Assessment of Correlation between Craniofacial Proportions and Genetic Indicators

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ABSTRACT

Aim and objective: To assess correlation among craniofacial proportions and genetic indicators using estrogen receptors (ESR1 and ESR2).

Materials and methods: A total of 128 patients undergoing orthodontic treatment with age range 12–18 years of both genders were included. Lateral cephalogram of all subjects were taken. Vertical and sagittal parameters were studied on these cephalogram. Saliva was used for DNA extraction. Real-time polymerase chain reaction was performed for assessment of genetic indicators in ESR1 (rs9340799 and rs2234693) and in ESR2 (rs4986938 and rs1256049).

Results: The mean SN cranial base was 68.4 mm, ANB (sagittal jaw relationship) was 2.8°, Ptm-A maxillary length was 46.2 mm, Go-Pg (mandibular body length) was 68.2 mm, Co-Gn (total mandibular length) was 112.8 mm, lower anterior facial height (ANS-Me) was 58.4 mm, N-Me (total anterior facial height) was 108.4 mm, lower posterior facial height (Co-Go) was 58.7 mm, and S-Go (total posterior facial height) was 72.4 mm. It was found that rs4986938 in ESR2 was linked with S-N dimension, with patients having CC genotype possessing negative correlation values (p value 0.05). Similarly, CC genotype possessed minimum mandibular body dimension, and it was found that rs4986938 in ESR2 was also linked with Go-Pg dimension (p value = 0.02). We found reduction in the ANS-Me values in patients with CC genotype in ESR1 rs2234693 (p value = 0.02), whereas there was no correlation of rest genotype with other craniofacial measurements (p value > 0.05).

Conclusion: Evaluation of ESR1 and ESR2 may show role of genetic markers in disparity of craniofacial dimensions in individuals.

Clinical significance: This study provides an outlay and supports the concept of possible correlation between genetic markers and craniofacial measurements.

Keywords: Craniofacial dimensions, Genes, Markers, Estrogen receptors.

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INTRODUCTION

The maturation of craniofacial complex demands the harmonization of various mechanisms.[1] For orthodontic tooth movements (OTM), timely movement and communication of various cell types are of paramount important. OTM requires an optimal lightest force that provides maximum or a near-maximum response.[2] The tooth should move through alveolar bone resulting from periodontal ligament (PDL) remodeling and the bone *per se*.[3] With heavy orthodontic forces, the chances of external apical root resorption (EARR), enhanced hyalinization, and excessive tipping increases. Thus, there should be well coordination between forces applied and type of movements' required.[4]

Hormones and genes located on it are responsible for jaw bone growth. Márquez Hernández et al.[5] in their study on animals mentioned that hormones such as estrogen play a significant function in maturation of the maxillary and mandibular arches after birth. Other studies have also advocated that role of hormones in body regulatory function.[6,7]

Estrogens are steroid sex hormones responsible for growth and development neuroendocrine, skeletal, adipogenesis, and cardiovascular systems. Estrogen receptors (ER) control cellular signaling of estrogens via its α and β forms. ESR1 codes ERα and ESR2 codes ERβ. This gene is located on chromosome 6 at 6q25.1, and its expression is regulated by ERs perform bone modeling, cardiovascular system functioning, and behavior in both genders. ERα and ERβ are G protein-coupled receptors that is family of intracellular receptors. ERα is present in ovarian stromal cells, endometrium, and hypothalamus. It is present in efferent ducts in males. ERβ is found in heart, bone, brain, granulosa cells of ovary, intestine, lungs, and prostate.

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Robinson et al.[8] in their article demonstrated the occurrence of ERα in condyle of mandible fibro cartilage, whereas cartilage tissue and trabecular bone possesses ERβ frequently. Gennari et al.[9] revealed that numerous conditions are linked with genes coding ERs, but relation of these genetic markers with maxillary and mandibular measurements has not been extensively studied. Single-nucleotide polymorphisms (SNPs) in the estrogen receptor gene (ESR1) can increase the risk of various abnormalities, such as craniofacial measurement variation and breast cancer, etc. Influence

of a functional variation or genetic polymorphism can result in a localized skeletal asymmetry. It has been observed that rs858339 has a significant association with temporomandibular disorders. ESR1 rs1643821 polymorphisms associated with skeletal Class II malocclusions and asymmetry. Genetic disparity of the estrogen metabolism pathway, particularly the genes concerned in the creation of estrogen through androgen conversion persuade the risk for the advancement of estrogen-sensitive breast cancer. This could be also factual for craniofacial malformation.[10] The present study evaluated the possible correlation between genetic markers and craniofacial measurements and role of variation in genetic indicator in ESR1 and ESR2 as a potential marker for disparity in the craniofacial dimensions.

MATERIALS AND METHODS

This randomized controlled study was conducted in the Department of Orthodontics, Al Ameen Dental College, Vijayapura, Karnataka, on 128 patients of both genders undergoing orthodontic treatment. The age ranged from 12 to 18 years. The study period was from May 2018 to July 2019. The ethics committee of the institute approved this study, and the study was commenced after obtaining approval. All the patients were explained about the study, and consent was obtained from their parents/guardians in vernacular language. Inclusion criteria were patients within specified age-group, non-syndromic patients, and patients with no history of previous orthodontic treatment. Exclusion criteria were those not willing to participate and patients with syndromes.

Demographic data were recorded in case history per forma. A good-quality digital lateral cephalogram was obtained, and parameters such as linear measurements of the maxillary length, that is Ptm-A, anterior cranial base (S-N), Co-Gn (mandibular total) and Go-Pg (body length), ANS-Me (lower anterior facial length), N-Me (full length) S-Go (posterior facial length) and Co-Go (mandibular ramus length), were traced. All pretreatment lateral cephalograms were recruited from the departmental computer and were digitally traced by two individual orthodontists with the help of Newtom New technology software. Two orthodontists traced and studied each lateral cephalogram twice, and their mean values were considered final.

Genomic DNA Assessment

Unstimulated saliva was collected in test tubes from all patients. After obtaining 5 mL of saliva, incubation was done and centrifugation was performed. After discarding supernatant, 1 mL of Tris–HCl 10 mM and EDTA 5 mM were added to remaining saliva. All test tubes were further subjected to incubation overnight, and to this, 10 M ammonium acetate in the amount of 500 μL were poured over it, and centrifugation was done. Isopropyl alcohol of 700 μL was added for DNA precipitation. Again, draining of the supernatant was done, and 70% ethanol was used for washing the pellet. Decontamination was done, and the DNA was r-balanced in TE buffer and stored.

Intronic genetic polymorphisms in ESR1 (rs9340799 and rs2234693) and ESR2 (rs4986938 and rs1256049) were genotyped (Table 1) with TaqMan polymerase chain reaction (PCR) method using kit ABI PRISM Sequence Detection System, USA.

The specificity of PCR method increased with TaqMan probes. These probes were comprised of fluorophore linked to the 5'-end of the oligonucleotide probe with covalent bond and a quencher at the 3'-end. The quencher molecule quenched the fluorescence

Table 1: Genetic markers used for the study

Characteristics	ESR1	ESR2
Locus	6q25.1	14q23.2
Reference sequence	rs2234682	rs1256054
	rs9340787	rs4986932
Base change in the sequence	AGC[C/T]GTT	CCG[C/T]ACT
	TCT[A/G]GAG	AGC[C/T]TGT
Minor allele frequency	0.436/2325	0.124/652
	0.274/1508	0.248/1306

Table 2: Demographic data of patients

Gender	Number	Percentage
Male	56	43.8
Female	72	56.2

emitted by the fluorophore when excited by the cycler's light source. Fluorescence signals were inhibited till the fluorophore and the quencher are in close proximity. These probes were annealed within a DNA region amplified by a specific set of primers. The 5' to 3' exonuclease activity of the Taq polymerase degraded the probe when the primer was being extended by Taq polymerase and synthesized the nascent strand. Degradation of the probe released the fluorophore from it and broke the proximity to the quencher, therefore relieving the quenching effect and allowed fluorescence of the fluorophore. Thus, fluorescence detected in the PCR cycle is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Hence, by this method, ESR1 (rs2234693 and rs9340799) and ESR2 (rs1256049 and rs4986938) were genotyped focusing loci, references sequence, base change in the sequence, and minor allele frequency.[11]

Data thus obtained were tabulated and statistically analyzed. The level of significance was p value < 0.05. All analyzes were done using Kolmogorov–Smirnov method. The linear dimensions were matched between genotypes, where one genotype of every gene was taken as an indication of control genotype.

RESULTS

Table 2 shows that of 128 patients, males were 56 (43.8%) and females were 72 (56.2%). Table 3 shows sagittal measurement values according to the genotypes. It shows that mean SN cranial base was 68.4 mm, ANB (sagittal jaw relationship) was 2.8°, Ptm-A maxillary length was 46.2 mm, Go-Pg (mandibular body length) was 68.2 mm, Co-Gn (total mandibular length) was 112.8 mm, ANS-Me (lower anterior facial length) was 58.4 mm, N-Me (total anterior facial length) was 108.4 mm, Co-Go (lower posterior facial length) was 58.7 mm, and S-Go (total posterior facial height) was 72.4 mm.

Table 4 shows vertical measurements. It was found that rs4986938 in ESR2 was linked with S-N dimension with patients having CC genotype possessed negative correlation values (p value = 0.05). CC genotype possessed minimum mandibular body dimension, and rs4986938 in ESR2 was also linked with Go-Pg dimension (p value = 0.02). There was nonsignificant (p value > 0.05) association of other parameters with genotypes. Figure 1 shows linear measurements done according to genotypes. ANS-Me Line is a junction of the Me and ANS landmarks. ANS-Me reflects the anteroinferior height of the face (lower anterior face height). We found reduction in the ANS-Me values in patients

Table 3: Assessment of Sagittal measurements

Genotype		S-N	p value	ANB°	p value	Ptm-'A'	p value	Go-Pg	p value	Co-Gn	p value
ESR1 rs2234693	CC (28)	66.8	0.61	3.1	0.2	50.2	0.21	67.2	0.52	55.6	0.41
	CT (73)	66.7	0.65	3.0	0.13	49.3	0.43	68.1	0.67	56.7	0.32
	TT (25)	67.1	–	3.2	–	49.6	–	67.2	–	57.2	–
ESR1 rs9340799	AA (62)	67.4	0.52	2.7	0.31	49.6	0.44	68.3	0.61	57.8	0.24
	AG (50)	66.8	0.57	2.9	0.21	49.6	0.42	67.1	0.72	56.5	0.31
	GG (14)	67.2	–	2.7	–	50.4	–	68.0	–	58.1	–
ESR2 rs1256049	CC (110)	66.5	0.52	2.9	0.15	51.2	0.28	67.8	0.81	57.4	0.23
	CT (10)	67.1	–	2.8	–	51.4	–	65.9	–	57.9	–
	TT (0)	–	–	–	–	–	–	–	–	–	–
ESR2 rs4986938	CC (45)	62.4	**0.05**	3.0	0.13	50.2	0.21	68.1	**0.02**	56.2	0.14
	CT (61)	66.5	0.63	3.1	0.2	50.4	0.34	68.5	0.13	56.8	0.15
	TT (14)	67.3	–	3.0	–	50.1	–	68.1	–	57.1	–

CC, AG, GG, TT, CT—genotypes; p value = 0.05

with CC genotype in ESR1 rs2234693 (*p* value = 0.02), whereas rest of the genotype had no correlation with other craniofacial measurements (*p* value > 0.05). Reduction in the ANS-Me values indicates decreased facial height.

DISCUSSION

The study of genes in orthodontics is a topic of concern. Growth hormone (GH) brings about maturation of the craniofacial region.[12] Growth is responsible for major increase in bony as well as soft tissue dimensions. Pilecka et al.[13] found that the attachment of GH occurs via its receptors, i.e., GHR. Bayram et al.[14] established correlation of various craniofacial parameters and genetic polymorphisms in GHR. Boys and girls in their teenage are under the influence of estrogen which is a sex hormone. It is also responsible for enhancing the release of GH in adolescent which provoke skeletal growth.[15] Estrogen has major role in controlling growth and development in young adults by its action of ERα and ERβ. It is contributing in juvenile development and growth of epiphyseal in both genders.[16] We found reduction in the ANS-Me values in patients with CC genotype in ESR1 rs2234693. There was association of genetic markers with cranial dimensions.

Omori et al.[17] in their study tried to establish correlation of craniofacial measurements and genetic markers in ESR1 and ESR2 on 146 ongoing orthodontic patients using sagittal and vertical measurements with the use of lateral cephalograms. In this, DNA extraction and assessment of genetic indicator in ESR1 and in ESR2 were performed. The authors found ESR1 and ESR2 show variations in the craniofacial dimensions. Hence, there is need to study this relation extensively.

We observed that rs4986938 in ESR2 was linked with S-N dimension and in patients with CC genotype reduced values were recorded. Decreased mandibular body dimensions were observed in CC genotype and also rs4986938 in ESR2 was linked with Go-Pg dimension. Linear measurements were done, and it was observed that there was reduction in the ANS-Me values in patients with CC genotype in ESR1 rs2234693 (*p* value = 0.02), whereas rest genotype had no correlation with other craniofacial measurements.

Mondockova et al.[18] assessed the associations of rs9340799:A > G (XbaI) and rs2234693:T > C (PvuII) polymorphisms in the ESR1 with femoral neck and lumbar spine bone mineral density in 343 postmenopausal females. Results of this study revealed that rs9340799 polymorphism may contribute to decreased BMD in postmenopausal women.

Kückler et al.[19] included 139 teenagers and 93 adults to assess if there is any relation of temporomandibular disorders (TMDs) with genetic polymorphisms in ESR1 and ESR2. Rats were also included to see if ERα and ERβ are expressed in the temporomandibular joint (TMJ) during adolescence. Results of the study showed more disk displacement and arthralgia in teenagers when compared to adults (*p* value < 0.05). There was association of genetic polymorphism rs1256049 in ESR2 with disk displacement and arthralgia in adults. The ERα and ERβ were expressed in rat TMJ tissues.

Possible role of ESR1 and ESR2 in craniofacial dimension variation have not been extensively studied. This study provides an outlay and supports the concept of possible correlation between two. However, large-scale studies on long-term follow-up is necessary to support the results obtained in our study. The limitation of the study is its small sample size. Moreover, the role of gender and age was not studied.

Table 4: Assessment of vertical measurements

Genotype		ANS-Me	p value	N-Me	p value	Co-Go	p value	S-Go	p value
ESR1 rs2234693	CC (28)	57.6	**0.02**	107.4	0.78	55.6	0.41	70.1	0.62
	CT (73)	60.7	0.15	106.3	0.71	56.4	0.31	73.2	0.58
	TT (25)	60.5	–	110.3	0.92	58.4	–	74.5	–
ESR1 rs9340799	AA(62)	60.7	0.21	108.4	0.62	56.8	0.15	74.2	0.56
	AG(50)	59.8	0.31	109.5	0.70	56.2	0.28	73.4	0.57
	GG(14)	57.9	–	107.2	0.72	57.8	–	74.1	–
ESR2 rs1256049	CC(110)	59.2	0.34	109.1	0.69	56.3	0.35	72.8	0.52
	CT (10)	61.0	–	108.6	–	59.2	–	73.2	–
	TT (0)	–	–	–	–	–	–	–	–
ESR2 rs4986938	CC (45)	59.3	0.28	111.2	0.94	56.6	0.36	72.1	0.50
	CT (61)	58.6	0.30	106.4	0.72	56.7	0.14	73.7	0.61
	TT (14)	59.2	–	105.2	0.74	57.2	–	–	–

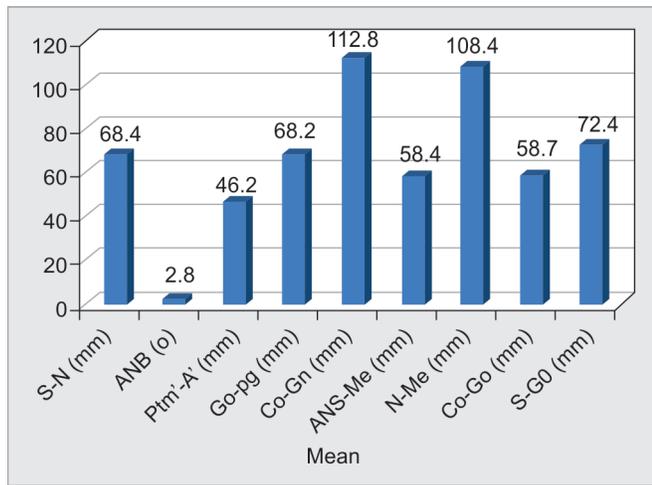CC, AG, GG, TT, CT—genotypes; *p* value = 0.02

Fig. 1: Assessment of linear measurements

CONCLUSION

The authors found that ESR1 and ESR2 variation may show potential markers for disparity in the craniofacial dimensions in individuals. This study provides an outlay and supports the concept of possible correlation between genetic markers and craniofacial measurements. Genetic markers in ESR1 and ESR2 can be used for evaluation of craniofacial discrepancy with genetic influence.

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