	None from immediate family
13	5	F	One mandibular permanent incisor	Distal step of primary molars, fusion of primary B and C	No ceph	Yes: mother
14	12	F	Two mandibular incisors, upper 3rd molars	Class I molar, blocked out cuspids, shovel shaped incisor	Skeletal class I	Yes: 2 of 3 sisters
15	30	M	Two mandibular incisors	Class III molar, extraction of lower right 1st molar, lower left 3rd molar	Skeletal class I	Yes: mother and sister
16	11	F	Two mandibular incisors	Full molar class III, class III cuspid, blocked out maxillary cuspids	Skeletal class III,	No
17	32	M	One mandibular incisor	Class I molar,	No ceph	Yes: son, daughter
18	7	M	One mandibular incisor	Class I molar	No ceph	Yes: father
19	30	F	Two mandibular incisors	Class III molar	No ceph	Yes: both mother and father, 3 of 4 sibs
20	27	F	One mandibular incisor	Class I molar, minimal crowding in maxillary and mandibular arches	No ceph	Yes: two aunts

Summary of affected people with congenitally missing teeth, other dental findings, cephalometric analysis, and family history of lower incisor agenesis. No ceph (cephalometric analysis), no lateral head film available. Yes, the subject has an affected relative.

calculations (table 2) were performed with the program Simlink¹⁹ assuming an autosomal dominant model with 90% penetrance.

Polymerase chain reaction and sequencing

Peripheral blood samples or buccal swabs were obtained from probands and family members, and DNA extractions were performed for affected probands and one affected relative for three independent families (families 1, 2, and 3) using a Pure-gene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). DNA was amplified by polymerase chain reaction (PCR) with primer sets for exons 2 to 4 of human *PAX9*, and with primer sets for exons 1 and 2 of *MSX1* (described previously⁶). The PCR and purification were carried out as described previously for *PAX9*²⁰ and for *MSX1*.¹² Amplified products were subsequently sequenced in both directions using ABI Big Dye terminator reagents (Applied Biosystems, Foster City, CA) using an ABI PRISM 377 DNA sequencer.

RESULTS

Clinical diagnosis

We found a unique form of incisor hypodontia that occurs as an isolated pattern, predominantly in Vietnamese people. All patients were otherwise medically healthy, and reported no syndromic involvement. Initially, 20 cases of incisor agenesis were identified, comprising 12 female and eight male patients. In 19 of the 20 Vietnamese patients with mandibular incisor hypodontia, all the remaining teeth were present, including the third molars. The only exception was a patient who was missing maxillary third molars (table 1). Ten out of 19 patients

showed bilateral incisor agenesis. For 16 of 20 patients, at least one first degree relative was also affected with mandibular incisor hypodontia. Clinical examination further disclosed that the remaining teeth of affected patients seemed morphologically normal with the following exception; 12 patients had “shovel shaped incisors” (fig 3), and one patient had a fusion of primary teeth B and C in the left lower quadrant (fig 4). Finally, cephalometric and occlusal analysis disclosed that 15 patients had a class III dental relationship, possibly resulting from mesial migration of the mandibular posterior teeth subsequent to the missing anterior teeth.

Previous studies have established the hereditary basis of tooth agenesis.^{21–22} We therefore questioned whether mandibular incisor agenesis in this population was transmitted as an inherited trait. Extension of six pedigrees and analysis of these pedigrees by visual inspection suggest that mandibular incisor hypodontia is inherited in an autosomal dominant manner with incomplete penetrance, although we could not exclude a pseudodominant pattern of inheritance.

Mutational analysis

The coding regions and exon splice boundaries of *MSX1* and *PAX9* were sequenced using DNA from affected probands and unaffected relatives in three unrelated families. No alterations were identified in the genes (data not shown).

Power calculations

We used Simlink¹⁹ to estimate the amount of power for linkage which was available with this set of families. Based on the

Figure 1 (A-F) Pedigrees of families with incisor hypodontia suggest that the condition is inherited as an autosomal dominant trait with incomplete penetrance. Darkened symbols indicate bilateral or unilateral incisor agenesis, clear symbols indicate normal unaffected; (?) indicates affection status unknown.

Table 2 Power analysis of six pedigrees based on an autosomal dominant model confirms that summed pedigrees provide sufficient power to detect linkage

II Model*	III Marker type	Theta		
		0.0	0.1	
Autosomal dominant, 90% Penetrance	Biallelic	1.926	0.905	Summed pedigrees Pedigree 2
	Biallelic	0.845	0.405	

* Assumes no effect of sex.

pedigree inspection, we assumed an autosomal dominant pattern of inheritance. We considered a 90% penetrance rate, and based on the clinical information, we did not consider the penetrance to be age dependent. We simulated with equal frequencies of biallelic marker alleles and found that the estimated mean lod score was 1.96, with family 2 providing the largest contribution of the overall lod score.

DISCUSSION

Previous epidemiological studies have shown that tooth agenesis varies with each class of tooth and for ethnic groups.²²⁻²⁷

The most commonly missing teeth in the white population are the third molars (wisdom teeth), followed by the maxillary lateral incisor or mandibular second premolar.²⁸⁻³³ In our study, we identify a unique pattern of missing teeth that occurs at a much higher rate in the Asian population^{16 17} (including Vietnamese, Japanese, Chinese, and Malaysian people) compared with white or black populations. Interestingly, the third molar, which is typically absent in association with missing molars or premolars of the same quadrant,³⁴ is seen to develop normally in patients with congenitally missing mandibular incisors in the Vietnamese population. We explored the question of whether this very unique pattern of familial tooth agenesis is

Figure 2 (A) Occlusal view of an 18 year old female patient showing the mandibular arch with unilateral mandibular incisor agenesis. (B) Frontal intraoral photograph of the same patient. All other teeth are present and are normal in shape and position.

Figure 3 Panoramic view of a patient with shovel shaped maxillary incisors and unilateral mandibular incisor agenesis.

caused by similar mutations in two genes known to cause other forms of tooth agenesis. Our analysis failed to identify mutations in the coding regions of either *MSX1* or *PAX9*. Moreover, the power calculations show that our families, although underpowered for conclusive findings, were of sufficient power to determine a suggestion of linkage. Because only mutations in the coding regions of *PAX9* or *MSX1* have been described in previous studies,^{3 5 6 8–10 12 13 20 23} our mutational analysis was limited to these regions. However, we cannot rule out the possibility that mutations may exist in the intronic or regulatory regions of these genes.

The studies define and describe a unique form of hypodontia present in people of Vietnamese descent, and excluded genes known to cause other forms of hypodontia as the cause of this condition. Future studies will identify the gene or genes responsible. Finally, as we continue to identify the genes

Figure 4 Fusion of primary mandibular teeth (B and C) in a 5 year old female patient.

responsible for various forms of tooth agenesis, we will further our understanding of the genes that control the patterning and formation of teeth.

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