Relationship of the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene with non-syndromic mandibular prognathism in the Iranian population

Kazem Dalaie^{1,A,D,F}, Vahid Reza Yassaee^{2,A,C,E,F}, Mohammad Behnaz^{1,A,D}, Mohsen Yazdani^{3,A,D}, Farbod Jafari^{4,B,D}, Reza Morvaridi Farimani^{1,A,D}

¹ Dentofacial Deformities Research Center, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

² Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Research Center for Prevention of Oral and Dental Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran

⁴ Department of Oral and Maxillofacial Surgery, School of Dentistry, Islamic Azad University, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl. 2020;57(4):433–440

Address for correspondence

Reza Morvaridi Farimani

E-mail: Morvaridi.reza@gmail.com

Funding sources

School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of interest

None declared

Received on February 10, 2020

Reviewed on March 15, 2020

Accepted on May 4, 2020

Published online on December 31, 2020

Cite as

Dalaie K, Yassaee VR, Behnaz M, Yazdani M, Jafari F, Morvaridi Farimani R. Relationship of the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene with non-syndromic mandibular prognathism in the Iranian population. *Dent Med Probl.* 2020;57(4):433–440. doi:10.17219/dmp/122004

DOI

10.17219/dmp/122004

Copyright

© 2020 by Wrocław Medical University

This is an article distributed under the terms of the

Creative Commons Attribution 3.0 Unported License (CC BY 3.0)

(<https://creativecommons.org/licenses/by/3.0/>).

Abstract

Background. The myosin 1H (*MYO1H*) gene, located on chromosome 12, encodes the unconventional MYO1H protein, which is involved in the intracellular movement and morphology of chondrocytes, and plays a vital role in the prognathism or retrognathism of the mandible.

Objectives. The objective of this study was to assess the relationship between the polymorphisms of the *MYO1H* gene and mandibular prognathism in the Iranian population.

Material and methods. The current project evaluated 64 patients with mandibular prognathism requiring orthognathic surgery and 60 controls with skeletal class I occlusion. Genome amplification was performed using specific primer pairs to assess the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene through the polymerase chain reaction (PCR). The restriction fragment length polymorphism (RFLP) technique was used to detect single-nucleotide polymorphisms. The data was analyzed using the χ^2 test.

Results. The patient and control groups were not significantly different in terms of age or gender ($p > 0.05$). In all, 3.1% of patients and 6.7% of controls had the rs10850110 polymorphism ($p = 0.680$), and 1.6% of patients and 5% of controls had the rs11611277 polymorphism ($p = 0.602$).

Conclusions. No significant correlation was noted between the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene and mandibular prognathism in the Iranian population. However, the lower frequency of these polymorphisms in the patient group suggests a possible association with mandibular retrognathism, which needs to be investigated with a larger sample size.

Key words: polymorphism, class III malocclusion, *MYO1H* gene, single-nucleotide polymorphism, prognathism

Introduction

Several environmental and genetic factors are implicated in the development of malocclusion. Despite a high prevalence of craniofacial disorders,¹ the role of genetic factors in their occurrence is not well understood.

Skeletal class III malocclusion can be caused by mandibular prognathism, maxillary deficiency or the combination of both, and is among the most severe skeletal deformities in orthodontics.² Class III malocclusion can cause both functional and social disabilities.^{3–5} It is believed to be a polygenetic disorder, which occurs as the result of the interaction of genetic and environmental factors.⁶ Genes play a prominent role in mandibular prognathism.⁷ Evidence shows that the prevalence of mandibular prognathism is the highest in East Asia (15–23%), moderate in Africa (3–8%) and the lowest in the European ethnicity (0.48–4%).^{8,9} The prominent role of genes in the class III phenotype has been documented, and human studies have demonstrated an autosomal dominant genetic pattern in class III malocclusion.¹⁰ The myosin 1H (*MYO1H*) gene encodes a protein that plays a role in the cellular movement, phagocytosis and the vesicular transfer. This gene is composed of 107,121 base pairs and is located on human chromosome 12. It is also involved in the mandibular growth by producing cartilage at the condylar head and determining the morphology of chondrocytes.^{11,12} Moreover, the expression of this gene in the masseter muscle of patients with class II malocclusion is higher than its expression in the masseter muscle of class III patients.¹³

Tassopoulou-Fishell et al. were the first to report an association between mandibular prognathism and *MYO1H* (rs10850110) in a Hispanic group.¹⁴ This association was also confirmed by Cruz et al. and Burnheimer et al. in Brazilian and African American groups.^{15,16}

However, recent studies by Cunha et al. and Arun et al. could not find any significant association between rs10850110 and mandibular prognathism and retrognathism in Brazilian and Hispanic populations.^{17,18}

Since the original linkage result, which pointed to the *MYO1H* locus as associated with skeletal malocclusion, referred to a group of Hispanic families,¹⁴ the aim of this project was to evaluate this result in the Iranian population.

Material and methods

Subjects of both sexes between 16 and 30 years of age were included in this study. This case–control study evaluated skeletal class III malocclusion patients (41 females and 23 males) who were candidates for orthognathic surgery, and were selected from among those presenting to the university hospitals affiliated to the Shahid Beheshti University of Medical Sciences in Tehran, Iran,

and 5 orthodontic clinics. The control subjects (38 females and 22 males) were skeletal class I occlusion patients who were apparently healthy and had a socioeconomic status comparable to that of the patient group. Patients were recruited for this study from January 2015 to January 2017. This study was approved by the ethics committee of the Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1396.49).

The inclusion criteria for the patient group were as follows: patients >16 years old whose growth and development had been terminated; and skeletal class III malocclusion patients with mandibular prognathism (SNB > 82°).

The exclusion criteria for the patient group were as follows: patients with other types of malocclusion, syndromic and metabolic conditions or endocrine disorders; patients with skeletal class III malocclusion due to maxillary retrognathism; and patients with dental class III malocclusion.

The inclusion criteria for the control group were the following: patients aged >16 years with class I occlusion or malocclusion and the orthognathic profile; patients without syndromic, congenital, systemic, or endocrine anomalies; ANB of 2–4°; and the Wits appraisal of 0–2 mm.

Subjects were selected using convenience sampling. The minimum sample size was calculated to be 60, assuming $\alpha = 0.05$ and a study power of 80%.

The demographic information about the patients and the controls was collected using a questionnaire. The patients and the controls were briefed about the study and written informed consent was obtained from them. Next, 5 mL of peripheral venous blood was obtained from the subjects and collected in tubes containing ethylenediaminetetraacetic acid (EDTA).

Extraction of genomic DNA

DNA was extracted from the peripheral blood samples using the salting-out technique.

Quantitative and qualitative assessment of the extracted DNA

After the extraction of DNA, its quality and quantity were evaluated using BioPhotometer® (Eppendorf AG, Hamburg, Germany). Table 1 presents the names and sequences of the specific primers used. Using primer pairs, the *MYO1H* gene was amplified with the polymerase chain reaction (PCR) program.

Assessment of the quality of the PCR products on 1.5% agarose gel

The PCR products along with the markers were electrophoresed to assess their quality, size and quantity. If the PCR products had favorable quality and the respective segment had been amplified, they were

Table 1. Sequences of the primers used for the amplification of the rs10850110 and rs11611277 polymorphisms

Primer name	Sequence	Length	Annealing	Extension	Product size [bp]
Sau96I	TCTTAACAGTGTCTTCTAATGAG	23	58°C	72°C	380
	GATTGTCTAAAGCCAGGAGTTG	22	30 s	40 s	
Hpy188I	TCCCAGGGTTTAGCATCTTG	20	60°C	72°C	386
	GAGTGCGCCTCAGTATCTC	20	30 s	40 s	

subjected to sequencing. The PCR restriction fragment length polymorphism (RFLP) technique was then employed to detect the genotypes.

Statistical analysis

Eventually, a cephalometric analysis was performed to determine SNA, SNB, ANB, and the Wits appraisal in the patient and control groups.

The data was analyzed using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA) with the *t* test and the χ^2 test. The level of significance was set at $p < 0.05$.

Results

A total of 64 patients and 60 controls were evaluated. Table 2 presents the cephalometric findings as well as the sex and mean age of the subjects. According to the *t* test, the 2 groups were significantly different in terms of SNA, SNB, ANB, and Wits appraisal ($p < 0.001$). Table 3 presents

the frequency of different genotypes with respect to the 2 polymorphisms in the 2 groups. In all, 3.1% of patients and 6.7% of controls had the rs10850110 polymorphism ($p = 0.680$), and 1.6% of patients and 5% of controls had the rs11611277 polymorphism ($p = 0.602$). The χ^2 test revealed no significant differences in the frequency of the polymorphisms between the 2 groups. In other words, the 2 polymorphisms showed no significant correlation with mandibular prognathism ($p > 0.05$).

Figures 1 and Figures 2 show the PCR products of *MYO1H* on 1.5% agarose gel for the detection of the rs10850110 and rs11611277 polymorphisms, respectively. As shown, the PCR products had acceptable quality and quantity, and were selected for sequencing. The rs10850110 polymorphism was not detected in the majority of the subjects except for 2 patients and 4 controls. Figure 3 shows the PCR products of *MYO1H* on 1.5% agarose gel for the detection of the rs11611277 polymorphism in the control group. Figures 4–8 show the chromatography results of the sequencing of the *MYO1H* gene containing the rs10850110 and rs11611277 polymorphisms.

Table 2. Cephalometric findings, and the sex and mean age of the subjects

Data	Group	<i>n</i>	<i>M</i> ± <i>SD</i>	<i>p</i> -value
Wits appraisal [mm]	case	64	−6.70 ± 4.32468	<0.001*
	control	60	0.76 ± 0.76495	
ANB [°]	case	64	−3.20 ± 2.28129	<0.001*
	control	60	1.03 ± 0.61753	
SNA [°]	case	64	83.40 ± 4.37822	<0.001*
	control	60	80.90 ± 0.81184	
SNB [°]	case	64	86.60 ± 3.82949	<0.001*
	control	60	80.01 ± 0.60013	
Age [years]	case	64	24.64 ± 5.86	–
	control	60	22.41 ± 7.40	
Sex	case	41F + 23M	–	–
	control	38F + 22M		

M – mean; *SD* – standard deviation; F – female; M – male; * statistically significant.

Table 3. Frequency of different genotypes with respect to the 2 polymorphisms in the 2 groups

Polymorphism	Genotype	Case group <i>n</i> (%)	Control group <i>n</i> (%)	<i>p</i> -value
rs10850110	GG	40 (62.5)	37 (61.7)	0.680
	GA	22 (34.4)	19 (31.7)	
	AA	2 (3.1)	4 (6.7)	
rs11611277	CC	40 (62.5)	37 (61.7)	0.602
	CA	23 (35.9)	20 (33.3)	
	AA	1 (1.6)	3 (5.0)

Fig. 1. Polymerase chain reaction (PCR) products of the *MYO1H* gene on 1.5% agarose gel for the detection of the rs10850110 polymorphism in the patient group TAE – tris-acetate-EDTA.

Fig. 2. PCR products of the *MYO1H* gene on 1.5% agarose gel for the detection of the rs11611277 polymorphism in the patient group

Fig. 3. PCR products of the *MYO1H* gene on 1.5% agarose gel for the detection of the rs11611277 polymorphism in the control group

Fig. 4. DNA sequencing chromatogram of the *MYO1H* gene containing the sites of the rs10850110 and rs11611277 polymorphisms

Fig. 5. DNA sequencing chromatogram of the heterozygote rs10850110 polymorphism

Fig. 6. DNA sequencing chromatogram of the normal heterozygote rs10850110 polymorphism

Fig. 7. DNA sequencing chromatogram of the heterozygote rs11611277 polymorphism

Fig. 8. DNA sequencing chromatogram of the normal heterozygote rs11611277 polymorphism

Discussion

The role of genetic factors in mandibular prognathism has been documented and the single-nucleotide polymorphisms of different genes may play a part in this respect. The *GHR* and *MYO1H* genes are 2 examples of such genes. This study aimed to assess the relationship between the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene and mandibular prognathism in the Iranian population.

Sun et al. used the whole-mount in situ hybridization (WISH) technique to analyze the pattern of expression of *MYO1H*.¹⁹ They concluded that *MYO1H* plays an important role in the mandibular growth, which was expected, considering the involvement of this gene in the proliferation and morphology of the mandibular condyle chondrocytes. They also found an association between the rs3825393 polymorphism and mandibular prognathism.¹⁹ Cruz et al. used the TaqMan method of the real-time PCR to amplify the genome, and demonstrated that the *MYO1H*, *GHR* and *FGF* genes play a role in

mandibular prognathism and maxillomandibular discrepancies.¹⁵ Tassopoulou-Fishell et al. used PCR along with TaqMan chemistry to amplify the genome and trace polymorphisms.¹⁴ They found no significant correlation between the rs2503243, rs972054, rs1413533, rs1490055, rs2101560, rs1601948, rs1387168, rs2940913, rs7718944, rs3016534, and rs9458378 polymorphisms and mandibular prognathism, but confirmed a correlation between the rs10850110 polymorphism of the *MYO1H* gene and mandibular prognathism in their study population.¹⁴ Arun et al. evaluated the rs10850110, rs11611277 and rs3825393 polymorphisms of the *MYO1H* gene and their correlation with mandibular retrognathism.¹⁸ The researchers extracted DNA by means of the modified salting-out method. They used the same enzymes as we did, and no cephalometric variable or growth measure showed a statistical difference between the genotypes for the rs10850110 and rs11611277 single-nucleotide polymorphisms in both studies. They confirmed a correlation between the rs3825393 polymorphism of the *MYO1H* gene and mandibular retrognathism.¹⁸ Cunha et al. studied

the relationship between *ACTN3* and *MYO1H* (rs10850110) and facial patterns on 4 different Brazilian samples with the TaqMan method of the real-time PCR.¹⁷ There was no significant correlation between *MYO1H* and mandibular prognathism in any sample, but in 1 of the 4 samples, they confirmed an association between *MYO1H* (rs10850110) and class II skeletal pattern.¹⁷ Ghergie et al. confirmed a correlation between the rs10850110 polymorphism of the *MYO1H* gene and mandibular prognathism.²⁰ Considering all the above, a significant correlation exists between the rs10850110 polymorphism and mandibular prognathism in the Romanian²⁰ and American⁹ populations, while no such correlation was noted in the Brazilian sample¹⁷ and our Iranian population. The controversy regarding the results of Arun et al.¹⁸ and Sun et al.¹⁹ is also understandable, considering their different study populations and sample sizes. Arun et al. found a strong correlation between the rs3825393 polymorphism and mandibular retrognathism,¹⁸ while Sun et al. found a strong correlation between the rs3825393 polymorphism and mandibular prognathism in a larger population.¹⁹ Future studies with larger sample sizes on both class II and class III patients from different populations are required to better elucidate the relationship between the rs3825393 polymorphism of the *MYO1H* gene and the position of the mandible. It should be noted that the genetic correlations found in a certain population cannot be generalized to other populations due to inherently different genetic backgrounds.²¹

In total, it should be emphasized that the *MYO1H* gene plays a fundamental role in the growth of the mandibular condyle cartilage, and there is evidence regarding the effect of the muscular system on the skeletal growth of the maxillofacial region. The rate of expression of this gene in the masseter muscle also affects the mandibular growth and development.^{22,23} Evidence shows a higher expression of this gene in the masseter muscle of distal occlusion subjects as compared to its expression in mesial occlusion individuals. In other words, the upregulation of this gene is more often noted in the masseter muscle of class II patients in comparison with class III individuals.^{24,25} It is assumed that *MYO1H* may take part in the intracellular transport of glucose with *MYO1C* to influence the masseter metabolism and fiber types. It, therefore, plays a role in the development of class I, II and III malocclusion.²⁶ Although the prognathic and retrognathic facial phenotypes may be similar vertically, the masseter muscle gene expression levels of the myosin heavy chain genes *MYH1*, *MYH2*, *MYH3*, *MYH6*, *MYH7*, and *MYH8* demonstrated that the prognathic and retrognathic facial phenotypes had different gene expression profiles.²⁷

Our study had some limitations. For instance, we evaluated only 1 city and 1 ethnic group of patients, and therefore the effect of ethnicity could not be evaluated. Also, we assessed the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene, while other polymorphisms

related to this gene were not evaluated. Future multicenter studies with larger sample sizes are required on different races and ethnic groups. Other single-nucleotide polymorphisms of this gene should also be assessed in future studies.

Conclusions

No significant correlation was noted between the rs10850110 and rs11611277 polymorphisms of the *MYO1H* gene and mandibular prognathism in the Iranian population. However, the lower frequency of the polymorphisms in the patient group suggests a possible association with mandibular retrognathism, which needs to be investigated with a larger sample size.

ORCID iDs

Kazem Dalaie <https://orcid.org/0000-0002-5430-3301>

Vahid Reza Yassaee <https://orcid.org/0000-0002-1879-3905>

Mohammad Behnaz <https://orcid.org/0000-0002-2793-3292>

Mohsen Yazdani <https://orcid.org/0000-0002-9109-8968>

Farbod Jafari <https://orcid.org/0000-0003-3899-4891>

Reza Morvaridi Farimani <https://orcid.org/0000-0002-6697-6837>

References

- Harris EF, Johnson MG. Heritability of craniometric and occlusal variables: A longitudinal sib analysis. *Am J Orthod Dentofacial Orthop.* 1991;99(3):258–268. doi:10.1016/0889-5406(91)70007-J
- Bayram S, Basciftci FA, Kurar E. Relationship between P561T and C422F polymorphisms in growth hormone receptor gene and mandibular prognathism. *Angle Orthod.* 2014;84(5):803–809. doi:10.2319/091713-680.1
- Bui C, King T, Proffit W, Frazier-Bowers S. Phenotypic characterization of Class III patients. *Angle Orthod.* 2006;76(4):564–569. doi:10.1043/0003-3219(2006)076[0564:PCOCIP]2.0.CO;2
- Frazier-Bowers S, Rincon-Rodriguez R, Zhou J, Alexander K, Lange E. Evidence of linkage in a Hispanic cohort with a Class III dentofacial phenotype. *J Dent Res.* 2009;88(1):56–60. doi:10.1177/0022034508327817
- Kataoka K, Ekuni D, Mizutani S, et al. Association between self-reported bruxism and malocclusion in university students: A cross-sectional study. *J Epidemiol.* 2015;25(6):423–430. doi:10.2188/jea.JE20140180
- Ko JM, Suh YJ, Hong J, Paeng JY, Baek SH, Kim YH. Segregation analysis of mandibular prognathism in Korean orthognathic surgery patients and their families. *Angle Orthod.* 2013;83(6):1027–1035. doi:10.2319/021313-130.1
- Xue F, Wong RWK, Rabie ABM. Genes, genetics, and Class III malocclusion. *Orthod Craniofac Res.* 2010;13(2):69–74. doi:10.1111/j.1601-6343.2010.01485.x
- Wolff G, Wienker TF, Sander H. On the genetics of mandibular prognathism: Analysis of large European noble families. *J Med Genet.* 1993;30(2):112–116. doi:10.1136/jmg.30.2.112
- Cruz RM, Krieger H, Ferreira R, Mah J, Hartsfield J Jr., Oliveira S. Major gene and multifactorial inheritance of mandibular prognathism. *Am J Med Genet A.* 2008;146A(1):71–77. doi:10.1002/ajmg.a.32062
- El-Gheriani AA, Maher BS, El-Gheriani AS, et al. Segregation analysis of mandibular prognathism in Libya. *J Dent Res.* 2003;82(7):523–527. doi:10.1177/154405910308200707
- Xue F, Rabie ABM, Luo G. Analysis of the association of *COL2A1* and *IGF-1* with mandibular prognathism in a Chinese population. *Orthod Craniofac Res.* 2014;17(3):144–149. doi:10.1111/ocr.12038
- Yamaguchi T, Park SB, Narita A, Maki K, Inoue I. Genome-wide linkage analysis of mandibular prognathism in Korean and Japanese patients. *J Dent Res.* 2005;84(3):255–259. doi:10.1177/154405910508400309

13. Desh H, Gray SL, Horton MJ, et al. Molecular motor *MYO1C*, acetyltransferase *KAT6B* and osteogenetic transcription factor *RUNX2* expression in human masseter muscle contributes to development of malocclusion. *Arch Oral Biol.* 2014;59(6):601–607. doi:10.1016/j.archoralbio.2014.03.005
14. Tassopoulou-Fishell M, Deeley K, Harvey EM, Sciote J, Vieira AR. Genetic variation in myosin 1H contributes to mandibular prognathism. *Am J Orthod Dentofacial Orthop.* 2012;141(1):51–59. doi:10.1016/j.ajodo.2011.06.033
15. Cruz CV, Mattos CT, Maia JC, et al. Genetic polymorphisms underlying the skeletal Class III phenotype. *Am J Orthod Dentofacial Orthop.* 2017;151(4):700–707. doi:10.1016/j.ajodo.2016.09.013
16. Burnheimer J, Deeley K, Vieira AR. Myosin 1H and the soft tissue profile of African American females with mandibular prognathism. *Revista Científica do CRO-RJ (Rio de Janeiro Dent J).* 2019;4(2):35–41. doi:10.29327/24816.4.2-6
17. Cunha A, Nelson-Filho P, Marañón-Vásquez GA, et al. Genetic variants in *ACTN3* and *MYO1H* are associated with sagittal and vertical craniofacial skeletal patterns. *Arch Oral Biol.* 2019;97:85–90. doi:10.1016/j.archoralbio.2018.09.018
18. Arun RM, Lakkakula BVKS, Chitharanjan AB. Role of myosin 1H gene polymorphisms in mandibular retrognathism. *Am J Orthod Dentofacial Orthop.* 2016;149(5):699–704. doi:10.1016/j.ajodo.2015.10.028
19. Sun R, Wang Y, Jin M, Chen L, Cao Y, Chen F. Identification and functional studies of *MYO1H* for mandibular prognathism. *J Dent Res.* 2018;97(13):1501–1509. doi:10.1177/0022034518784936
20. Ghergie M, Feștilă D, Kelemen B, Lupan I. *Myo1H* gene single nucleotide polymorphism rs10850110 and the risk of malocclusion in the Romanian population. *Acta Medica Transilvanica.* 2013;2(4):281–281.
21. Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. *Nat Rev Genet.* 2009;10(4):241–251. doi:10.1038/nrg2554
22. Mew JR. Factors influencing mandibular growth. *Angle Orthod.* 1986;56(1):31–48. doi:10.1043/0003-3219(1986)056<0031:FIMG>2.0.CO;2
23. Ueda HM, Ishizuka Y, Miyamoto K, Morimoto N, Tanne K. Relationship between masticatory muscle activity and vertical craniofacial morphology. *Angle Orthod.* 1998;68(3):233–238. doi:10.1043/0003-3219(1998)068<0233:RBMMMA>2.3.CO;2
24. Gedrange T, Büttner C, Schneider M, Oppitz R, Harzer W. Myosin heavy chain protein and gene expression in the masseter muscle of adult patients with distal or mesial malocclusion. *J Appl Genet.* 2005;46(2):227–236.
25. Gedrange T, Büttner C, Schneider M, et al. Change of mRNA amount of myosin heavy chain in masseter muscle after orthognathic surgery of patients with malocclusion. *J Craniomaxillofac Surg.* 2006;34(Suppl 2):110–115. doi:10.1016/S1010-5182(06)60023-1
26. Stedman HH, Kozyak BW, Nelson A, et al. Myosin gene mutation correlates with anatomical changes in the human lineage. *Nature.* 2004;428(6981):415–418. doi:10.1038/nature02358
27. Moawad HA, Sinanan ACM, Lewis MP, Hunt NP. Grouping patients for masseter muscle genotype-phenotype studies. *Angle Orthod.* 2012;82(2):261–266. doi:10.2319/122810-750.1