Analyzing specific candidate genes implicated in the development of Class III skeletal malocclusion

By Shreya Srikanth

Abstract:

Jaw malocclusion is characterized by a misalignment between the upper and lower jaws, and is a situation that poses a range of challenges, including difficulty in chewing, speech issues, and aesthetic concerns. This study researched the roots of the Class III skeletal malocclusion specifically; By researching and gathering information from genetic databases, candidate genes associated with this mandibular prognathism were identified and analyzed. The findings of this research were deemed statistically significant, and showed a very strong correlation between the mandibular prognathism and genes in the Fibroblast Growth Factor (FGF) family, suggesting that these genes play a role in the craniofacial development seen in an underbite. After further investigation into the molecular pathways controlled by the FGF family genes, it was determined that these genes may have involvement in processes like facial morphogenesis, bone growth, and tooth development. I hypothesized that disturbances in these pathways, possibly due to genetic mutations, could lead to malocclusion. This study is useful because it allows for potential therapeutic solutions to underbites, and early detection of the disorder from genes. This allows orthodontists to develop more personalized treatment strategies, based on specific individual genetics profiles. This will offer better treatment outcomes and improved quality of life for patients with Class III skeletal malocclusion. However, I recognize that malocclusions are influenced by various factors, including genetics, environment, and development. Further research is needed to understand how these factors work together to shape malocclusion phenotypes.

Introduction

Jaw malocclusion, characterized by misalignment between the upper and lower jaws, poses a range of challenges, including difficulty in chewing, speech issues, and aesthetic concerns. Recognizing its genetic basis is crucial for diagnosis, prevention, and effective treatment (Jackson, Maria et al). Candidate genes, linked to malocclusion development, play a vital role in unraveling the genetic complexities associated with this condition.

The impact of jaw malocclusion on an individual's quality of life cannot be overstated, with complications extending to temporomandibular joint disorders, dental problems, and psychological distress. Early diagnosis and intervention are essential to address these issues, emphasizing the need to decode the genetic underpinnings of malocclusion.

Understanding jaw malocclusion is of utmost importance because it can significantly impact an individual's quality of life. Complications associated with malocclusion include temporomandibular joint disorders, dental problems, and psychological distress. Early diagnosis and intervention are essential for addressing these issues, making it crucial to decipher the genetic underpinnings of this condition.

Understanding these specific genetic factors could revolutionize craniofacial genetics, precision orthodontics, and personalized treatment strategies, enhancing the quality of life for individuals with Class III malocclusion. The findings from this study may pave the way for advancements in craniofacial genetics, precision orthodontics, and personalized treatment modalities, ultimately benefiting individuals with Class III malocclusion and improving their quality of life. By understanding the specific genetic factors contributing to this condition, our research aims to provide valuable insights that can advance the field of orthodontics and

craniofacial genetics, leading to more effective and personalized treatment strategies for individuals with Class III skeletal malocclusion.

Methods

The initial phase of our research focused on scrutinizing candidate genes to identify patterns and extract significant data related to mandibular prognathism (MP). To compile the list of candidate genes, we conducted an extensive literature review and delved into genetic databases, emphasizing genes associated with craniofacial development, tooth formation, and bone growth. **The full list of candidate genes analyzed can be found in the appendix, Table 2.** Subsequently, we performed a Gene Ontology (GO) Enrichment Analysis using the EnrichR tool. This analysis compared our identified gene list to a reference gene set, typically encompassing all genes in the genome. The goal was to pinpoint overrepresented GO terms. A high enrichment score for a term indicated that the input genes were significantly associated with that particular biological process or molecular function. Our analysis, visually represented in a graph, revealed significant associations of multiple gene families with mandibular prognathism.

Statistical analyses, inclusive of p-values, were executed to ascertain the significance of our findings. The results were systematically presented through graphs and tables. The integration of data across various analyses, enriched with GO terms and corresponding p-values, contributed to a holistic understanding of the genetic basis underlying Class III skeletal malocclusion.

Ensuring the robustness of our methodology, validation efforts were undertaken through collaboration with independent research groups and meticulous comparison with existing datasets. This collaborative approach reinforced the reliability and credibility of our findings, enhancing the overall strength of our research methodology.

Discussion

Linked Genes and Genome-wide Linkage Analysis:

Linked genes are two genes that sit together on a chromosome, making them more likely to be inherited.

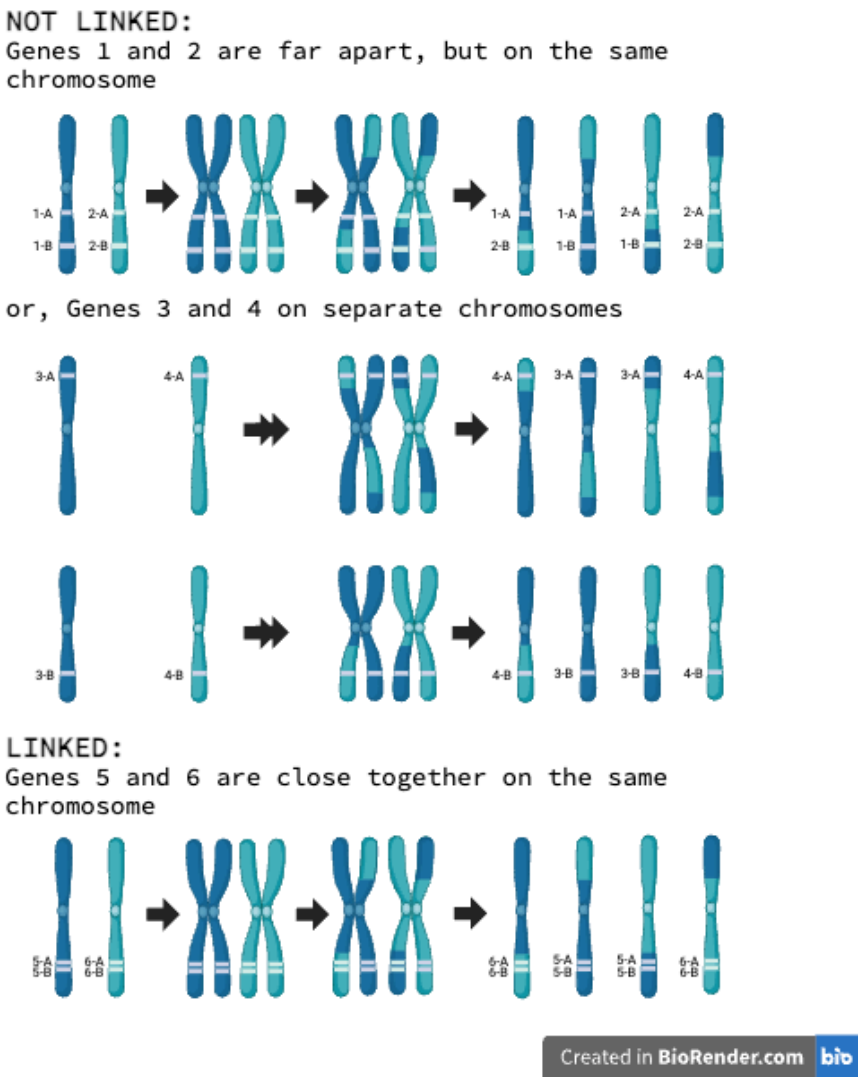

FIGURE 1: An illustration of crossing over with Unlinked vs Linked Genes, Created with BioRender.com

As seen in Figure 1, Linked genes are two genes that are located close together on the same chromosome. This proximity makes them more likely to be inherited together as a unit during the process of genetic recombination, which occurs during meiosis. In Figure 2, the concept of crossing over is illustrated, showing both unlinked and linked genes.

1. Genes 1 and 2 are far apart on the same chromosome and are not linked. This means that during meiosis, when genetic material is exchanged between homologous chromosomes, there is a higher probability of crossing over occurring between these genes. Consequently, alleles of genes 1 and 2 can assort independently into gametes, leading to a wide variety of genetic combinations in the offspring.
2. Genes 3 and 4 are on different chromosomes and are not linked. This means that they are on separate chromosomes and thus assort independently during meiosis. The inheritance of alleles of gene 3 is not dependent on the inheritance of alleles of gene 4, and vice versa.
3. Genes 5 and 6 are close together on the same chromosome and are linked. This means that crossing over between genes 5 and 6 is less likely to occur during meiosis because of their close proximity. As a result, alleles of genes 5 and 6 tend to be inherited together as a unit more often.

Crossing over is the exchange of genetic material between homologous chromosomes during meiosis. It occurs when chromosomes pair up during prophase I of meiosis and portions of chromatids are exchanged between them. This process leads to genetic recombination, where new combinations of alleles are formed, contributing to genetic diversity among offspring.

Understanding the concept of linked genes and crossing over is crucial in genetic studies for several reasons:

1. Mapping of genes: Linked genes can be used to map the relative positions of genes on a chromosome. The frequency of crossing over between linked genes can be used to estimate the distance between them.
2. Inheritance patterns: Linked genes can exhibit non-Mendelian inheritance patterns, such as incomplete linkage or linkage disequilibrium, which can provide insights into genetic inheritance.
3. Evolutionary studies: Analysis of linked genes and crossing over can provide information about the evolutionary history of species and the relationships between different organisms.

In genetic studies, researchers often employ techniques such as genome-wide linkage analysis to identify regions of the genome that may harbor genes associated with a particular trait or condition, in this case, mandibular prognathism (MP) or Class III skeletal malocclusion (National Human Genome Research Institute).

Genome-wide linkage analysis involves examining the inheritance pattern of genetic markers (typically polymorphisms) in families with a history of the trait or condition of interest. In the context of the research paper, the study "Towards Genetic Dissection of Skeletal Class III Malocclusion" utilized genome-wide linkage analysis to identify significant linkage to mandibular prognathism at specific chromosomal locations.

The identification of linked genes through linkage analysis provides researchers with potential candidate genes that may contribute to the observed trait. (Bailey-Wilson et al). In the

case of jaw malocclusion, understanding the genetic markers linked to the condition can guide the selection of candidate genes for further investigation.

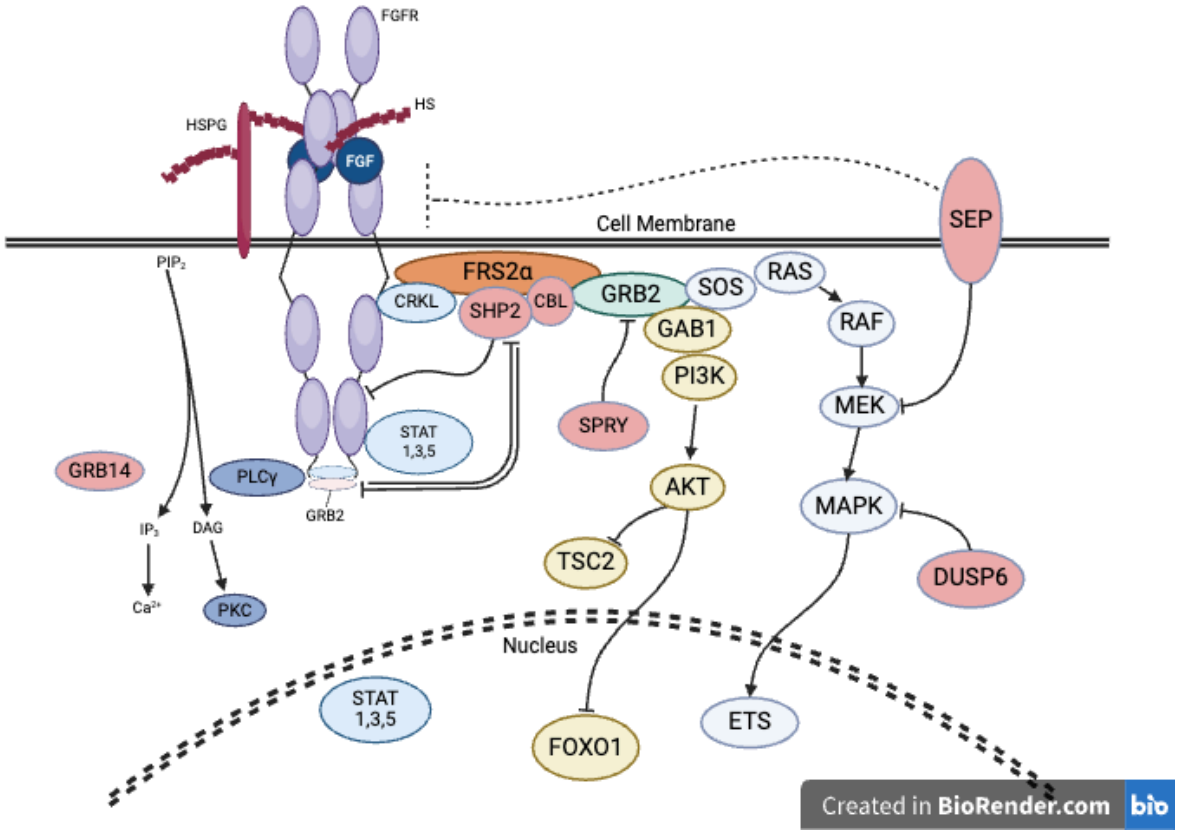

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Figure 2: The FGF (Fibroblast Growth Factor) signaling pathway plays a critical role in craniofacial development, and its connection to jaw malocclusions can be understood through several key points in the pathway:

FGF Family Signaling Pathways

1. Binding and Activation: FGF binds to FGFR with HSPG as a cofactor, initiating the signaling cascade. This initial step is crucial because it sets off a series of events that influence cell behavior and tissue development.

2. Phosphorylation: Upon binding, FGFR undergoes phosphorylation, which activates its intracellular tyrosine kinase domain. This activated receptor state is central to transmitting signals into the cell.

Intracellular Pathways.

3. RAS-MAPK Pathway: Phosphorylated FRS2α recruits GRB2, initiating the RAS-MAPK pathway. This pathway is known for its role in cell proliferation and differentiation. In craniofacial development, it could influence the growth and patterning of jaw structures, potentially contributing to malocclusions when dysregulated.

4. PI3K-AKT Pathway: The recruitment of GAB1 by GRB2 activates PI3K, which, in turn, phosphorylates AKT. The PI3K-AKT pathway is involved in cell survival, growth, and metabolism. Dysregulation of this pathway could affect the balance of tissue development in the jaw region, potentially leading to malocclusions.

5. PLCγ Pathway: Activation of PLCγ generates IP3 and DAG, triggering calcium release and PKC activation. Calcium signaling is essential for muscle contraction and tissue patterning. Dysfunctional calcium signaling may disrupt the proper development of jaw muscles and skeletal structures, contributing to malocclusions.

6. STAT Pathway: FGFR kinase activation of STAT proteins regulates gene expression in the nucleus. Altered gene expression patterns can impact various aspects of craniofacial development, including jaw formation and alignment.

7. Gene Regulation: FGF signaling pathways ultimately converge on gene regulation in the nucleus. Genes involved in craniofacial development, including those responsible for jaw formation and alignment, can be influenced by the activity of FGF signaling pathways.

8. Negative Regulators: Molecules like SPRY, SHP2, CBL, SEF, and DUSP6 act as negative regulators, fine-tuning the FGF signaling pathway.

I hypothesize that dysregulation of these negative regulators, through genetic mutation, environmental effects, or heredity, can lead to aberrant pathway activity, potentially affecting craniofacial development and contributing to malocclusions.

Results

Term	Overlap	P-value	Odds Ratio	Combined Score	Genes
Receptor Ligand Activity (GO:0048018)	7/317	6.12E-06	11.72051282	140.6951926	JAG1; WNT3A; TGFB3; FGF20; IGF1; FGF23; FGF3
Fibroblast Growth Factor Receptor Binding (GO:0005104)	3/22	1.56E-05	75.4002448	834.6529797	FGF20; FGF23; FGF3
Notch Binding (GO:0005112)	3/24	2.04E-05	68.21262458	736.6599333	NCOR2; JAG1; NOTCH4
Growth Factor Activity (GO:0008083)	4/85	3.86E-05	24.07054674	244.6094308	JAG1; FGF20; FGF23; FGF3
Type I Transforming Growth Factor Beta Receptor Binding (GO:0034713)	2/9	1.74E-04	133.4155844	1154.996359	TGFB3; SMAD6
Histone H3 Acetyltransferase Activity (GO:0010484)	2/13	3.75E-04	84.88429752	669.7057178	KAT6B; EP300
DNA-binding Transcription Factor Binding (GO:0140297)	5/279	3.83E-04	9.025725476	71.01535203	HDAC4; TCF21; EP300; NFATC1; TBX5
Transforming Growth Factor Beta Receptor Binding (GO:0005160)	2/18	7.30E-04	58.34375	421.4174356	TGFB3; SMAD6
Fibroblast Growth Factor Binding (GO:0017134)	2/20	9.03E-04	51.85606061	363.4728781	FGFR2; FGFR1
Sequence-Specific DNA Binding (GO:0043565)	7/713	9.54E-04	5.045725285	35.0896615	HDAC4; TCF21; RORA; NFATC1; FOXO3; TBX5; GLI2
Transforming Growth Factor Beta Binding (GO:0050431)	2/21	9.97E-04	49.12440191	339.4792176	TGFB3; LTBP2
Nuclear Androgen Receptor Binding (GO:0050681)	2/25	0.001416438825	40.57312253	266.143837	TCF21; EP300
RNA Polymerase II-specific DNA-binding Transcription Factor Binding (GO:0061629)	4/226	0.001604573437	8.722007722	56.12522419	HDAC4; EP300; NFATC1; TBX5

Histone Acetyltransferase Activity (GO:0004402)	2/27	0.00165253381	37.32363636	239.0745196	KAT6B; EP300
Transcription Coactivator Binding (GO:0001223)	2/34	0.002615122156	29.14914773	173.3337883	EP300; RORA
Transcription Cis-Regulatory Region Binding (GO:0000976)	5/463	0.00360473639	5.350676323	30.10026516	HDAC4; TCF21; RORA; FOXO3; SMAD6
Sequence-Specific Double-Stranded DNA Binding (GO:1990837)	6/705	0.004593710136	4.260515021	22.93463898	HDAC4; TCF21; NFATC1; FOXO3; SMAD6; GLI2
Transmembrane Receptor Protein Tyrosine Kinase Activity (GO:0004714)	2/50	0.005582386826	19.41761364	100.7412756	FGFR2; FGFR1
DNA-binding Transcription Activator Activity, RNA Polymerase II-specific (GO:0001228)	4/342	0.007038409807	5.695970696	28.23135545	TCF21; NFATC1; FOXO3; TBX5

Table 1: A Gene Ontology Analysis on Candidate Genes

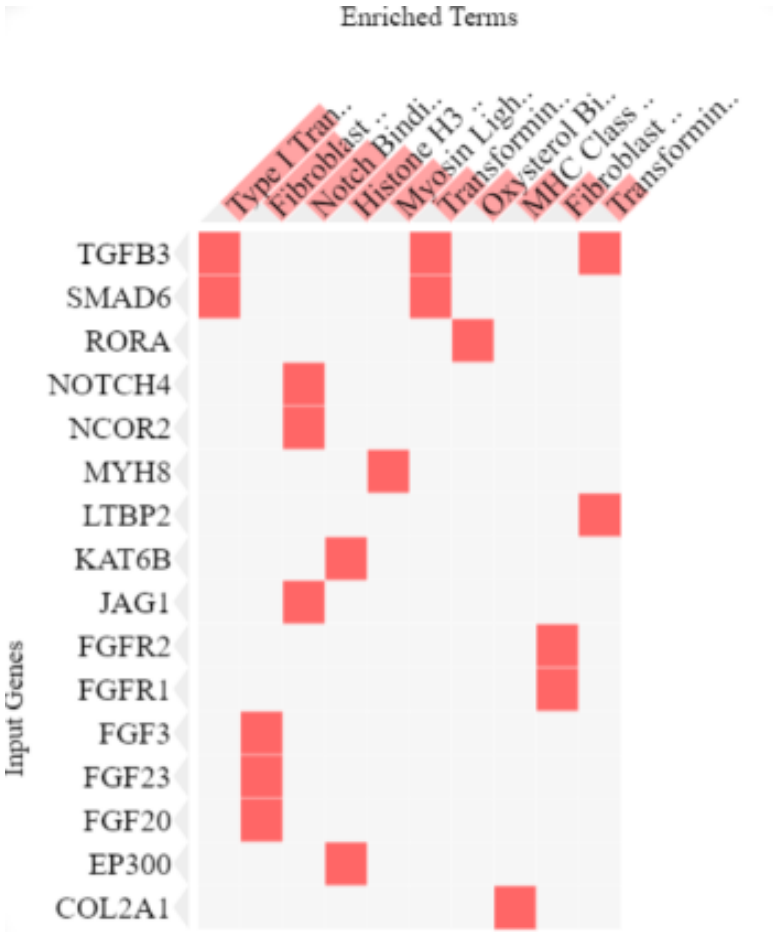

Figure 3: A Gene Ontology Analysis on Candidate Genes

As seen in both graph 1 and figure 3, multiple families are significantly associated with mandibular prognathism (not all data is shown, and the rest of the gene families can be found in the appendix, Table 3). However, when analyzing the candidate genes, I found that there was an unusually large amount of candidate genes from the fibroblast growth factor family. Utilizing EnrichR, the correlation between FGF family genes and jaw malocclusions was assessed to ascertain its significance. Specifically, the Fisher's exact test was employed to calculate the p-value, elucidating the strength of the association between the FGF family and mandibular prognathism.

Table 1 provides an overview of the correlation coefficients and corresponding p-values for all candidate genes implicated in the study. Notably, the analysis revealed an unusually high representation of FGF family genes among the candidate genes associated with mandibular prognathism. To quantify the significance of this observation, statistical analysis was conducted, with the results demonstrating a remarkably low p-value, indicative of a robust statistical significance. Additionally, Figure 3 illustrates in a more visual way the distribution of candidate genes associated with mandibular prognathism, with emphasis on the notable representation of the FGF family. This visual representation aids in elucidating the prominence of FGF family genes within the dataset, further highlighting their potential role in the development of mandibular prognathism.

Six members of the FGF family - Fgf-1, -2, -4, -5, -8, and -12 - are expressed in developing facial primordia. Fgf-2, -4, and -8 are expressed in the epithelium, specifically in restricted regions. They can partially substitute for epithelial signals, promoting outgrowth of the facial primordia. This hints at their potential role in controlling facial development, akin to their role in limb bud development.

Understanding the intricate association between the Fibroblast Growth Factor (FGF) family and malocclusions marks a significant advancement in dental science, carrying profound implications for precision medicine. This comprehension enables the tailoring of treatment strategies to individuals' distinct genetic compositions, signifying a transformative shift toward personalized therapeutic interventions. By leveraging this knowledge, therapeutic approaches can transcend conventional standardized methods, allowing for optimization of treatment outcomes through precise calibration based on genetic predispositions. The implications of this understanding extend beyond the realm of orthodontics, offering a promising avenue for targeted

therapeutic interventions. With a comprehensive understanding of the molecular underpinnings of malocclusions, interventions can be designed to address specific genetic factors, thereby enhancing treatment efficacy. By elucidating the role of FGF family genes in malocclusions, we gain valuable insights into the broader landscape of craniofacial development. This includes the intricate processes governing jaw formation, facial structure alignment, and dental arch emergence.

However, it is imperative to acknowledge the multifactorial nature of malocclusions. These conditions arise from a complex interplay of genetic, environmental, and developmental factors. While FGF family genes represent a critical component, they are just one element within a complex network of genetic determinants influencing craniofacial morphology. The manifestation of malocclusions is caused by many genetic variations, where FGF family genes may interact with numerous other genetic factors involved in jaw development, tooth formation, and facial alignment. In this respect, much more research could be done for us to fully catalog enough data and analyses for precision medicine to have all the information it needs.

Appendix

Gene	Function Description	Role	Implications	Chromosomal Location
WNT3A	Involved in Wnt signaling, crucial for embryonic development	Crucial in craniofacial development, including jaw	May have relevance to jaw malocclusions	1p34.3
MATN1	Matrilin, involved in extracellular matrix organization	Extracellular matrix, cartilage development	Implications in cartilage-related disorders	1p35.3
HSPG2	Encodes perlecan, a heparan sulfate proteoglycan, involved in cell adhesion	Extracellular matrix, cell signaling	Potential role in extracellular matrix disorders	1p36.12
EPB41	Cytoskeletal protein, essential for maintaining cell shape	Cell membrane organization	Implications in erythrocyte-related disorders	1p36.13
NBPF9	Neural-specific gene, role in brain development and function	Brain development	Neurodevelopmental disorders	1q21.1
NBPF8	Neural-specific gene, implicated in brain development	Brain development	Neurodevelopmental disorders	1q21.1
PLXNA2	Semaphorin receptor, involved in axon guidance and cell migration	Cell adhesion, axon guidance	Potential role in neural development	1q32.1
PSEN2	Part of the γ-secretase complex, involved in Notch signaling	Alzheimer's risk factor	Alzheimer's disease progression	1q42.13
KAT6B	Histone acetyltransferase, implicated in chromatin remodeling	Histone acetylation, gene regulation	Potential role in epigenetic disorders	10q22.2
FGFR2	**Fibroblast growth factor receptor, essential for skeletal development**	**Cell growth, tissue development**	**Implications in tissue-specific disorders**	**10q26.13**
FGF3	**Fibroblast growth factor, involved in embryonic development**	**Cell growth, tissue development**	**Implications in tissue-specific disorders**	**11q13.3**
MMP13	Matrix metalloproteinase, involved in extracellular matrix remodeling	Extracellular matrix	Tissue remodeling, cancer	11q22.3
FGF23	**Regulator of phosphate and vitamin D metabolism**	**Phosphate homeostasis, bone development**	**Implications in metabolic bone disorders**	**12p13.32**
COL2A1	Collagen type II alpha 1 chain, critical for cartilage formation	Extracellular matrix, cartilage development	Implications in genetic cartilage disorders	12q13.11
HOXC	Part of the HOX gene family, crucial for embryonic development	Developmental patterning	Implications in morphological anomalies	12q13.3
DUSP6	Dual-specificity phosphatase, regulates MAPK signaling pathway	MAPK signaling regulation	Implications in cellular signaling and diseases	12q21.33
IGF1	Insulin-like growth factor, plays a role in cell growth and development	Growth regulation	Growth disorders, metabolism, bone development	12q23.2
TBX5	Critical for heart and limb development	Heart development	Congenital heart defects	12q24.21
NCOR2	Functions as a transcriptional corepressor	Gene regulation	Metabolic disorders, cancer	12q24.31
TGFB3	Part of the TGF-beta pathway, regulates cell growth and differentiation	Regulates growth and differentiation of facial structures	May be involved in jaw malocclusions	14q24.3
NUMB	Regulates cell fate determination during development	Cell fate determination	Cancer, neurodevelopmental disorders	14q24.3
LTBP2	Part of the TGF-beta pathway, regulates the bioavailability of TGF-beta	Tissue elasticity	Connective tissue disorders	14q24.3
RORA	Involved in transcriptional regulation and circadian rhythm	Circadian rhythm	Sleep disorders, metabolic health, development	15q22.2
SMAD6	Negative regulator in the TGF-beta signaling pathway	Bone development	Osteoporosis, bone disorders, Jaw malocclusions	15q22.31
MYH8	Encodes a myosin protein, involved in muscle contraction; About 100k bp from MYH1	Muscle contraction	Muscle disorders, myopathies	17p13.1

MYH1	Encodes a myosin protein, crucial for muscle contraction	Muscle contraction	Muscle disorders, myopathies	17p13.1
MYO1H	Myosin motor protein, involved in intracellular transport	Actin-based motor activity	Implications in cellular dynamics and functions	17p13.1
COL1A1	Collagen type I alpha 1 chain, a major component of connective tissue	Extracellular matrix, tissue development	Implications in genetic connective tissue disorders	17q21.33
ERLEC1	Involved in endoplasmic reticulum (ER) membrane protein targeting	Protein folding, ER function	Potential role in ER-related disorders	17q23.2
NFATC1	Plays a role in immune response and cardiac development	Immune response	Immunological disorders, cancer	18q23
NOTCH3	Critical for vascular smooth muscle cell development	Vascular function	CADASIL syndrome, vascular disorders	19p13.2
JAG1	Ligand for Notch receptors, crucial for cell communication	Cell communication	Alagille syndrome, cancer	20p12.2
EP300	Histone acetyltransferase, involved in gene transcription	Epigenetic regulation	Potential role in epigenetic disorders	22q13.2
GLI2	Transcription factor, crucial in Hedgehog signaling pathway	Hedgehog signaling, development	Implications in Hedgehog-related disorders	2q14.2
HDAC4	Histone deacetylase, regulates gene expression and cell cycle	Epigenetic regulation	Potential role in epigenetic disorders	2q37.3
RASA2	Acts as a GTPase activating protein, regulating cellular signaling	Cell signaling control	Cancer, cell behavior regulation	3q22.1
FGF12	**Fibroblast growth factor, involved in nervous system function**	**Neural development**	**Potential role in neurodevelopmental disorders**	**3q28**
EVC2	Part of the EVC-EVC2 complex, implicated in ciliogenesis; About 130k bp from EVC2	Ciliogenesis, skeletal development	Implications in skeletal ciliopathies	4p16.2
EVC	Part of the EVC-EVC2 complex, implicated in ciliogenesis	Ciliogenesis, skeletal development	Implications in skeletal ciliopathies	4p16.2
GHR	Growth hormone receptor, involved in growth and development	Growth regulation	Implications in growth-related disorders	5p13.1
NOTCH4	Part of the Notch signaling pathway, involved in cell communication	Cell communication	Cardiovascular disease, cancer	6p21.32
FOXO3	Transcription factor, regulates cell cycle and apoptosis	Cellular stress response, longevity	Links to aging, longevity, and metabolic disorders	6q21
TCF21	Plays a role in cardiovascular development and tissue homeostasis	Involved in craniofacial development, including the jaw	Potential contribution to the etiology of malocclusions	6q23.2
CALN1	Calneuron 1, involved in calcium-dependent processes	Calcium signaling, neuronal function	Potential role in neurological	7p14.2
FGFR1	**Fibroblast growth factor receptor, plays a role in cell growth**	**Cell growth, tissue development**	**Implications in tissue-specific disorders**	**8p11.23**
FGF20	**Fibroblast growth factor, implicated in neural development**	**Neural development**	**Potential role in neurodevelopmental disorders**	**8p22**
SSX2IP	Scaffold protein, implicated in cell adhesion and migration	Cellular transport, nuclear localization	Implications in cellular localization processes	Xp11.23

Table 2: An analysis of Candidate Genes found to be related to Class III Skeletal Jaw Malocclusions

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Receptor Ligand Activity (GO:0048018)	7/317	6.12E-06	6.36E-04	0	0	11.72051282	140.6951926	JAG1;WNT3A;TGFB3;FGF20;IGF1;FGF23;FGF3
Fibroblast Growth Factor Receptor Binding (GO:0005104)	3/22	1.56E-05	7.08E-04	0	0	75.4002448	834.6529797	FGF20;FGF23;FGF3
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Type I Transforming Growth Factor Beta Receptor Binding (GO:0034713)	2/9	1.74E-04	0.003616750647	0	0	133.4155844	1154.996359	TGFB3;SMAD6
Histone H3 Acetyltransferase Activity (GO:0010484)	2/13	3.75E-04	0.00568670754	0	0	84.88429752	669.7057178	KAT6B;EP300
DNA-binding Transcription Factor Binding (GO:0140297)	5/279	3.83E-04	0.00568670754	0	0	9.025725476	71.01535203	HDAC4;TCF21;EP300;NFATC1;TBX5
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Sequence-Specific DNA Binding (GO:0043565)	7/713	9.54E-04	0.009427663999	0	0	5.045725285	35.0896615	HDAC4;TCF21;RORA;NFATC1;FOXO3;TBX5;GLI2
Transforming Growth Factor Beta Binding (GO:0050431)	2/21	9.97E-04	0.009427663999	0	0	49.12440191	339.4792176	TGFB3;LTBP2
Nuclear Androgen Receptor Binding (GO:0050681)	2/25	0.001416438825	0.01227580315	0	0	40.57312253	266.143837	TCF21;EP300
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Sequence-Specific Double-Stranded DNA Binding (GO:1990837)	6/705	0.004593710136	0.0281026973	0	0	4.260515021	22.93463898	HDAC4;TCF21;NFATC1;FOXO3;SMAD6;GLI2
Transmembrane Receptor Protein Tyrosine Kinase Activity (GO:0004714)	2/50	0.005582386826	0.03225379055	0	0	19.41761364	100.7412756	FGFR2;FGFR1
DNA-binding Transcription Activator Activity, RNA Polymerase II-specific (GO:0001228)	4/342	0.007038409807	0.03852603263	0	0	5.695970696	28.23135545	TCF21;NFATC1;FOXO3;TBX5
DNA Binding (GO:0003677)	6/840	0.01052988394	0.05255214019	0	0	3.546582734	16.14949916	HDAC4;EP300;RORA;FOXO3;TBX5;GLI2

RNA Polymerase II Cis-Regulatory Region Sequence-Specific DNA Binding (GO:0000978)	7/1106	0.01079044751	0.05255214019	0	0	3.177200719	14.38984079	HDAC4;TCF21;RORA;NFATC1;FOXO3;TBX5;GLI2
Myosin Light Chain Binding (GO:0032027)	1/5	0.01111679889	0.05255214019	0	0	114.1611111	513.6448477	MYH8
Transcription Regulatory Region Nucleic Acid Binding (GO:0001067)	3/216	0.01239385512	0.05363878522	0	0	6.662299378	29.25118841	HDAC4;FOXO3;SMAD6
Oxysterol Binding (GO:0008142)	1/6	0.01332561583	0.05363878522	0	0	91.32444444	394.3450784	RORA
MHC Class II Protein Binding (GO:0042289)	1/6	0.01332561583	0.05363878522	0	0	91.32444444	394.3450784	COL2A1
Chromatin DNA Binding (GO:0031490)	2/82	0.01446538565	0.05363878522	0	0	11.63238636	49.27475002	EP300;FOXO3
RNA Polymerase II Transcription Regulatory Region Sequence-Specific DNA Binding (GO:0000977)	7/1209	0.01696049716	0.05363878522	0	0	2.889564401	11.78037361	HDAC4;TCF21;RORA;NFATC1;FOXO3;TBX5;GLI2
Peptide N-acetyltransferase Activity (GO:0034212)	1/8	0.01772878286	0.05363878522	0	0	65.22539683	263.0257052	EP300
Histone H3K14 Acetyltransferase Activity (GO:0036408)	1/8	0.01772878286	0.05363878522	0	0	65.22539683	263.0257052	KAT6B
Type II Transforming Growth Factor Beta Receptor Binding (GO:0005114)	1/8	0.01772878286	0.05363878522	0	0	65.22539683	263.0257052	TGFB3
STAT Family Protein Binding (GO:0097677)	1/8	0.01772878286	0.05363878522	0	0	65.22539683	263.0257052	EP300
Growth Factor Receptor Binding (GO:0070851)	2/95	0.01909577774	0.05363878522	0	0	10	39.58288029	FGF20;FGF3
Potassium Ion Binding (GO:0030955)	1/9	0.0199231532	0.05363878522	0	0	57.06944444	223.4766821	HDAC4
MAP Kinase Tyrosine Phosphatase Activity (GO:0033550)	1/9	0.0199231532	0.05363878522	0	0	57.06944444	223.4766821	DUSP6
MAP Kinase Tyrosine/Serine/Threonine Phosphatase Activity (GO:0017017)	1/9	0.0199231532	0.05363878522	0	0	57.06944444	223.4766821	DUSP6
co-SMAD Binding (GO:0070410)	1/9	0.0199231532	0.05363878522	0	0	57.06944444	223.4766821	SMAD6
Protein Tyrosine/Threonine Phosphatase Activity (GO:0008330)	1/9	0.0199231532	0.05363878522	0	0	57.06944444	223.4766821	DUSP6
Transcription Coregulator Binding (GO:0001221)	2/99	0.02063030201	0.05363878522	0	0	9.585754452	37.20225851	EP300;RORA
Histone Deacetylase Binding (GO:0042826)	2/99	0.02063030201	0.05363878522	0	0	9.585754452	37.20225851	HDAC4;NCOR2
Protein Tyrosine Kinase Activity (GO:0004713)	2/99	0.02063030201	0.05363878522	0	0	9.585754452	37.20225851	FGFR2;FGFR1

Platelet-Derived Growth Factor Binding (GO:0048407)	1/10	0.02211272829	0.0547553272	0	0	50.72592593	193.3470354	COL2A1
Semaphorin Receptor Activity (GO:0017154)	1/10	0.02211272829	0.0547553272	0	0	50.72592593	193.3470354	PLXNA2
Nuclear Receptor Binding (GO:0016922)	2/115	0.02725809316	0.0659265509	0	0	8.222043443	29.61912881	TCF21;EP300
Insulin-Like Growth Factor Receptor Binding (GO:0005159)	1/13	0.02865278366	0.06621976668	0	0	38.03888889	135.1333308	IGF1
I-SMAD Binding (GO:0070411)	1/13	0.02865278366	0.06621976668	0	0	38.03888889	135.1333308	SMAD6
1-Phosphatidylinositol Binding (GO:0005545)	1/14	0.03082327931	0.06678377183	0	0	35.11111111	122.1685863	EPB41
Alkali Metal Ion Binding (GO:0031420)	1/14	0.03082327931	0.06678377183	0	0	35.11111111	122.1685863	HDAC4
MAP Kinase Phosphatase Activity (GO:0033549)	1/14	0.03082327931	0.06678377183	0	0	35.11111111	122.1685863	DUSP6
Cis-Regulatory Region Sequence-Specific DNA Binding (GO:0000987)	6/1081	0.03215061245	0.0682380346	0	0	2.717860465	9.342166404	HDAC4;RORA;NFATC1;FOXO3;TBX5;GLI2
Histone Deacetylase Activity (GO:0004407)	1/18	0.03945791778	0.08129910487	0	0	26.84444444	86.77521825	HDAC4
Zinc Ion Binding (GO:0008270)	3/339	0.03986783027	0.08129910487	0	0	4.19788206	13.52635487	HDAC4;MMP13;GLI2
R-SMAD Binding (GO:0070412)	1/19	0.04160479179	0.08320958359	0	0	25.35185185	80.60722529	SMAD6
Histone H4 Acetyltransferase Activity (GO:0010485)	1/20	0.04374697166	0.0858431142	0	0	24.01637427	75.15522985	EP300
bHLH Transcription Factor Binding (GO:0043425)	1/22	0.04801728905	0.08917496538	0	0	21.72698413	65.96734198	TCF21
Low-Density Lipoprotein Particle Receptor Binding (GO:0050750)	1/22	0.04801728905	0.08917496538	0	0	21.72698413	65.96734198	HSPG2
N-acetyltransferase Activity (GO:0008080)	1/22	0.04801728905	0.08917496538	0	0	21.72698413	65.96734198	EP300
Protein Tyrosine/Serine/Threonine Phosphatase Activity (GO:0008138)	1/23	0.05014544656	0.08991597314	0	0	20.73838384	62.06640681	DUSP6
Mitogen-Activated Protein Kinase Binding (GO:0051019)	1/23	0.05014544656	0.08991597314	0	0	20.73838384	62.06640681	NFATC1
SUMO Transferase Activity (GO:0019789)	1/24	0.0522689499	0.09213509812	0	0	19.83574879	58.54229227	HDAC4
Double-Stranded DNA Binding (GO:0003690)	4/646	0.05549471637	0.09361559104	0	0	2.953716066	8.5405739	TCF21;NFATC1;FOXO3;GLI2
NF-kappaB Binding (GO:0051059)	1/26	0.05650203381	0.09361559104	0	0	18.24711111	52.43268411	EP300

Acetyltransferase Activity (GO:0016407)	1/26	0.05650203381	0.09361559104	0	0	18.24711111	52.43268411	EP300
Lipoprotein Particle Receptor Binding (GO:0070325)	1/27	0.05861163422	0.09361559104	0	0	17.54444444	49.77046713	HSPG2
MHC Protein Binding (GO:0042287)	1/27	0.05861163422	0.09361559104	0	0	17.54444444	49.77046713	COL2A1
Cytokine Activity (GO:0005125)	2/177	0.0594043598	0.09361559104	0	0	5.292987013	14.94415421	WNT3A;TGFB3
Protein Homodimerization Activity (GO:0042803)	4/661	0.05940989431	0.09361559104	0	0	2.88410524	8.14267845	GHR;COL2A1;FGFR2;FGFR1
Frizzled Binding (GO:0005109)	1/33	0.07117267635	0.1088523285	0	0	14.25069444	37.65954485	WNT3A
Sodium Channel Regulator Activity (GO:0017080)	1/33	0.07117267635	0.1088523285	0	0	14.25069444	37.65954485	FGF12
N-acyltransferase Activity (GO:0016410)	1/36	0.07739156408	0.1166481546	0	0	13.02730159	33.33526886	EP300
Transition Metal Ion Binding (GO:0046914)	3/453	0.08017554663	0.119117955	0	0	3.116744186	7.865218386	HDAC4;MMP13;GLI2
Tau Protein Binding (GO:0048156)	1/39	0.08356971348	0.1224119747	0	0	11.99707602	29.77763172	EP300
Transcription Corepressor Binding (GO:0001222)	1/42	0.08970738544	0.1278023025	0	0	11.11761518	26.80681793	RORA
Promoter-Specific Chromatin Binding (GO:1990841)	1/42	0.08970738544	0.1278023025	0	0	11.11761518	26.80681793	GLI2
Damaged DNA Binding (GO:0003684)	1/45	0.09580483926	0.134644639	0	0	10.35808081	24.29427861	EP300
DNA-binding Transcription Repressor Activity, RNA Polymerase II-specific (GO:0001227)	2/242	0.1016679291	0.1409795283	0	0	3.847159091	8.794772548	TCF21;FOXO3
E-box Binding (GO:0070888)	1/51	0.1078801215	0.1476254294	0	0	9.112444444	20.29099583	TCF21
Myosin Binding (GO:0017022)	1/54	0.1138584605	0.1537828557	0	0	8.595387841	18.67605163	MYH8
Protein Serine/Threonine Phosphatase Activity (GO:0004722)	1/55	0.1158425172	0.1544566896	0	0	8.435802469	18.18357148	MYH8
Hydrolase Activity, Acting On Carbon-Nitrogen (But Not Peptide) Bonds, In Linear Amides (GO:0016811)	1/56	0.1178222281	0.1551077433	0	0	8.282020202	17.71174895	HDAC4
Sterol Binding (GO:0032934)	1/59	0.1237353782	0.1608559917	0	0	7.852490421	16.40864283	RORA
Acyltransferase Activity, Transferring Groups Other Than Amino-Acyl Groups (GO:0016747)	1/64	0.1335045755	0.1714132822	0	0	7.227513228	14.55346177	EP300

Hormone Activity (GO:0005179)	1/73	0.1508213348	0.1912855954	0	0	6.321296296	11.95773928	IGF1
Protein Tyrosine Phosphatase Activity (GO:0004725)	1/76	0.1565179997	0.1928662534	0	0	6.067555556	11.25279304	DUSP6
Cytokine Receptor Activity (GO:0004896)	1/76	0.1565179997	0.1928662534	0	0	6.067555556	11.25279304	GHR
Cadherin Binding (GO:0045296)	2/317	0.1576310725	0.1928662534	0	0	2.92034632	5.395333871	NOTCH3;NUMB
Amyloid-Beta Binding (GO:0001540)	1/79	0.1621772741	0.1950010909	0	0	5.833333333	10.611214	HSPG2
Endopeptidase Activity (GO:0004175)	2/324	0.1631259126	0.1950010909	0	0	2.855872388	5.178361792	MMP13;PSEN2
Calcium Ion Binding (GO:0005509)	2/343	0.1782124727	0.2106147405	0	0	2.694214876	4.64692463	MMP13;NOTCH4
Metalloendopeptidase Activity (GO:0004222)	1/99	0.1989678001	0.232501699	0	0	4.638321995	7.489091634	MMP13
Phosphatidylinositol Binding (GO:0035091)	1/106	0.2114672833	0.2443621941	0	0	4.327619048	6.723756719	EPB41
Serine-Type Endopeptidase Activity (GO:0004252)	1/120	0.2358964921	0.2676326823	0	0	3.815873016	5.511502603	MMP13
Protease Binding (GO:0002020)	1/122	0.2393253793	0.2676326823	0	0	3.752433425	5.365721755	COL1A1
Metallopeptidase Activity (GO:0008237)	1/122	0.2393253793	0.2676326823	0	0	3.752433425	5.365721755	MMP13
Serine-Type Peptidase Activity (GO:0008236)	1/137	0.2645666256	0.2927120113	0	0	3.336111111	4.435900728	MMP13
G Protein-Coupled Receptor Binding (GO:0001664)	1/140	0.2695156339	0.2945188694	0	0	3.263629097	4.27903836	WNT3A
Kinase Binding (GO:0019900)	2/457	0.2718635717	0.2945188694	0	0	2.007792208	2.615058825	GHR;FOXO3
Protein Kinase Binding (GO:0019901)	2/508	0.3141974441	0.3368714864	0	0	1.800844413	2.084898243	GHR;FOXO3
Metal Ion Binding (GO:0046872)	2/517	0.3216316547	0.3413233887	0	0	1.768578994	2.006184606	MMP13;NOTCH4
Ubiquitin-Like Protein Transferase Activity (GO:0019787)	1/239	0.4157493836	0.4367468272	0	0	1.896825397	1.66479176	HDAC4
Ubiquitin Protein Ligase Binding (GO:0031625)	1/268	0.4528679372	0.4709826547	0	0	1.688389513	1.337465731	SMAD6
ATP Binding (GO:0005524)	1/274	0.4602544362	0.4739253601	0	0	1.650793651	1.280975957	MYH8
Ubiquitin-Like Protein Ligase Binding (GO:0044389)	1/285	0.4735438197	0.4828289926	0	0	1.58599374	1.185547492	SMAD6

Adenyl Ribonucleotide Binding (GO:0032559)	1/304	0.4957481843	0.5005612734	0	0	1.485148515	1.042109665	MYH8
Purine Ribonucleoside Triphosphate Binding (GO:0035639)	1/469	0.6537552255	0.6537552255	0	0	0.9537037037	0.4053453136	MYH8

Table 3: A Gene Ontology Analysis on Candidate Genes (full list)

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