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Short communication

Non-syndromic severe hypodontia caused by a novel frameshift insertion mutation in the homeobox of the *MSX1* gene

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ABSTRACT

Objective: Inherited congenital anomalies in tooth number, particularly hypodontia are relatively common. Although substantial progress has been made that permits a better understanding of the causes of tooth agenesis, overall knowledge of the phenotype:genotype correlations in this anomaly are still lacking. The aim in this study was to identify the causal gene mutation(s) in a family of two sisters with severe hypodontia (oligodontia) including 2nd premolars and 1st and 3rd molars, using whole exome sequencing (WES).
Methods: WES was performed using in-solution hybridization, followed by massively parallel sequencing.
Results: A frameshift insertion of 7 basepairs (GCAAGTT) in the homebox of *MSX1* gene located in the exon 2 in heterozygous state has been identified in both sisters (NM_002448:exon2:c.572_573ins GCAAGTT: p. F191fs).
Conclusion: We conclude that this frameshift mutation in the homeodomain (which plays an essential role in DNA binding) of *MSX1* gene is responsible for tooth agenesis in this family. This expands the phenotype-genotype correlation associated with *MSX1* mutations.

1. Introduction

Tooth agenesis is one of the most common craniofacial anomalies seen in human populations, characterized by the developmental absence of one or more teeth. For clinicians this type of anomaly can constitute a challenging clinical problem in many cases. Different terms have been used to describe missing primary and permanent teeth. Hypodontia is used to describe absence of less than six teeth excluding the third molar. An absence of more than six teeth excluding the third molar is termed oligodontia. In more severe cases the term anodontia is used to describe a complete absence of teeth (Chhabra, Goswami, & Chhabra, 2014; Cobourne & Sharpe, 2012; Matalova, Fleischmannova, Sharpe, & Tucker, 2008).

In Caucasians, tooth agenesis is relatively common, one or two teeth, in particular incisors and/or premolars and it is seen in up to 8% of the population (Arte, Nieminen, Pirinen, Thesleff, & Peltonen, 1996), wheras oligodontia is rarer (0.25%) (Schalk-van der Weide, Beemer, Faber, & Bosman, 1994) and anadontia is very rare (Gorlin, Herman, & Moss, 1980). Both genetic and environmental factors are thought to play a role in the etiology of tooth agenesis. In the majority of cases, tooth agenesis is inherited as an autosomal dominant trait (Goldenberg et al., 2000; Vastardis, Karimbux, Guthua, Seidman, & Seidman, 1996), recessive (Pirinen, Kentala, Nieminen, Varilo, & Thesleff, 2001) or sex-linked trait (Erpenstein & Pfeiffer 1967) with varying degrees of penetrance and expressivity. A number of genes have been identified in individuals and families with tooth agenesis. These include msh homeobox 1 (*MSX1*) (Vastardis et al., 1996), paired box 9 (*PAX9*) (Stockton, Das, Goldenberg, D'souza, & Patel, 2000), (*WNT10A*) (Kantaputra & Sripathomsawat 2011), latent transforming growth factor β binding protein (*LTBP3*) (Noor et al., 2009), ectodysplasin A (*EDA*) (Tao et al., 2006), *EDAR*-associated death domain (*EDARADD*) (Bergendal, Klar, Stecksén-Blicks, Norderyd, & Dahl, 2011) and *AXIN2* (Lammi et al., 2004).

MSX1 plays an important role during the early stages of odontogenesis, where it is highly expressed in the mesenchyme of the developing tooth germ especially during bud and cap stages (MacKenzie, Ferguson, & Sharpe, 1991; Tucker, Al, & Sharpe, 1998).

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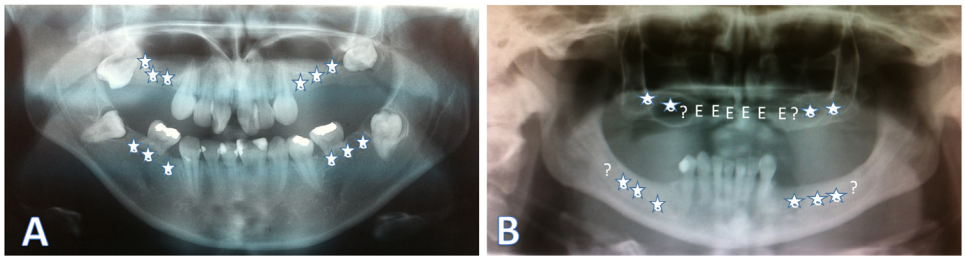

Fig. 1. Dental panoramic radiographs for both sisters. Shows missing teeth in both sisters, (A) sibling KA (proband), (B) is sibling SA (sister). Star indicates missing teeth,? the diagnosis was not confirmed and E is extracted teeth.

Table 1
Summary of missing and present teeth in both sisters.

Subject	JAWS	8R	7R	6R	5R	4R	3R	2R	1R	1L	2L	3L	4L	5L	6L	7L	8L
Sibling 1 (KA)	Upper	*	–	*	*	*	–	–	–	–	–	–	*	*	*	–	*
	Lower	*	–	*	*	*	–	–	–	F		–	–	*	*	–	*
Sibling 2 (SA)	Upper	*	?	*	*	?	**E**	**E**	**E**	**E**	**E**	**E**	?	*	*	?	*
	Lower	*	?	*	*	–	–	–	–	–	–	–	*	*	*	?	*

1: central incisors, 2: lateral incisors, 3: canine, 4 and 5: first and second premolars, respectively; 6,7 and 8: first, second and third molars respectively. * indicates missing teeth, – present teeth, F is fusion, L is left, R is right? the diagnosis was not confirmed and E is extracted teeth.
The "*" is an additional clarification regarding the mutation in this reference.

It has also been associated with different types of syndromic and non-syndromic tooth agenesis. *MSX1* is a member of the muscle segment homebox family that contains a highly conserved sequence encoding 60 amino acids that have the ability to bind DNA at specific sequences (Hewitt, Clark, Ivens, & Williamson, 1991). *MSX1* is located on chromosome 4 and consist of two exons, the second exon includes the homeodomain. In humans the first connection between *MSX1* gene and tooth agenesis was reported in a family with second premolar and third molar hypodontia through genetic linkage analyses that identified the 4p16.1 locus where the *MSX1* gene is located. Further analysis revealed an arg31-to-pro missense mutation in the homeodomain of the *MSX1* gene (Vastardis et al., 1996). Since then a total of 15 different mutations in *MSX1* have been identified. Exome sequencing has become a powerful and well established strategy for discovering rare alleles underlying Mendelian disorders. In this study we used whole exome sequencing to identify the causative gene mutation (s) in a family of 2 sisters with severe hypodontia.

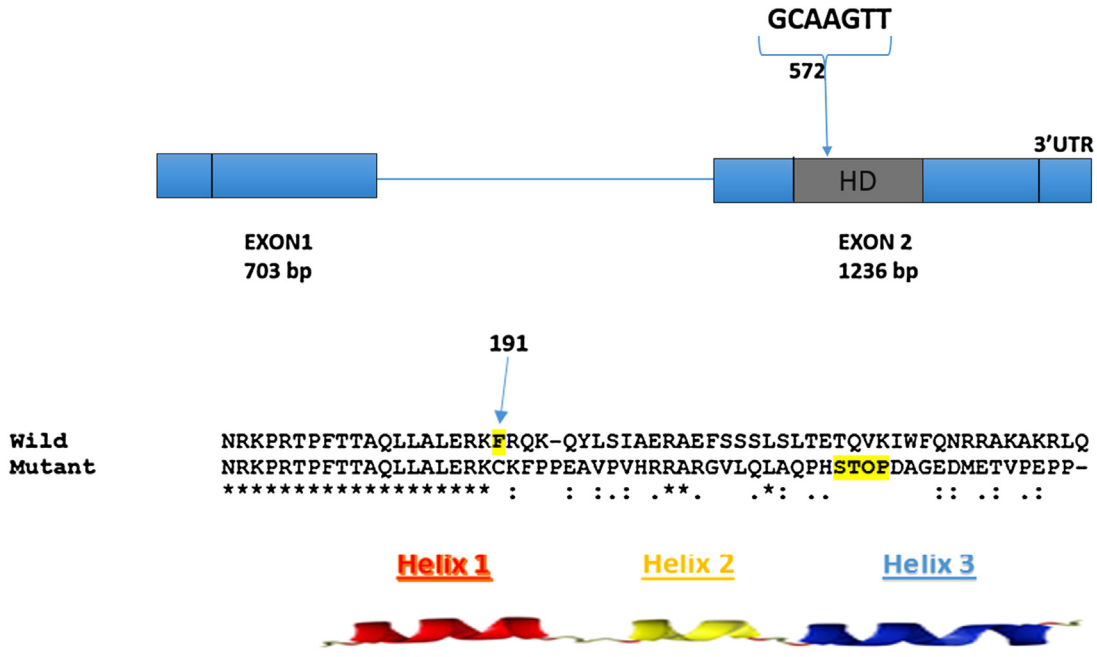

Fig. 2. Structure of human MSX1 gene and the site of mutation. The upper diagram shows the location of the detected mutation within the homeodomain (HD). The lower diagram shows the amino acid sequence and the exact location of the amino acid change 191 (arrowed) and the three helices.

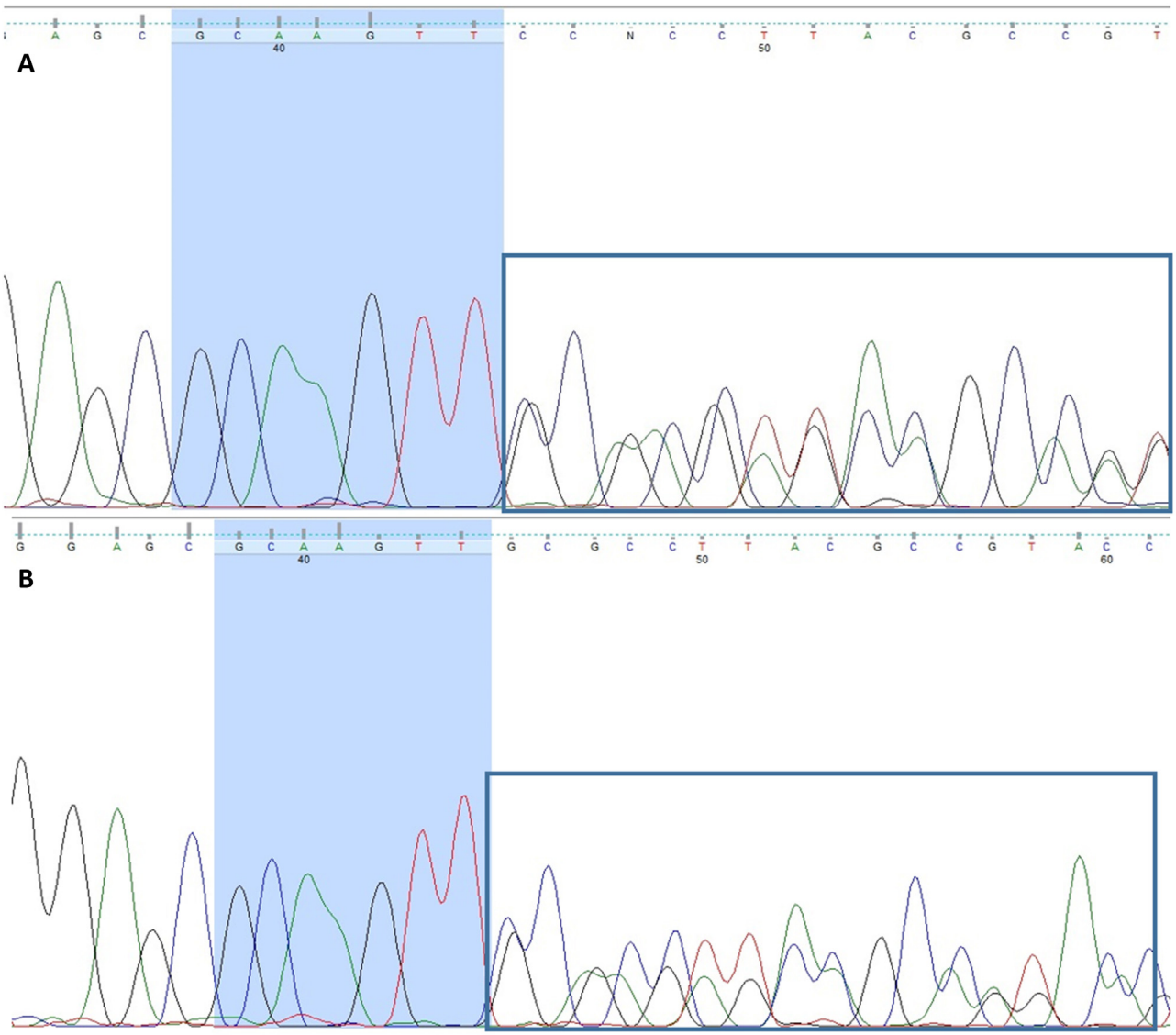

Fig. 3. Chromatogram of Sanger sequencing. Shaded area shows the 7 bp insertion, rectangular area shows the frameshift (multiple abnormal peaks) occurred after the insertion of the 7 bp (A) proband and (B) sister.

2. Materials and methods

2.1. Sample (Fig. 1, Table 1)

A 24 year old female (proband) was referred to the orthodontic department at Guy's Hospital seeking treatment. After diagnosis and clinical examination, severe oligodontia was verified in addition to congenital anomaly (fusion) in the lower left central and lateral incisors which was subsequently confirmed by panoramic dental radiograph examination. She was immediately questioned about other members of her family and her 44 year sister also had hypodontia although she did not know whether her parents were affected. The sister was recruited, clinical examination and radiographical examination revealed severe tooth agenesis. Both sisters medical histories revealed no other congenital anomalies.

Exome sequencing was performed to identify genes that might be responsible for this severe oligodontia phenotypes. Written and oral consent was obtained from the patients

2.1.1. Sibling 1 (proband)

clinical and radiographical examination indicated that this patient had severe oligodontia with 11 missing teeth excluding the third molars, including all the lower and upper first and second premolars, except the lower left first premolar. Additionally, this patient had fusion in the lower central and lateral incisors, and retained lower first primary molars.

2.1.2. Sibling 2

clinical and radiographical examination revealed that this patient had tooth agenesis with multiple missing teeth including upper and lower premolars and molars. However, the patient did not recall or confirm extraction of some of her teeth.

2.2. DNA extraction

Blood samples were taken from the patients. Genomic DNA was isolated from peripheral blood leukocytes using the standard salt extraction procedure.

Table 2
Table summarizes MSX1 mutations, location, mode of inheritance and consequence in non-syndromic tooth agenesis.

Author	Location	Effect on amino acid, mutation type	Mode of inheritance	Consequence
Vastardis et al. (1996)	Exon 2 (HD)	Arg196Pro missense	Autosomal recessive	Reduced MSX1 interaction with DNA
De Muynck et al. (2004)	Exon 2 (HD)	Gln187Stop nonsense	Autosomal dominant	uncertain
Chishti et al. (2006)	Exon 2 (HD)	Ala219Thr missense	Autosomal recessive	uncertain
Mostowska et al. (2006)	Exon 2 (HD)	Ala194val transition	Autosomal dominant	uncertain
Mostowska et al. (2012)	Exon 2 (HD)	Lue224Pro transition	Autosomal dominant	uncertain
Yamaguchi et al. (2014)	Exon 2 (HD)	Thr174lle nonsense	Sporadic	(Directly impair DNA binding)
Wong et al. (2014)	Exon 2	p.304Tyrext48 dup. TA	Autosomal dominant	Haploinsufficiency
Kamamoto et al. (2011)	Exon 2	Arg151Ser substitution	Autosomal dominant	Haploinsufficiency
AlFawaz et al. (2015)	Exon 2	F251PfsX92 Frameshift insertion	Autosomal dominant	uncertain
Van den Boogaard et al. (2000) *	Exon1	Ser104X nonsense	Autosomal dominant	uncertain
Lidral and Reising (2000)	Exon 1	Met61Lys substitution	Autosomal dominant	uncertain
Kim et al. (2006)	Exon 1	G22RfsX168 Frameshift dup.	Autosomal dominant	Haploinsufficiency
Kimura et al. (2014)	Exon 1	Trp139X nonsense	Autosomal dominant	Truncating the amino acid.
Pawlowska et al. (2009)	Intronic	Del740-451, deletion	sporadic	uncertain
Tatematsu et al. (2015)	Intronic	c.452-9G > A substitution	Autosomal dominant	Truncating amino acid at (HD)
This study family I, 2015	Exon 2 (HD)	p.F191 fs frameshift insertion	uncertain	uncertain

HD is homeodomain.
*This mutation has been identified in a family with cleft lip-palate patients with tooth agenesis including those with tooth agenesis without cleft.

2.3. Whole exome sequencing and sanger sequencing

To identify the causal mutation whole exome sequencing was performed using in-solution hybridization, followed by massively parallel sequencing using the all exon 50Mb Target Enrichment System (Agilent)(Coffey et al., 2011), and sequenced on the Illumina Hiseq 2500 system. The resulting sequencing reads were aligned against the human reference genome hg-19 using novoalign software (Novocraft Technologies). More than 5 Gb of sequencing data were generated for each individual. More than 85% of the coding bases of the GENCODE-defined exome were represented by at least 20 reads. Variants were annotated with respect to genes and transcripts with the Annovar tool (Wang, Li, & Hakonarson, 2010). Variants were then filtered according to their class (synonymous, missense, nonsense), but also based on their frequency on public databases such as the 1000 Genomes (www.1000genomes.org) and the exome variant server (http://evs.gs.washington.edu/EVS/).

2.4. Sanger sequencing

In order to validate the identified *MSX1* mutation, primers were designed using the Primer3 tool; forward primer (5′ CGCCAAGGCAAAGAGACTAC 3′) and reverse primer (5′ CTCTTCCAGCCACTTTTTGG 3′). A standard PCR was performed and products were sequenced by Source BioScience www.sourcebioscience.com.

3. Results

Exome sequencing data generated a list of variants (24,000) for each patient that was then filtered on the basis of novelty of the shared variants, down to 13 to include only truncating mutations. Out of the remaining variants a novel frameshift insertion of 7 base pairs in *MSX1* (*MSX1* NM_002448) gene in the heterozygous state in both sisters was identified. This mutation was found in exon 2 in the highly conserved homeodomain (c.572_573ins GCAAGTT: p. F191 fs) at position 191 at the end of helix I, changing the amino acid sequence and creating a stop codon at the beginning of helix III (Fig. 2). Sanger sequencing confirmed the presence of the mutation in the *MSX1* gene in both sisters (Fig. 3). The percentage of exome capture for the proband at 5×, 10× and 20× were 96.06, 93.74 and 87.81 respectively, for sibling 2 they were 96.34, 94.11 and 88.07.

4. Discussion

In this study a novel *MSX1* frameshift insertion was identified following whole exome sequencing of DNA samples obtained from two sisters with severe hypodontia including the first and second premolars and first molars. This frameshift insertion of 7 basepairs (GCAAGTT) is located in exon 2 at the end of helix I of the homeodomain (phenylanine). This domain is demonstrated to play an essential role in DNA binding.

MSX1 plays an important role during odontogenesis and it is expressed in the mesenchyme at sites where epithelial-mesenchymal interactions occur during the bud and cap stage, (Thesleff, 2006). The *MSX1* homeodomain is a DNA binding domain that is composed of 3 helical amino acid chains (60 amino acids), which are highly conserved among mammals, and an N-terminal arm consisting of 9 amino acids with 2 loops (one between helix 1 and 2 and the other between helix 2 and 3). In this study, a novel frameshift insertion of 7 bp (GCAAGTT) in the homedomain of *MSX1* gene (NM_002448) was identified, specifically in exon 2 at

c.572-573 affecting the amino acid sequence of the homeodomain at phenylanine (an amino acid that residue of the homeodomain) at the position 191 at the end of helix 1 changing the amino acid sequence after this position and creating a stop codon at the beginning of helix 3 (recognition helix) that is predicted to severely impair DNA binding (Fig. 3). We speculate this novel mutation in the homeodomain of *MSX1* has a significant impact on the structure of the resulting protein, leading to severe consequences in protein interactions and DNA binding of this gene. Therefore it is likely that this mutation resulted in selective tooth agenesis. Further functional analysis of this mutation should assist in the revelation of the molecular mechanism of action.

Functional analysis of the first discovered *MSX1* mutations revealed that the mutated protein has unstable structure and reduced thermostability, and illustrated reduced or no ability to bind with DNA or interact with other proteins (Hu et al., 1998; Vastardis et al., 1996). Furthermore, there is some evidence that most of the mutant protein can still potentiate *PAX9*-induced *Bmp4* and *MSX1* promoter activation, proving the ability of *MSX1* to cause tooth agenesis independent of any synergism with *PAX9* (Wang, Kong, Mues, & D'souza, 2011). To our knowledge, 15 *MSX1* mutations had been identified in association with non-syndromic tooth agenesis. Several are in exon 2 (AlFawaz, Plagnol, Wong, & Kelsell, 2015 ;Chishti, Muhammad, Haider, & Ahmad, 2006; De Muynck, Schollen, Matthijs, Verdonck, & Devriendt, 2004; Kamamoto et al., 2011; Mostowska, Biedziak, & Jagodzinski, 2012; Mostowska, Biedziak, & Trzeciak, 2006; Vastardis et al., 1996; Wong, Liu, & Han, 2014), others are in exon 1(Kim, Simmer, Lin, & Hu, 2006; Lidral & Reising, 2000; Van den Boogaard, Dorland, Beemer, & van Amstel, 2002; Kimura et al., 2014) and two are intronic mutations (Pawlowska, Janik-Papis, Wisniewska-Jarosinska, Szczepanska, & Blasiak, 2009; Tatematsu, Kimura, Nakashima, & Machida, 2015). Most of those mutations in exon 2 are in the homeodomain indicative of the important role of this domain for MSX1 function. Interestingly, this is the first frameshift insertion mutation described in the homeodomain, since all others are substitutions (missense mutations), except (AlFawaz et al., 2015) which was a frameshift insertion in exon 2 but not in the homeodomain (Table 2). The pattern of tooth agenesis in both sisters

in this study is upper and lower second premolars and third molars agenesis which is a phenotype consistent with previous phenotypes associated with *MSX1* mutation. However, it is worth noting that upper and lower 1st molars were missing too, which is not normally affected in many of the *MSX1* mutations described.

5. Conclusion

In conclusion, the current study indicates that this novel heterozygous frameshift insertion mutation in the homeodomain of *MSX1* gene is responsible for tooth agenesis in this family. This is the first frameshift insertion that has been recorded in this domain.

Conflict of interest

The authors declare no conflicts of interests.

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