Twin Research and Human Genetics
Volume 16 ▌ Number 6 ▌ pp. 1112–1116 ▌ © The Authors 2013 ▌ doi:10.1017/thg.2013.69

MSX1 and *PAX9* Investigation in Monozygotic Twins With Variable Expression of Tooth Agenesis

Sofia I. N. Lopez,[1] Karina S. Mundstock,[1] Vanessa R. Paixão-Côrtes,[2] Lavínia Schüler-Faccini,[2] Carlos A. Mundstock,[1] Maria Cátira Bortolini,[2] and Francisco M. Salzano[2]

[1]Department of Orthodontics, School of Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

[2]Genetics Department, Biosciences Institute, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Non-syndromic agenesis of permanent teeth is one of the most common anomalies in human development, a multifactorial characteristic caused by genetic and environmental factors. We describe a pair of monozygotic twins who showed second premolar and third molar agenesis, albeit with different expressions. We studied the DNA of two genes, paired domain box gene 9 (*PAX9*) and muscle segment homeodomain-homeobox1 (*MSX1*), encoding transcription factors that earlier studies found were involved in the manifestation of this condition. No specific causative mutation was found. However, we detected a C→T change in *MSX1* exon 2 in both twins, suggesting that this polymorphism might be involved in the trait's expression.

■ **Keywords:** dental agenesis, *MSX1* and *PAX9*, monozygotic twins, gene expression

Non-syndromic permanent dentition agenesis is clearly a multifactorial trait, determined by both genetic and environmental factors (Brook, 2009). The paired domain box gene 9 (*PAX9*) and the muscle segment homeodomain-homeobox1 (*MSX1*) have 16 and 11 identified mutations that lead to non-syndromic tooth loss in humans (see http://www.ncbi.nlm.nih.gov/omim — OMIM#167416; OMIM#142893). For several years, we have conducted research on the correlations between genotype and dental agenesis phenotypes involving these genes (Pereira et al., 2006; Paixão-Côrtes et al., 2011a, 2011b). Relevant information about them is provided in Table 1. During our ongoing research we came across a pair of monozygotic (MZ) twins who showed a different pattern of second premolar and third molar agenesis. *PAX9* and *MSX1* analyses of the twins and their father revealed interesting results.

Subjects and Methods

The 12-year-old male twins (patients 1 and 2) discussed in this article are of predominantly European descent, although phenotypic evaluation suggests some level of African ancestry. A detailed clinical and radiographic study of the twins and their father was conducted at the Orthodontic Clinic of the Federal University of Rio Grande do Sul (the mother was not available for study). The three had missing, non-erupted teeth. No other anomaly was found, thereby excluding other syndromes and associated pathologies. We extracted and studied their DNA as described below.

DNA was extracted from saliva using the QIAamp DNAminikit (Qiagen) according to the manufacturer's recommendations.

Amplification of the *PAX9* exons 2, 3, 4 and the *MSX1* exon 2 (those previously verified to be more prone to variation) was performed in a total volume of 25 μL per reaction, 50 pmol of each primer (Table 2), PCR Master Mix following manufacturer's specifications, and 100 ng of genomic DNA. The components of the reaction were mixed and the sample was placed in a thermal cycler. Polymerase chain reaction (PCR) was performed under the following conditions: 35 cycles of DNA denaturation at 94°C, annealing of primers at 58–59°C, and DNA chain extension (polymerization) at 72°C.

RECEIVED 18 April 2013; ACCEPTED 4 September 2013. First published online 9 October 2013.

ADDRESS FOR CORRESPONDENCE: Dr. Maria Cátira Bortolini, Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, CEP 91501-970 Porto Alegre, Rio Grande do Sul, Brazil. E-mail: maria.bortolini@ufrgs.br

TABLE 1
Information About the Genetic Systems Tested in This Study

Symbol	Description	Chr	Start	End	Size (bp)	Exon	Domain	Known polymorphisms	OMIM mutations	Summary	Phenotype associations
MSX1	Msh homeobox 1; Msh homeobox 1-like protein; msh homeobox homolog 1; homeobox 7	4	4912293	4916564	4,271	2	Homeobox	182	9	It may have roles in limb-pattern formation, craniofacial development, particularly odontogenesis, and tumor growth inhibition.	Mutations in this gene, which was once known as homeobox 7, have been associated with non-syndromic cleft lip with or without cleft palate 5, Witkop syndrome, Wolf–Hirschorn syndrome, and autosomal dominant hypodontia.
PAX9	Paired domain gene 9; paired box gene 9; paired box 9	14	36196533	36216763	20,230	4	Paired	406	15	This gene is a transcription factor required for normal development of thymus, parathyroid glands, ultimobranchial bodies, teeth, skeletal elements of skull and larynx as well as distal limbs.	Mutations in this gene are known to cause hypodontia (all primary and permanent molars; twins with molar agenesia) and oligodontia.

Note: Chr = Chromosome; bp = base pair.
Sources of data: the Gene Ontology — AmiGO (http://www.geneontology.org/); Genecards (http://www.genecards.org/index.shtml); Ensemble (http://www.ensembl.org/); OMIM (http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim); and NextBio (http://www.nextbio.com/b/nextbio.nb).

To determine whether the twins were monozygotic or dizygotic (DZ), the AmpFℓ STRIdentifiler^{TM} PCR Amplification kit (Applied Biosystems) was used. The kit tested 15 short tandem repeats (STR) loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. The results were analyzed using the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems) via the GeneMapper Software (Applied Biosystems).

The present research was approved by the Federal University of Rio Grande do Sul Ethics Committee as well as by the Brazilian National Ethics Commission (CONEP protocol no. 9365).

Results and Discussion

Clinical and radiographic tests revealed that patients 1 and 2 had tooth agenesis, and excluded other causes for the missing teeth, such as dental caries, periodontal disease, or aggressive trauma. Patient 1 had a congenital absence of lower second premolars and upper and lower third molars (Figure 1). In contrast, patient 2 revealed agenesis of upper and lower second premolars, as well as that of upper and lower third molars (Figure 2). Their father also had unilateral agenesis of a lower permanent incisor (data not shown).

The twins' genotypes for the STR markers revealed an identical composition: D8S1179:13/14; D21S11:30/30; D7S820:8/12; CSF1PO:11/12; D3S11358:15/15; THO1:6/9; D13S317:8/12; D16S539:11/12; D2S1338:20/21; D19S433:13/14; vWA:17/17; TPOX:8/8; D18S51:14/17; D5S818:9/11; and FGA:19/25. These results provide a negligible probability value for dizygosis (see, e.g., Yang et al., 2006), thereby establishing their MZ nature.

No new mutation in any of the sequenced segments among the three family members was detected. However, in exon 2 of the *MSX1* gene we found an already described polymorphism in heterozygosis (rs1095, an exchange of C→T at position 4915839 of chromosome 4; see Supplementary Information in Table S1). Figure 3 presents the sequences of the twins and their father. The mutation was not found in the twins' father, and therefore must have been inherited from their mother. In an earlier research (Paixão-Côrtes et al., 2011a) we found this mutation in 20% of agenesis patients, while it did not appear among the controls. Third molar agenesis is a common trait found among affected individuals analyzed in the present and aforementioned studies.

In the databank of the 1000 Genomes Project, the rs1095 T allele was present among Asians and Latin Americans (15% and 7%, respectively), but not among Africans or Europeans (http://www.1000genomes.org/; 1000 Genomes Project Consortium et al., 2010). We could, therefore, infer a probable Native American origin for this allele among Brazilians and other Latin Americans. The twins' mother

TABLE 2

Primers Utilized in the Amplification Reaction (PCR)

Primers	Sequences	Expected size (bp)	Design
PAX9 Exon 3	Forward 5′-GTGGGTCAGAGAATTTGGAA-3′ Reverse 5′-CACGAAGGATCTGGCTCGT-3′	0 589	Pereira et al. (2006)
PAX9 Exon 2	Forward 5′-CCAGCCTTCGGGGAGGTGAA-3′ Reverse 5′-GACGCTGCACATCCACACG-3′	0 640	Paixão-Côrtes et al. (2011a)
PAX9 Exon4	Forward 5′-AGGCACCAAATGGTCTCCCAGCTGT-3′ Reverse 5′-GAAGCCGTGACAGAATGACTA-3′	0 247	Paixão-Côrtes et al. (2011a)
MSX1 Exon 2	Forward 5′-AAGTTCCGCCAGAAGCAGTA-3′ Reverse 5′-ACATCTGTGTTTTCCCTGCC-3′	0 698	Xuan et al. (2008)

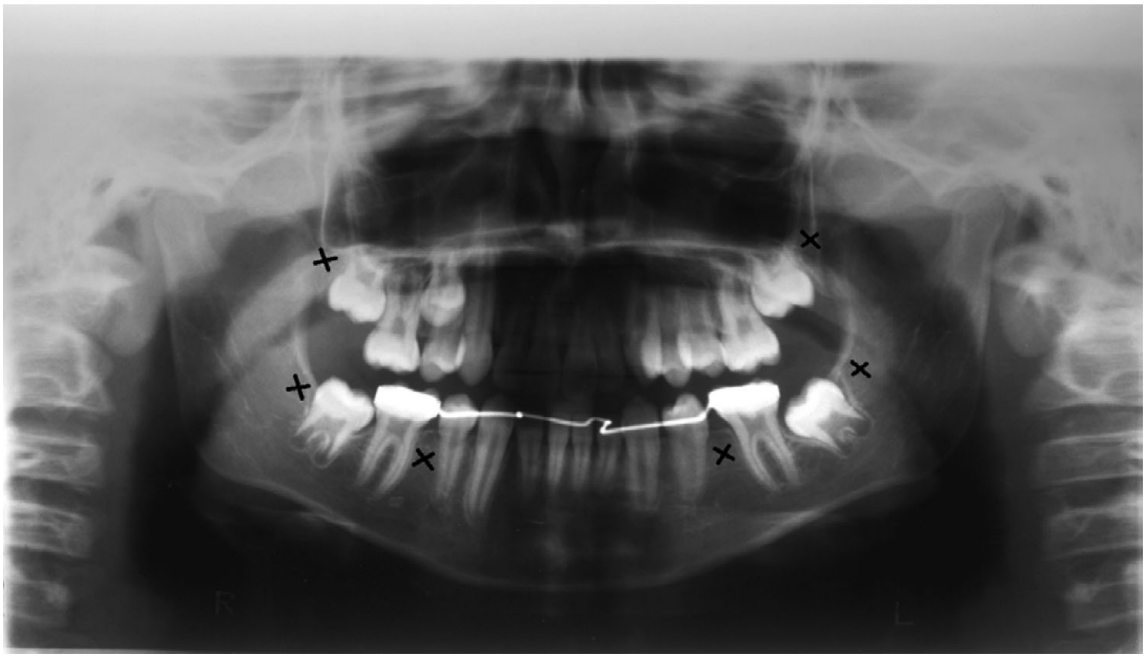

FIGURE 1

Panoramic radiograph of patient 1 teeth in 2011; those missing are indicated with an x.

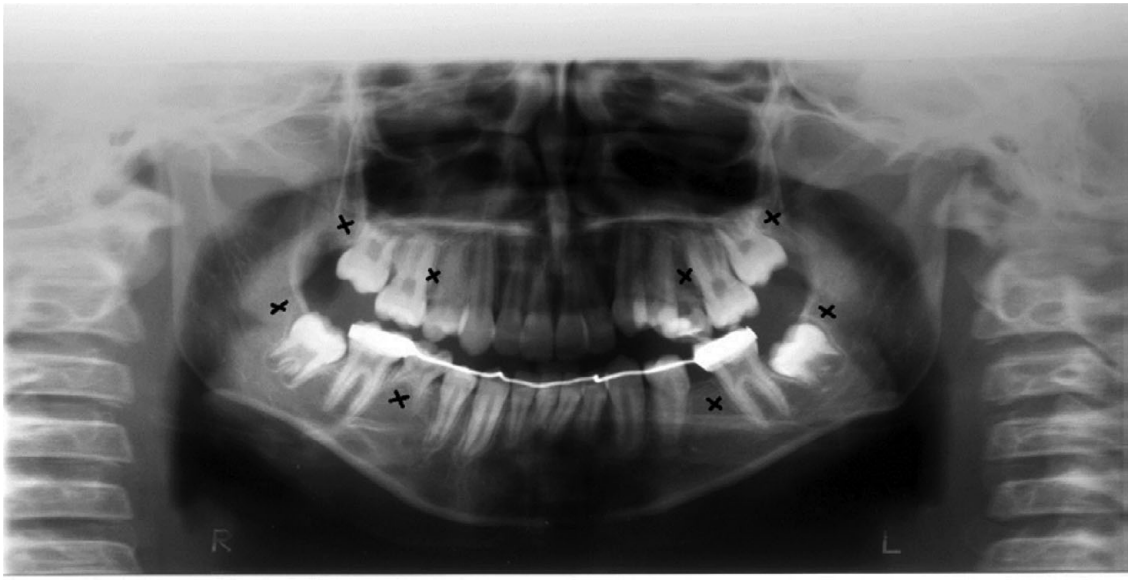

FIGURE 2

Panoramic radiograph of patient 2 teeth in 2011; those missing are indicated with an x.

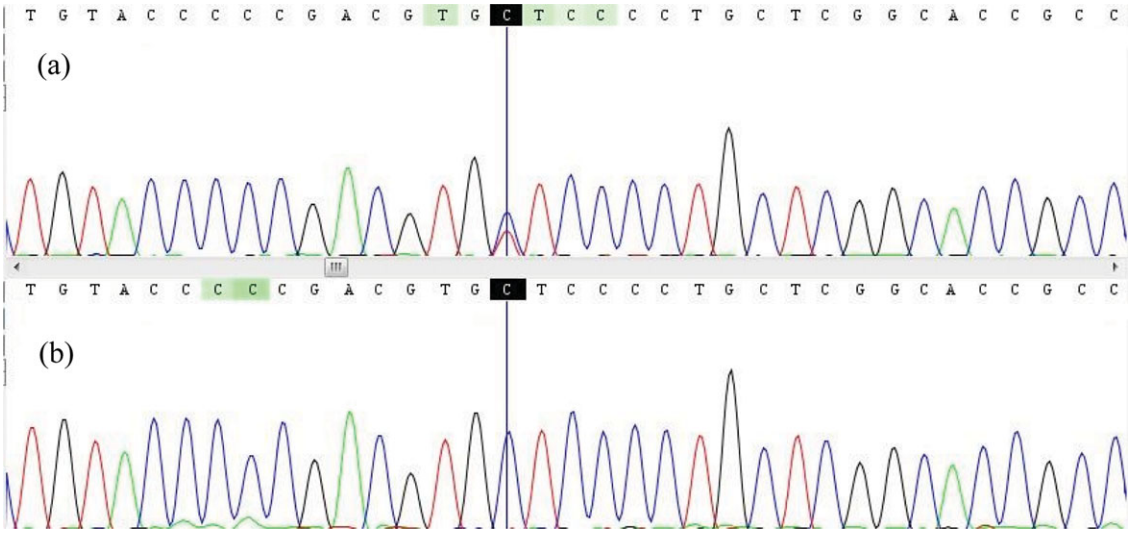

FIGURE 3
(Colour online) MSX1 exon 2 chromatograms of (a) the twins (they are identical); and (b) their father.

(who had no tooth agenesis) would have received this mutation independent of any agenesis susceptibility. However, due to the fact that the agenesis present in the twins was distinct from that of their father, and considering also its independent presence among other patients (Paixão-Côrtes et al., 2011a), it is not possible to discard the hypothesis that rs1095 T allele can contribute to agenesis tooth manifestation, particularly of third molars.

Paixão-Côrtes et al. (2011a) identified at least 44 genes that are possibly involved in the dental development network. This and other results suggest that tooth agenesis is influenced by complex population or individual-specific genetic backgrounds (Paixão-Côrtes et al., 2011b). Table S1 presents the SNPs of the *MSX1* and *PAX9* genes that have been identified and described up to the present, indicating the degree of variability found among human populations. Furthermore, even among MZ twins, differential expression is not unlikely if they were differently influenced by epigenetic factors. Townsend et al. (2005) found that 21 out of 24 pairs of MZ twins with tooth agenesis also revealed discordant agenesis expression, while Hughes et al. (2013) suggested that differences in methylation status at a whole-genome level are related to discordance in the number of teeth found in MZ twins. Indeed, distinct and often undetectable environmental agents may also play a role (Machin, 2009).

Acknowledgments

We thank the twins and their father for allowing us to conduct this study. Financial support was granted by Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Programa de Apoio a Núcleos de Excelência).

Supplementary Material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/thg.2013.69.

References

Brook, A. H. (2009). Multilevel complex interactions between genetic, epigenetic and environmental factors in the aetiology of anomalies of dental development. *Archives of Oral Biology*, *54*, S3–S17.

Hughes, T., Bockmann, M., Mihailidis, S., Bennett, C., Harris, A., Seow, W. K., . . . Townsend, G. C. (2013). Genetic, epigenetic, and environmental influences on dentofacial structures and oral health: Ongoing studies of Australian twins and their families. *Twin Research and Human Genetics*, *16*, 43–51.

Machin, G. (2009). Non-identical monozygotic twins, intermediate twin types, zygosity testing: A review. *American Journal of Medical Genetics*, *151C*, 110–127.

1000 Genomes Project Consortium, Durbin, R. M., Abecasis, G. R., Altshuler, D. L., Auton, A., Brooks, L. D., . . . McVean, G. A. (2010). A map of human genome variation from population-scale sequencing. *Nature*, *467*, 1061–1073.

Paixão-Côrtes, V. R., Braga, T., Salzano, F. M., Mundstock, K., Mundstock, C. A., & Bortolini, M. C. (2011a). PAX9 and MSX1 transcription factor genes in non-syndromic dental agenesis. *Archives of Oral Biology*, *56*, 337–344.

Paixão-Côrtes, V. R., Meyer, D., Pereira, T.V., Mazières, S., Elion, J., Krishnamoorty, R., . . . Bortolini, M. C. (2011b). Genetic variation among major geographic groups supports a peculiar evolutionary trend in PAX9. *PLoS One*, *6*, e15656.

Pereira, T. V., Salzano, F. M., Mostowska, A., Trzeciak, W. H., Ruiz-Linares, A., Chies, J. A. B., . . . Bortolini, M. C. (2006). Natural selection and molecular evolution in primate *PAX9* gene, a major determinant of tooth development.

Proceedings of the National Academy of Sciences (USA), *103*, 5676–5681.

Townsend, G. C., Richards, L., Hughes, T., Pinkerton, S., & Schwerdt, W. (2005). Epigenetic influences may explain dental differences in monozygotic twin pairs. *Australian Dental Journal*, *50*, 95–100.

Xuan, K., Jin, F., Liu, Y-L., Yuan, L-T., Wen, L-Y., Yang, F-S., ... Jin, Y. (2008). Identification of a novel missense mutation of *MSX1* gene in Chinese family with autosomal-dominant oligodontia. *Archives of Oral Biology*, *53*, 773–779.

Yang, M.-J., Tzeng, C.-H., Tseng, J.-Y., & Huang, C.-Y. (2006). Determination of twin zygosity using a commercially available STR analysis of 15 unlinked loci and the gender-determining marker amelogenin — A preliminary report. *Human Reproduction*, *21*, 2175–2179.