


Class III Malocclusion: Missense Mutations in DUSP6 Gene

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Abstract

Objective: To determine the DUSP6 gene mutation in three generations of Malaysian Malay subjects having Class III malocclusion. **Material and Methods:** Genetic analyses of DUSP6 gene were carried out in 30 subjects by selecting three individuals representing three generations, respectively, from ten Malaysian Malay families having Class III malocclusion and 30 healthy controls. They were submitted Clinical Evaluation to clinical examination, lateral cephalometric radiographs, dental casts, and/ or facial and intra-oral photographs. Buccal cell was taken from each participant of Class III malocclusion and control groups. DNA extractions from buccal cell were carried out using Gentra puregene buccal cell kit. Bio Edit Sequence Alignment Editor software was used to see the sequencing result. **Results:** A heterozygous missense mutation c.1094C>T (p. Thr 365 Ile) was identified in DUSP6 gene in three members of one family with Class III malocclusion, whereas no mutation was found in the control group. **Conclusion:** Current study successfully identified a missense mutation in DUSP6 gene among one Malaysian Malay family affected by Class III malocclusion. The outcome of this study broadened the mutation spectrum of Class III malocclusion and the importance of DUSP6 gene in skeletal functions.

Keywords: Genetic Variation; Mutation, Missense; Malocclusion, Angle Class III.

Introduction

Class III malocclusion is generally regarded as mandibular prognathism with reverse overbite, overjet and concave profile. It is a dominant inherited slowly progressive dento-skeletal disharmony. However, it is a complicated skeletal phenotype may ground either due to retrusion of maxilla, protrusion of mandible or simultaneous occurrence of both. The prevalence of Class III malocclusion has been described between 1% [1,2] to over 10% [3], depending on ethnic backgrounds [2], gender [3, 4] and age [5].

It has been reported that approximately 75% of Class III malocclusion cases in male Caucasians have a skeletal origin and were a result of mandibular prognathism or macrognathia [6]. The prevalence of Class III malocclusion among Caucasian people ranges from 0.48% to 4% [2]. However, compared to Caucasian people the prevalence of Class III malocclusion is higher in Japanese population reaching up to 10% [7]. In Malaysia, among the three races (Malay, Chinese and Indian) Malaysian Malay and Malaysian Chinese showed the higher prevalence of Class III malocclusion than the Malaysian Indian [8-12].

The etiology of skeletal Class III malocclusion is an interesting topic and there is still much to understand. Environmental and genetic factors play an important role in occurrence of Class III malocclusion. Endocrine imbalances, enlarged tonsils, congenital anatomic defects, nasal breathing, pituitary gland disease, habitual protrusion of mandible, and early loss of deciduous incisors are the most common environmental factors associated with Class III malocclusion or mandibular prognathism [13-18].

Several human and animal studies have been carried out to validate the influence of heredity in the development of Class III malocclusion. Animal study on mouse also established that extent of mandible is related with the chromosome number 10 and 11 [19]. Since long it has been known that, the Class III malocclusion follows the autosomal-dominant mode of inheritance. However, unfortunately few family studies have been conducted relating with Class III malocclusion. This phenotype follows the autosomal dominant mode of inheritance and was demonstrated in different studies [20,21].

To find out the specific gene or genes responsible for Class III malocclusion, limited genome wide family based linkage studies have been conducted [22-24]. For identifying the genetic variation, different mutation on candidate gene were checked between the case and the control groups. Genetic variations are the most common hereditary transformations in human beings that affect protein expressions and functions, and they can be related to a disease [25]. Among the dental diseases, malocclusion is very common and it may be suggested that mutations are the major genetic variations causing malocclusion [26]. Recently, one study established a candidate gene DUSP6 (Dual Specificity Protein Phosphatases) for Class III malocclusion in an Estonian family. Whole exome sequencing was carried out among affected five siblings from one single family and a rare missense mutation c.545C>T (p.Ser182Phe) was found. This candidate gene spans 4.46 kb of genomic DNA on chromosome 12q22-q23 [27].

The aim of our study was to determine the DUSP6 gene mutation in Malaysian Malay family with Class III malocclusion.

Material and Methods

Study Design and Sample

A total of 60 Malaysian Malay consenting subjects participated in this case-control study. The subjects were distributed into patient and control groups, consisting of 30 patients and 30 controls. Patient group consisted of 10 families with one individual from three consecutive generations. The mean ages for Class III malocclusion group were 22.50 (\pm 5.30), 53.50 (\pm 10.06) and 79.20 (\pm 9.35) years old of each generation, respectively. Moreover, healthy controls were chosen with same ethnicity and age between 18 to 29 years old.

Clinical Evaluation

We took Class III malocclusion subjects who were verified by clinical examination, lateral cephalometric radiographs, dental casts, and/ or facial and intra-oral photographs. Romexis™ Software 2.3.1.R (Planmeca OY, Helsinki, Finland) was used for cephalometric analysis to decide the level of skeletal malocclusion. Class III malocclusion considered when they showed concave profile, negative ANB angle. Mandibular plane angle was also used for verification, as low value of this angle may represent the predominant pattern of straight facial growth and high values may recommend the predominant pattern of vertical facial growth. All cephalometric measurements were presented previously [28].

Any participant with craniofacial congenital anomalies (i.e cleft lip or palate), pregnancy, uncontrolled medical problems are requiring antibiotics prophylaxis prior to any dental procedure, interracial marriage were excluded from this study. Thirty controls were taken who had been identified as Class I normal occlusion in our orthodontic department. All participants were resident in Malaysia.

Genetic Study

Buccal cell was taken from each participant of Class III malocclusion (diagnosed by specialized orthodontist) and control groups. DNA extractions from buccal cell were carried out using Gentra puregene buccal cell kit. We prepared 3 sets of primers for DUSP6 gene using Primer 3 web version 4. The specificity of these primers to the DUSP6 gene will be confirmed using the 'Basic Local Alignment Search Tool (BLAST)' program available online at <http://www.ncbi.nlm.nih.gov/blast>.

The upstream primers and downstream primers were designed for Exon 1, 5'-TTGAGAGCTAAGATGTGCCAA-3' and 5'-GTAAGGCGAGGCGGAATTAAA-3', for Exon 2, 5'-TTAAACTCTATGAATGGCTAGG-3' and 5'-AGGATGCTTGTGGTGTTCCTT-3', for Exon 3, 5'-TATCTATACAGCATGTCCTGTT-3' and 5'-GATACATTTTCTGCTGCTTGTA-

3'. All pellet form of primers was diluted with nucleus free water and make it 10 μ M as a final concentration. Polymerase Chain Reaction (PCR) was performed in a total volume of 50 μ l containing- PCR master mix 25 μ l, forward and reverse primers 4 μ l, DNA samples 4 μ l and Nucleus free water 13 μ l for Exon 1. For exon 2, PCR master mix 25 μ l, forward and reverse primers 3 μ l, DNA samples 2 μ l and nucleus free water 17 μ l. For exon 3, PCR master mix 25 μ l, forward and reverse primers 4 μ l, DNA samples 3 μ l and Nucleus free water 14 μ l.

Polymerase chain reaction (PCR) was carried out with an initial 5 minutes denaturation at 95°C, followed by 30 cycles of 95°C for 30 seconds, 56°C for 40 seconds, 72°C for 1 minute and a final extension period at 10 minutes using PCR thermo cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). Next PCR products were run in 1% agarose gel, DNA bands were estimated and photographed over UV-transilluminator (Bio-Rad Laboratories Inc., Hercules, CA, USA). Upon getting the desired size of band, all PCR products were sent to 1st base company Malaysia for sequencing to perceive the results. The sequencing was performed in all patients and control groups. Bio Edit Sequence Alignment Editor software was used to see the sequencing result.

Ethical Aspects

Written informed consent was obtained from all participants in line with the Helsinki Declaration before inclusion in the study, which was performed with the approval of the Human Research and Ethics Committee, Universiti Sains Malaysia.

Results

A missense mutation was found in exon 3 with DUSP6 gene in three members of a single Malaysian Malay family. Mutation was detected in amino acid position (p.Thr 365 Ile) where amino acid Threonine change to Isoleucine in nucleotide position c.1094 C> T in exon 3 (Figure 1). The rest of the families with Class III malocclusion showed no mutation in exon 3. There was no mutation found in exon 1 and exon 2 among all subjects with Class III malocclusion. Moreover, there was no mutation found in DUSP6 gene among the control group (Figure 2).

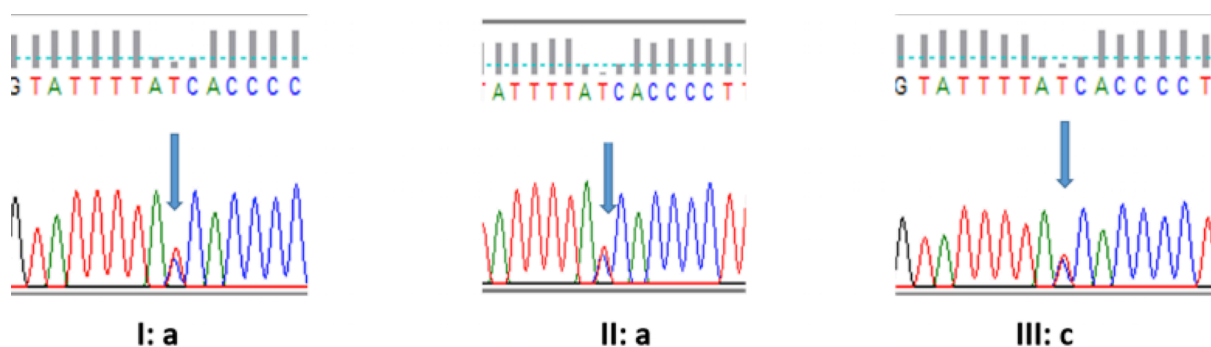


Figure 1. Partial DNA sequences of exon 3 of DUSP6 gene from the affected female I: a, II: a, III: c.

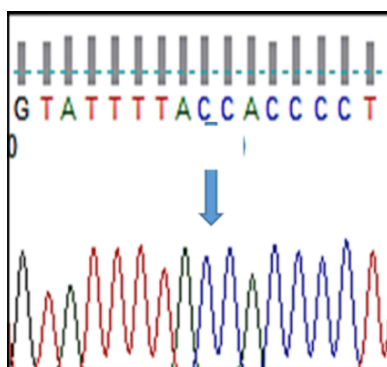


Figure 2. Partial DNA sequences of exon 3 of DUSP6 gene from the control group.

A missense mutation was found from the Ia, IIa and IIIc subjects (Figure 3).

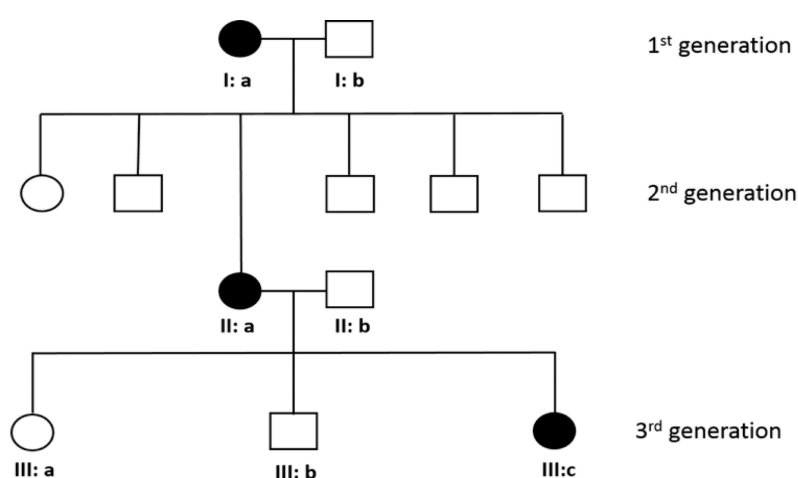


Figure 3. Pedigree of a Malaysian Malay family with Class III malocclusion. Symbols: male- squares; females- circles; affected- filled symbol; unaffected- empty symbol. I:a, I:b is 1st generation of patient, II:a, II:b is 2nd generation of patient, III:a, III:b is sibling of patient, III:c is patient

Discussion

In this study, the genetic analyses were performed for DUSP6 gene in 10 families with their 3 consecutive generations having Class III malocclusion in Malaysian Malay subjects and compared with the 30 healthy controls. A missense mutation was determined in exon 3 of DUSP6 gene. This missense mutation c.1094C>T (p. Thr 365 Ile) consequentially results in the replacement of amino acid Threonine to Isoleucine at position number 365. Three members of one family displayed the same mutation at amino acid position (p.Thr 365 Ile) and presented a concave facial profile, which represents Class III malocclusion that was further proved by cephalometric radiographs. Upon analyzing this family, same mutation was found from the Ia, IIa and IIIc subjects (Figure 3).

There was no genetic study on Class III malocclusion has been done yet in Malaysian Malay population. This is the first case from the Malay. However, after getting the genetic result, we performed another experiment on craniofacial morphology, which suggested that mandible is more deviated from cranial base in DUSP6 gene mutation group compare to non-mutation group in Class III malocclusion [28].

DUSP6 contrarily control individuals from the mitogen-initiated protein (MAP) kinase superfamily (MAPK/ERK, SAPK/JNK, p38), which is related with cell expansion and separation. This DUSP6 gene has significant role in development and organogenesis due to extracellular signal-regulated kinase (ERK)-specific phosphatase. Moreover, the period of embryonic development, phenotype of DUSP6 knockout and transgenic animals seem to be a result of inappropriate response of ERK signaling encouraged by members of fibroblast growth factor (FGF) family. DUSP6^{-/-} mice reveal some abnormalities like dwarfism and craniosynostosis, the premature fusion of the cranial sutures [29].

For skeletal development, FGF/FGFR (FGF receptor) signaling pathways play the significant role. Moreover, DUSP6 gene reflects as one of the key genes in FGF/FGFR (FGF receptor) signaling pathways that play the significant role in skeletal development [26]. FGFR2 and FGFR3 might be associated to maxillary retrognathism as proved by their participation in cranial suture biology and craniosynostosis that may be the result of Class III malocclusion due to abnormal premaxillary suture function [30].

Genetic variants on gene are claimed to exhibit possible pathogenesis in Class III malocclusion [27,31]. Different studies used different methods like linkage analyses, whole exome sequencing, polymorphism study or mutational studies to link the genetic association with Class III malocclusion [22-24,32]. Current study has hypothesized detection of mutations in Class III malocclusion for the prediction of developing the phenotype with the DUSP6 gene. Until now, only one mutational study has been done in relation with Class III malocclusion associated with DUSP6 gene. Whole exome sequencing showed significant association of DUSP6 gene in Class III malocclusion in an Estonian family and showed a rare missense mutation on c.545C>T in Exon 2 (p.Ser182Phe) associated with Class III malocclusion [27]. The same missense mutation (p.Ser182Phe) was informed before as a responsible variant in case of KS in another study [33].

DUSP6 is a member of the FGF8 synexpression module that determines pleiotropic roles during embryogenesis and in adulthood, and recent studies have delivered sign of an oligogenic model accounting for variable phenotypes in CHH/KS [34,35]. These findings can be interpreted as a clue of etiological heterogeneity: the infrequent allele in combination with other (more likely common) alleles constructing different phenotypic anomalies regarding CHH/KS and Class III malocclusion.

A missense mutation was identified in different position on c.1094 C>T in exon 3 (p.Thr365Ile) in 3 generations of Class III malocclusion. This missense variant (p.Thr365Ile) of DUSP6 gene exists in NCBI database (rs370130918), however no report had been found on (p.Thr365Ile) variants in DUSP6 gene associating it with any disease. Moreover, none of patients that participated in current study demonstrated any KS features, and no pubertal issues were stated during history taking.

More recently, a study found five missense mutations Tyr67Asn, Arg291Gln, Thr381Met, Val327Ile and Gly1121Ser in five different gene BMP3, ANXA2, FLNB, HOXA2, and ARHGAP21

associated with Class III malocclusion in a single family living in Italy. However, the genetic variant Gly1121Ser of ARHGAP21 gene was present in all members of the affected family [32]. In current study, we did not find any missense mutation other than (p.Thr365Ile) in three members of a single family.

To know the molecular pathogenesis of Class III malocclusion, recognize the vulnerable genetic factors is the main step. However, the advancement in identifying the gene or genes contributed in this dentofacial trait is very narrow.

Conclusion

Mutation of DUSP6 gene may causes Class III malocclusion in Malaysian Malay population. May be there is a significant relation of craniofacial growth and genetic linkage with Class III malocclusion at 12q22-q23 region. Further study should do to find out the molecular mechanism of Class III malocclusion and underlying genetic effects which response to dentofacial development.

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Conflict of Interest: The authors declare no conflicts of interest.

References

- [1] Hill I, Blayney J, Wolf W. The Evanston Dental Caries Study XIX. Prevalence of malocclusion of children in a fluoridated and control area. *J Dent Res* 1959; 38:782-94. <https://doi.org/10.1177/00220345590380040601>
- [2] Emrich R, Brodie A, Blayney J. Prevalence of Class 1, Class 2, and Class 3 malocclusions (Angle) in an urban population. An epidemiological study. *J Dent Res* 1965; 44(5):947-53. <https://doi.org/10.1177/00220345650440053301>
- [3] El-Mangoury NH, Mostafa YA. Epidemiologic panorama of dental occlusion. *Angle Orthod* 1990; 60(3):207-14.
- [4] Baccetti T, Reyes BC, McNamara Jr JA. Gender differences in Class III malocclusion. *Angle Orthod* 2005; 75(4):510-20.
- [5] Thilander B, Pena L, Infante C, Parada SS, de Mayorga C. Prevalence of malocclusion and orthodontic treatment need in children and adolescents in Bogota, Colombia. An epidemiological study related to different stages of dental development. *Eur J Orthod* 2001; 23(2):153-67.
- [6] Staudt CB, Kiliaridis S. Different skeletal types underlying Class III malocclusion in a random population. *Am J Orthod Dentofacial Orthop* 2009; 136(5):715-21. <https://doi.org/10.1016/j.ajodo.2007.10.061>
- [7] Nakasima A, Ichinose M, Nakata S. Genetic and environmental factors in the development of so-called pseudo-and true mesiocclusions. *Am J Orthod Dentofacial Orthop* 1986; 90(2):106-16. [https://doi.org/10.1016/0889-5406\(86\)90041-7](https://doi.org/10.1016/0889-5406(86)90041-7)
- [8] Woon K, Thong Y, Abdul KR. Permanent dentition occlusion in Chinese, Indian and Malay groups in Malaysia. *Aust Orthod J* 1989; 11(1):45-8.
- [9] Alam MK, Islam M, Haque F, Hossain MM, Jamil M, Islam R, et al. Class III malocclusion with anterior open bite, orthodontic treatment in an adult patient: A clinical note. *Int Med J* 2017; 24(4):324-5.
- [10] Nowrin SA, Alam MK, Basri R. Genetic effect and prevalence of Class III malocclusion in different population: An Overview. *Int J Pharma Bio Sci* 2015; 6:910-8.
- [11] Nowrin SA, Alam MK, Basri R. Genetic analysis: Future diagnostic tool in clinical Orthodontics. *Bangladesh J Med Sci* 2015; 14(3):310-5. <https://doi.org/10.3329/bjms.v14i3.20929>
- [12] Nowrin SA, Alam MK. Chin cup in class III malocclusion: An update. *Int Med J* 2017; 24(3):264-7.

- [13] Angle EH. Treatment of Malocclusion of the Teeth: Angle's System. Philadelphia: S.S. White Manufacturing Co., 1907.
- [14] Downs Jr WG. Studies in the causes of dental anomalies. *Genetics* 1927; 12(6):570-80.
- [15] Gold JK. A new approach to the treatment of mandibular prognathism. *Am J Orthod* 1949; 35(12):893-912.
- [16] Monteleone L, Duvigneaud J. Prognathism. *J Oral Surg* 1963; 21:190-5.
- [17] Pascoe JJ, Hayward JR, Costich ER. Mandibular prognathism: Its etiology and a classification. *J Oral Surg Anesth Hosp Dent Serv* 1960; 18:21-4
- [18] Rubbrecht O. A study of the heredity of the anomalies of the jaws. *Am J Orthod Oral Surg* 1939; 25(8):751-79. [https://doi.org/10.1016/S0096-6347\(39\)90224-3](https://doi.org/10.1016/S0096-6347(39)90224-3)
- [19] Dohmoto A, Shimizu K, Asada Y, Maeda T. Quantitative trait loci on chromosomes 10 and 11 influencing mandible size of SMXA RI mouse strains. *J Dent Res* 2002; 81(7):501-4.
- [20] El-Gheriani AA1, Maher BS, El-Gheriani AS, Sciote JJ, Abu-Shahba FA, Al-Azemi R, et al. Segregation analysis of mandibular prognathism in Libya. *J Dent Res* 2003; 82(7):523-7. <https://doi.org/10.1177/154405910308200707>
- [21] Cruz RM, Krieger H, Ferreira R, Mah J, Hartsfield J, Oliveira S. Major gene and multifactorial inheritance of mandibular prognathism. *Am J Med Genet A* 2008; 146A(1):71-7. <https://doi.org/10.1002/ajmg.a.32062>
- [22] Yamaguchi T, Park S, Narita A, Maki K, Inoue I. Genome-wide linkage analysis of mandibular prognathism in Korean and Japanese patients. *J Dent Res* 2005; 84(3):255-9. <https://doi.org/10.1177/154405910508400309>
- [23] 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. <https://doi.org/10.1177/0022034508327817>
- [24] Li Q, Li X, Zhang F, Chen F. The identification of a novel locus for mandibular prognathism in the Han Chinese population. *J Dent Res* 2011; 90(1):53-7. <https://doi.org/10.1177/0022034510382546>
- [25] Wang Z, Moulton J. SNPs, protein structure, and disease. *Hum Mutat* 2001; 17(4):263-70. <https://doi.org/10.1002/humu.22>
- [26] Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; 273(5281):1516-7.
- [27] Nikopensius T, Saag M, Jagomägi T, Annilo T, Kals M, Kivistik PA, et al. A missense mutation in DUSP6 is associated with class III malocclusion. *J Dent Res* 2013; 92(10):893-8. <https://doi.org/10.1177/0022034513502790>
- [28] Nowrin SA, Basri R, Alam MK, Yusa T, Nakano J, Jaafar S, et al. Craniofacial morphology of Class III malocclusion with DUSP6 gene: Mutation and non-mutation groups. *J Hard Tissue Biol* 2016; 25(3):247-56. <https://doi.org/10.2485/jhtb.25.247>
- [29] Li C, Scott DA, Hatch E, Tian X, Mansour SL. Dusp6 (Mkp3) is a negative feedback regulator of FGF-stimulated ERK signaling during mouse development. *Development* 2007; 134(1):167-76. <https://doi.org/10.1242/dev.02701>
- [30] Ruan WH, Winger JN, Yu JC, Borke JL. Induced premaxillary suture fusion: class III malocclusion model. *J Dent Res* 2008; 87(9):856-60. <https://doi.org/10.1177/154405910808700901>
- [31] Perillo L, Monsurrò A, Bonci E, Torella A, Mutarelli M, Nigro V. Genetic association of ARHGAP21 gene variant with mandibular prognathism. *J Dent Res* 2015; 94(4):569-76. <https://doi.org/10.1177/0022034515572190>
- [32] Xue F, Wong RW, Rabie AB. Genes, genetics, and Class III malocclusion. *Orthod Craniofac Res* 2010; 13(2):69-74. <https://doi.org/10.1111/j.1601-6343.2010.01485.x>
- [33] Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet* 2013; 92(5):725-43. <https://doi.org/10.1016/j.ajhg.2013.04.008>
- [34] Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U S A* 2010; 107(34):15140-4. <https://doi.org/10.1073/pnas.1009622107>
- [35] Quaynor SD, Kim HG, Cappello EM, Williams T, Chorich LP, Bick DP, et al. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril* 2011; 96(6):1424-30.e6. <https://doi.org/10.1016/j.fertnstert.2011.09.046>