UNIVERSIDADE FERNANDO PESSOA

GENETICS AND EPIGENETICS OF TEMPOROMANDIBULAR DISORDERS – CRITICAL REVIEW OF THE LITERATURE

[Genética e Epigenética das Disfunções Temporomandibulares – Revisão Crítica da Literatura]

Dissertação de Mestrado

[Mestrado Integrado em Medicina Dentária]

Emmy Cohen-Akenine

Orientadora:

Doutora Cláudia Barbosa

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RESUMO

As disfunções temporomandibulares (DTM) constituem um grupo de condições músculo-esqueléticas que afetam os músculos da mastigação, as articulações temporomandibulares (ATM) e estruturas associadas, apresentando uma etiologia multifatorial e complexa. Sendo a segunda causa mais frequente de dor orofacial, a DTM pode comprometer significativamente a qualidade de vida, sobretudo quando os sintomas se tornam crónicos e há presença de dor. A investigação recente tem-se centrado cada vez mais no papel dos fatores genéticos e epigenéticos no início, progressão e variabilidade das DTM. Esta revisão narrativa da literatura analisa criticamente os principais genes e polimorfismos associados à suscetibilidade às DTM, explora o seu impacto funcional na fisiopatologia e nos mecanismos de dor, e avalia a relevância clínica e as limitações dos estudos genéticos atuais no diagnóstico e tratamento. Genes envolvidos no processamento da dor, na inflamação e na modulação psicológica, como COMT, ESR1, TNF-α e 5-HT2A, influenciam a vulnerabilidade individual às DTM. As modificações epigenéticas, em particular a metilação do DNA de genes como ZFP57, RNF39, ZNF718 e PM20D1, podem regular a expressão génica em resposta a fatores ambientais e psicossociais, contribuindo para a persistência da dor e a sensibilização central. Embora estes avanços ofereçam perspetivas promissoras para um diagnóstico e tratamento personalizados das DTM, a sua aplicação clínica continua limitada pela variabilidade metodológica e pela reduzida dimensão das amostras estudadas. A revisão sublinha a necessidade de investigação padronizada, de larga escala, que permita compreender melhor os mecanismos causais e potenciar abordagens terapêuticas personalizadas.

Palavras-chave: “disfunções temporomandibulares”, “genética”, “epigenética”, “DNA/genoma”, “polimorfismo”, “expressão génica”.

ABSTRACT

Temporomandibular disorders (TMDs) are a category of musculoskeletal conditions affecting the masticatory muscles, temporomandibular joints (TMJ), and related structures, with a multifactorial and complex etiology. As the second most common cause of orofacial pain, TMD can significantly reduce quality of life, especially when symptoms become chronic and painful. Recent research has increasingly focused on the role of genetic and epigenetic factors in the onset, progression, and variability of TMD. This narrative literature review critically examines key genes and polymorphisms associated with TMD susceptibility, explores their functional impact on pathophysiology and pain mechanisms, and evaluates the clinical relevance and limitations of current genetic studies in diagnosis and treatment. Genes involved in pain processing, inflammation, and psychological modulation, such as COMT, ESR1, TNF-α, and 5-HT2A, influence individual vulnerability to TMD. Epigenetic modifications, especially DNA methylation of genes like ZFP57, RNF39, ZNF718, and PM20D1, may regulate gene expression in response to environmental and psychosocial stressors, contributing to pain persistence and central sensitization. Although these insights offer promising avenues for personalized diagnosis and management of TMD, their integration into clinical practice remains limited by methodological variability and small study cohorts. The review highlights the need for standardized, large-scale research to better understand causal mechanisms and enhance personalized therapeutic approaches.

Keywords: “temporomandibular joint disorders“, “genetic, epigenetic”, ”DNA/genome”, ”polymorphism”, “gene expression“.

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LIST OF ACRONYMS, INITIALISMS, AND ABBREVIATIONS

1082A/G IL-10	Single Nucleotide Polymorphism Position 1082, Adenine or Guanine of Interleukin-10
158Met	Catechol-O-Methyltransferase Gene val158met Polymorphism
174G/C IL-6	Single Nucleotide Polymorphism Position 174, Guanine or Cytosine of Interleukin-6
308G/A TNF-α	Single Nucleotide Polymorphism within Tumor Necrosis Factor Alpha Gene Promoter
5-HT1A	Serotonin 1A Receptor
5-HT2A	Serotonin 2A Receptor
5-HTT	Serotonin Transporter
AAOP	American Academy of Orthotists and Prosthetists
ACTN3	Alpha-Actinin-3 Gene
AGTGC	Adenine, Guanine, Thymine, Guanine and Cytosine
ANKH	Ankyrin Repeat and Kinase Domain Gene
ANKH-OR	Single Nucleotide Polymorphism Ankyrin Repeat and Kinase Domain Gene
APOL3	Apolipoprotein L3
BOLDSV	Blood-Oxygen-Level-Dependent Signal Variability
CAMK4	Calcium/Calmodulin-Dependent Protein Kinase IV
CCL20	Chemokine (C-C Motif) Ligand 20
CHRM2	Cholinergic Receptor Muscarinic 2
CHST11	Carbohydrate Sulfotransferase 11
COMT	Catechol-O-Methyltransferase
CT	Computed Tomography

CXCL1	Chemokine (C-X-C Motif) Ligand 1
CXCL8	Chemokine (C-X-C Motif) Ligand 8
DC/TMD	Diagnostic Criteria for Temporomandibular Disorders
DDWOR	Disc Displacement without Reduction
DDWR	Disc Displacement with Reduction
DEAF-1	Deformed Epidermal Autoregulatory Factor 1
dlPFC	Dorsolateral Prefrontal Cortex
DNA	Deoxyribonucleic Acid
DRD1	Dopamine Receptor D1
DRD2	Dopamine Receptor D2
DRD3	Dopamine Receptor D3
DRD4	Dopamine Receptor D4
DRD5	Dopamine Receptor D5
ENPP1	Ectonucleotide Pyrophosphatase / Phosphodiesterase 1
ESR1	Estrogen Receptor Alpha
ESRRB	Estrogen Related Receptor Beta
FMOD	Fibromodulin
GDF5	Growth/Differentiation Factor 5
GPCR	G Protein-Coupled Receptor
GRK5	G Protein-Coupled Receptor Kinase 5
GWAS	Genome-Wide Association Studies
HPA	hypothalamic-Pituitary-Adrenal
HTR2A	5-Hydroxytryptamine Receptor 2A
IFRD1	Interferon-Related Developmental Regulator 1
IL-10	Interleukin-10
IL-17A	Interleukin-17A

IL-17RC	Interleukin 17 Receptor C
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL6−174	Specific Location within the Interleukin-6 Gene's Promoter Region
IL-8	Interleukin-8
MAOB	Monoamine Oxidase B
MMP	Matrix Metalloproteinase
MMP-13	Matrix Metalloproteinase 13
MMP3	Matrix Metalloprotease 3
MRC2	Mannose Receptor C-Type 2
MRI	Magnetic Resonance Imaging
MTHFD1	Methylenetetrahydrofolate Dehydrogenase 1
MTRR	5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase
n	Number
NF-κB	Nuclear Factor-Kappa B
ng/mL	Nanograms per Milliliter
NR3C1	Nuclear Receptor Subfamily 3, Group C, Member 1
OPG	Osteoprotegerin
OPG/RANKL/RANK	Osteoprotegerin / Receptor Activator of Nuclear Factor Kappa-B / Receptor Activator of Nuclear Factor Kappa-B Ligand
OPPERA	Orofacial Pain: Prospective Evaluation and Risk Assessment
PCR	Polymerase Chain Reaction
PI3K/Akt	Phosphatidylinositol 3-Kinase / Protein Kinase B
PITX1	Paired-Like Homeodomain Transcription Factor 1

PITX2 Paired-Like Homeodomain Transcription Factor 2

PM20D1 Palmitoyl-Protein Thioesterase 1

PPi Inorganic Pyrophosphate

RANK Receptor Activator of Nuclear Factor Kappa-B

RANKL Receptor Activator of Nuclear Factor Kappa-B Ligand

RCD Rotator Cuff Disease

RDC/TMD Research Diagnostic Criteria for Temporomandibular Disorders

RNF39 Human RING Finger Protein 39

rs1406846 Single Nucleotide Polymorphism in the Runt-Related Transcription Factor 2

rs1800497 Single Nucleotide Polymorphism in the Ankyrin Repeat and Kinase Domain Containing 1 Gene

rs1800629 Single Nucleotide Polymorphism in the Tumor Necrosis Factor-Alpha Gene

rs2460300 Mannose Receptor C-Type 2

rs4646310 Catechol-O-Methyltransferase Genetic Polymorphisms

rs4680 Catechol-O-Methyltransferase Gene val158met Polymorphism

rs4818 Single Nucleotide Polymorphism in the Catechol-O-Methyltransferase Gene

rs6269 Single Nucleotide Polymorphism in the Catechol-O-Methyltransferase Gene

rs6280 Dopamine Receptor D3 Ser9Gly Polymorphism

rs80575 Apolipoprotein L3

rs878962 Intron Variant Allele t

rs9332377 3 Prime utr Variant Allele t

RUNX2 Runt-Related Transcription Factor 2

SHMT1	Serine Hydroxymethyltransferase 1
SMAD3	SMAD Family Member 3 Intracellular Signal Transducer Proteins
SNPs	Single Nucleotide Polymorphisms
TAK1	Transforming Growth Factor β-Activated Kinase 1
TGFβ	Transforming Growth Factor Beta
TGFβ1	Transforming Growth Factor Beta 1
Th17	T Helper 17
TMD	Temporomandibular Disorder
TMJ	Temporomandibular Joint
TMJOA	Temporomandibular Joint Osteoarthritis
TNF	Tumor Necrosis Factor
TNFA-308	Tumour Necrosis Factor-Alpha 308 Promoter Gene Polymorphism
TNF-α	Tumour Necrosis Factor Alpha
TSPAN9	Tetraspanin 9
Val158Met	Catechol-O-Methyltransferase Gene val158met Polymorphism
VD	Vitamin D
VDR	Vitamin D Receptor
ZFP57	Zinc Finger Protein 57
ZNF718	Zinc Finger Protein 718

1. INTRODUCTION

Temporomandibular disorders (TMDs) represent a significant cause of orofacial pain and functional impairment worldwide, affecting millions of individuals and posing ongoing challenges for diagnosis and treatment. These disorders encompass a range of musculoskeletal conditions involving the temporomandibular joints (TMJ), masticatory muscles, and associated structures (Sabsoob et al., 2022).

TMDs are among the most common causes of non-dental orofacial pain and rank second only to low back pain among musculoskeletal conditions (Wieckiewicz et al., 2018; Cao et al., 2022). The global prevalence of TMDs ranges between 25% and 35%, with an annual incidence of approximately 3.5% (Braga et al., 2021; Ferrillo et al., 2022).

These disorders primarily affect adults aged 18 to 60, with symptoms peaking between 20 and 40 years. Women are disproportionately affected, likely due to hormonal influences on pain perception and inflammatory regulation (Bonato et al., 2016; Cao et al., 2022). Prevalence also varies geographically, being highest in South America, followed by Asia and Europe, suggesting a role for environmental and regional factors in disease development (Zieliński et al., 2024).

Clinically, TMDs manifest with pain, limited jaw movement, and joint sounds, often accompanied by comorbid conditions such as headaches, neck pain, and psychological distress, including anxiety and depression (List & Jensen, 2017; Kandasamy & Greene, 2020). These symptoms can become chronic, especially in patients with high initial pain, psychological comorbidities, and female sex, significantly impacting quality of life (Cao et al., 2022; Sabsoob et al., 2022).

The clinical heterogeneity of TMDs reflects their complex, multifactorial etiology, which includes biomechanical factors such as occlusal abnormalities and internal joint derangements; psychological and behavioral factors like stress and parafunctional habits such as bruxism; as well as systemic conditions and trauma (Gui & Barbosa, 2015; List & Jensen, 2017; Rinchuse & Greene, 2018; Kandasamy & Greene, 2020). More recently, genetic factors influencing inflammation and pain modulation have emerged as important contributors to individual susceptibility and TMD chronicity (Braga et al., 2021; Campello et al., 2023).

Given this complexity, standardized diagnostic criteria such as the Diagnostic Criteria for TMD (DC/TMD) enable precise classification into pain-related and intra-articular disorders, facilitating targeted management (Schiffman et al., 2014; Klasser et al., 2023). Management requires a multidisciplinary approach integrating dental, medical, psychological, and physical therapies to address the diverse causes and symptoms of TMD (Wieckiewicz et al., 2018).

Among the various etiological factors, genetic and epigenetic influences have recently emerged as pivotal modulators of TMD susceptibility and clinical manifestation. Genetics encompasses inherited gene variants and polymorphisms that can irreversibly affect biological pathways, whereas epigenetics refers to reversible modifications in gene expression regulated by environmental and lifestyle factors, such as stress, that do not alter the underlying DNA sequence (Alshahrani et al., 2024; Murray & Sessle, 2024). These mechanisms interact closely with environmental and psychosocial factors, collectively influencing the musculoskeletal and neuroanatomical structures involved in pain perception and modulation in TMD (Toghill et al., 2015; Polonowita et al., 2024).

Several genetic factors have been identified as key contributors to TMD susceptibility and progression. These include genes related to inflammatory and structural changes within the TMJ, genes influencing the onset, chronicity, and intensity of pain in painful TMD subtypes, as well as genes affecting etiologic factors or an individual's adaptability and resilience to developing TMD (Alshahrani et al., 2024). This multifaceted genetic involvement highlights the complexity of TMD pathophysiology, and underlines the importance of considering genetic profiles in understanding patient variability (Li et al., 2023).

Building on this growing knowledge, emerging research suggests that targeting genetic and epigenetic mechanisms may open promising avenues for future treatments. Personalized therapies tailored to a patient's molecular and epigenetic profile could potentially improve diagnostic accuracy and therapeutic efficacy, moving beyond symptom management to address underlying biological processes (Ao et al., 2024). Such precision medicine approaches hold the potential to revolutionize TMD care by enabling more effective, individualized interventions that account for the interplay between genetic predisposition and environmental influences (List & Jensen, 2017). Realizing this potential will require deeper investigation into the functional consequences of specific polymorphisms and epigenetic modifications, paving the way for truly personalized

diagnostic and therapeutic approaches (Kandasamy & Greene, 2020).

Therefore, this critical literature review aims to (1) identify key genes and polymorphisms implicated in TMD susceptibility, (2) elucidate their functional consequences on TMD pathophysiology and pain mechanisms, and (3) assess the clinical relevance and limitations of current genetic research in diagnosis and treatment of TMDs, while proposing future research directions.

2. DEVELOPMENT

2.1. Temporomandibular disorders

2.1.1. Definition, epidemiology, etiology and symptoms

Temporomandibular disorders (TMDs) are a category of musculoskeletal conditions that impact the masticatory muscles, temporomandibular joints (TMJ), and related structures (Sabsoob et al., 2022). Initially considered a single entity, TMDs are now recognized as a complex and heterogeneous group of conditions. Ranking among the most common nondental pains in the orofacial area (Cao et al., 2022), they are the second most common musculoskeletal disorders after low back pain (Wieckiewicz et al., 2018).

The global impact of TMDs is significant, with a prevalence from 25.2% to 34.9% in the world population (Ferrillo et al., 2022) and an annual incidence of 3,5% (Braga et al., 2021).

However, the groundbreaking shift occurred in 2006 with the launch of the OPPERA project (Orofacial Pain: Prospective Evaluation and Risk Assessment). Initiated by the National Institute of Dental and Craniofacial Research, this series of longitudinal prospective studies marked a real turning point in the evolution of the field of TMD. Grounded in four american research centers, they highlighted the risk factors of the TMDs, but also notably theirs prevalences (Kandasamy & Greene, 2020).

Among the demographic findings, OPPERA study revealed a notable contrast compared to the US population, particularly based on the age, sex and race. For instance, women exhibited a significantly higher incidence rate than men (hazard ratio = 1.3, P=0.06) (Dubner et al., 2016).

Recent studies confirm that TMDs are more prevalent in women (Cao et al., 2022), with rates between 9% and 56% (Zieliński et al., 2024). This gender difference is likely due to hormonal influences; estrogen modulates pain responses, inflammatory regulation, and nociceptive signaling in the nervous system (Bonato et al., 2016). Additionally, TMD symptoms were twice as frequent in African Americans compared to whites, but symptoms persisted in 61% of white patients versus 35% of African Americans (Dubner et al., 2016).

Although symptoms peak between ages 20 and 40 (Cao et al., 2022), TMDs affect

individuals from 18 to 60 years (Zieliński et al., 2024). Prevalence is highest in South America (47%), followed by Asia (33%) and Europe (29%), highlighting environmental and regional influences (Zieliński et al., 2024). These differences underscore the multifactorial etiology of TMDs, involving biological, psychological, and environmental factors (Kandasamy & Greene, 2020).

Historically, occlusal factors were among the first to be recognized as potential etiologies. In 1934, Dr. J.C. Costen emphasized the relationship between occlusion and TMDs, suggesting that conditions like deep overbites, reduced vertical dimension, mandibular misalignment, and condylar malpositions were significant contributors to thesis disorders. This perspective dominated early TMD research and treatment strategies, focusing primarily on mechanical and structural abnormalities (Rinchuse & Greene, 2018; Kandasamy & Greene, 2020).

In the 1970s, developments in imaging techniques shifted the focus towards internal derangements of the TMJ. Studies revealed that disk displacements, particularly antero-medial dislocations, were frequently associated with TMJ dysfunction. This discovery highlighted the importance of joint mechanics and introduced a deeper understanding of TMD pathophysiology (Kandasamy & Greene, 2020).

Subsequent research expanded the scope of TMD etiology to include comorbid conditions and systemic factors. The OPPERA study demonstrated significant associations between TMDs and conditions such as fibromyalgia, irritable bowel syndrome, chronic headaches, tinnitus, and lower back pain. These coexisting disorders, often without clear structural abnormalities, pointed to shared underlying mechanisms like central sensitization (Kandasamy & Greene, 2020).

In recent years, the role of psychological and behavioral factors has gained prominence. Psychological distress, including anxiety, depression, somatization, and poor sleep quality, has been shown to significantly influence TMD onset and chronicity. Studies suggest that these factors, which alter pain processing, may have a stronger impact on TMD persistence than mechanical behaviors (Gui & Barbosa, 2015).

Trauma, both macro and microtrauma, is another well-documented etiological factor. Macrotrauma can result from injuries or prolonged mouth opening, such as during dental procedures or intubation. The OPPERA study suggested that trauma due to extended mouth opening may contribute to the onset of TMD pain, although the cause-effect

relationship remains unclear. Microtrauma often arises from parafunctional habits, such as bruxism, jaw bracing, tongue thrusting, nail biting, or chewing on pens (List & Jenser, 2017). Bruxism refers to involuntary clenching or grinding of the teeth, which may occur during sleep or while awake. It can lead to tooth wear, muscle fatigue, and jaw discomfort. This behavior is considered a potential cause of the onset of TMDs (Oporto et al, 2018).

Finally, genetic contributions to TMD etiology have become an area of increasing interest. Variants genes involved in pain modulation have been identified as potential risk factors for chronic TMD. These results highlighted the link between genetic predispositions, central and peripheral pain pathways, and other etiological factors, showing the multifaceted nature of TMDs (Braga et al., 2021).

Smith et al. (2011) synthesized these concepts and proposed an integrative model for the development of TMDs. This model is based on two major mechanisms: psychological distress (including anxiety, depression, and stress) and pain amplification (increased sensitivity to pain). These mechanisms are influenced by environmental factors, such as trauma and infections, and are regulated by genetic pathways related to neurotransmission, inflammation, and stress response (List & Jenser, 2017).

These causes lead to diverse symptoms affecting the masticatory system, primarily pain, limited jaw movement, and joint sounds. Pain, the most common complaint, is usually localized in the temple, cheek, or ear, and worsens with chewing, talking, or yawning. It may be moderate to severe, intermittent or persistent (List & Jensen, 2017). TMDs often coexist with tension-type headaches, neck and back pain, and psychosocial factors like anxiety and depression, underscoring their multifactorial nature (Kandasamy & Greene, 2020).

The transition from acute to chronic pain is common. Risk factors include myofascial pain, high baseline pain intensity, female gender, and greater pain-related disability. Psychological conditions such as depression and somatization also contribute to pain persistence (Sabsoob et al., 2022). Genetic predispositions may further explain individual susceptibility to TMD chronification (Campello et al., 2023).

Whether acute or chronic, TMDs significantly impact patients' quality of life, as reflected in OHRQoL scores (Cao et al., 2022). Thus, a multidisciplinary approach is essential for effective management (Wieckiewicz et al., 2018).

2.1.2. Classification, diagnostic criteria and management

Given the complexity and variability of TMDs, a standardized classification system is crucial to ensure accurate diagnosis and effective patient management. These systems are derived from an understanding of the disorder's etiology, pathophysiology, and management. Several organizations have developed TMD classification systems, typically focusing on musculoskeletal and neuromuscular conditions affecting the TMJs and related structures (Klasser et al., 2023).

The first version, Research Diagnostic Criteria for TMD (RDC/TMD) introduced in 1992, was updated as the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) in 2014. This classification is now the most widely recognized evidence-based system for diagnosing TMDs. It divides TMDs into two major categories: pain-related TMDs and intra-articular TMDs. The pain related TMds are subclassified into myalgia (including local myalgia, myofascial pain, and myofascial pain with referral), arthralgia, and headache attributed to TMD. In another hand, intra-articular TMDs, are divided into disc displacement (with or without reduction or locking), degenerative joint disease, and subluxation (Schiffman et al., 2014; Klasser et al., 2023).

Considering the pain-related TMDs, myalgia is the most common diagnosis for TMD, affecting approximately 80% of patients. It is characterized by muscle-origin pain that worsens with jaw movement, function, or parafunction. Diagnosis is based on both history and physical exam findings. Patients typically report pain in the jaw, temple, ear, or in front of the ear, which is modified by jaw movement or function. On examination, pain is confirmed in the temporalis or masseter muscles, and provocation tests, such as palpation of these muscles or testing maximum jaw opening, reproduce the pain (List & Jensen, 2017)

Moreover, arthralgia refers to joint pain, which can arise from various causes including mechanical, metabolic, infectious, neuropathic, or inflammatory factors. When inflammation is present, it is classified as arthritis, which can affect the TMJ in forms ranging from osteoarthritis and post-traumatic arthritis to more inflammatory conditions like infectious or rheumatoid arthritis (Ahmad & Schiffman, 2016).

Headache attributed to TMD involves pain in the temple area associated with jaw function and is reproducible by masticatory system provocation. It is diagnosed only after excluding other headache types and has strong diagnostic sensitivity and specificity,

aiding in proper treatment targeting the underlying TMD rather than mistaking it for a primary headache disorder (List & Jensen, 2017).

Considering the intra-articular TMDs, temporomandibular joint osteoarthritis (TMJOA) is the most common degenerative condition, involving cartilage breakdown, bone changes, and inflammatory responses, all of which contribute to joint damage and chronic symptoms. Degeneration of the TMJ could affect the articular cartilage, subchondral bone, joint capsule, ligaments, and synovial membrane, leading to joint dysfunction and pain (Yamaguchi et al., 2014).

Disc displacement disorders involve misalignment of the articular disc relative to the condylar head and glenoid fossa. Two main types are described: displacement with reduction (DDWR), where the disc returns to position during mouth opening, and without reduction (DDWOR), where it remains displaced. TMJ disc displacement classification includes four progressive stages based on MRI and tomography findings: Stage I, disc returns to position upon opening; Stage II, intermittent locking; Stage III, with persistent displacement limiting movement and causing pain; Stage IV, displacement with degenerative changes such as disc or posterior attachment perforation. Patients may be asymptomatic, but DDWR often presents with joint sounds (clicking or popping), while DDWOR typically causes jaw deviation, reduced contralateral movement, pain, and functional limitations like difficulty eating (Ahmad & Schiffman, 2016).

The updated DC/TMD classification made significant contributions by incorporating the influence of function, movement, and parafunction on TMDs. Refining the dual-axis model introduced by the RDC/TMD, it divides the classification into Axis I, which focuses on physical diagnoses, and Axis II, which integrates psychological and functional factors (Schiffman et al., 2014). The Axis II uses validated questionnaires and interpretation guidelines to assess the psychosocial effects of pain, pain behaviors, jaw function, and emotional distress Also, in clinical practice, the version improved diagnostic precision by introducing provocation tests to confirm that the pain reported during the clinical examination matched the patient's primary complaint. These tests, along with a structured clinical exam and patient questionnaires, ensure accurate diagnosis of common TMD conditions. The 30-day pain timeframe in the questionnaires ensures clinical relevance, and structured exams help reduce false positives (List & Jensen, 2017).

In addition to the DC/TMD, other classification systems, such as the one proposed by the

American Academy of Orofacial Pain (AAOP), further contribute to standardizing diagnostic criteria for TMD, offering complementary perspectives. Outlined in the AAOP Guidelines (6th edition, 2018), AAOP categorizes TMD into joint and muscle disorders, offering diagnostic criteria based on clinical features, history, and tests for common conditions. Developed in collaboration with international organizations, it includes 52 clinical entities and integrates ICD-10 codes for enhanced alignment with broader medical classifications (Klasser et al., 2023).

Despite their advancements, we must keep in mind that TMD classification systems have limitations, with issues such as overlapping symptoms. However, these diagnostic criteria remain essential for helping the clinicians with an accurate guide to patient care, providing a standardized classification system that ensures effective management. (Klasser et al., 2023).

The classification and diagnostic system must also consider psychological factors. Chronic TMD progression is marked by psychological distress and increased pain sensitivity, with evidence pointing to an interplay between these elements. Research indicates that psychological factors such as somatization, catastrophizing, depression, and poor sleep, which are linked to altered pain processing, are more strongly associated with the development and persistence of TMD than mechanical factors like clenching (Gui & Barbosa, 2015). Studies have found that individuals with TMD-related pain report significantly higher levels of stress, anxiety, pain catastrophizing, and kinesiophobia compared to controls. Psychological distress, including depression, perceived stress, mood disturbances, and life dissatisfaction, contributes significantly to both the onset and persistence of TMD pain. The OPPERA study identified perceived stress and prior stressful events as key predictors of TMD onset, emphasizing a multifactorial pathophysiological model (List & Jensen, 2017). Despite this recognition of the psychosocial dimension, no current diagnostic system yet incorporates genetic profiling, even though genetic and epigenetic factors are increasingly recognized as contributors to individual vulnerability and chronicity in TMD (Klasser et al., 2023).

The socio-economic impact of TMD treatment is very high and has an estimation of an annual $4 billion costs worldwide. Stowell et al. (2007) highlighted that conventional treatments often lead to unnecessarily high expenditures, whereas an early biopsychosocial intervention can be a cost-effective alternative. This approach, which integrates biological, psychological, and social factors, aims to optimize patient outcomes

while reducing long-term financial strain on health care systems (Stowell et al., 2007; Zieliński, et al., 2024).

These findings highlight the necessity of a multidisciplinary approach, not limited to symptom management but addressing the patient's psychological well-being. Chronic TMD pain can exacerbate emotional distress, reinforcing the need for care that incorporates both physical and psychological support (Cao et al., 2022). The biopsychosocial model had also led the dentist to work with other professionals, such as physicians, psychologists, physical therapists, and orofacial pain clinicians (Kandasamy & Greene, 2020).

The need for TMD treatment is significant, with some studies estimating that up to 15% of the adult population requires care. However, only a small proportion of patients with TMD-related pain receive treatment (List & Jensen, 2017).

The TMDs management process involves 2 distinct phases: a first reversible phase and a second irreversible one. The first phase refers to a conservative, reversible, and symptomatic therapy, such as physical therapy, pharmacological drugs, and occlusal splints, which aim to alleviate symptoms temporarily (Kandasamy & Greene, 2020; Ferrillo et al., 2022). If the initial phase of treatment proves effective, it could be followed by a second phase considered the "definitive" TMD treatment, involving invasive and permanent procedures, like orthodontics, occlusal adjustments, or even TMJ surgeries. However, these approaches are rarely necessary and should not be routinely implemented (Kandasamy & Greene, 2020).

Furthermore, keeping in mind the biopsychosocial model, studies show the importance of the patient expectations, the environment and the relationship between patient-professional, in influencing the overall management of the TMD patient. It is well-established that psychosocial elements can affect the perception of pain, with numerous studies examining the placebo effect as an example. These effects are mediated through the activation of the endogenous opioid system, driven by psychological processes that interact with emotions and motivations. This interplay is an essential factor to consider in our approach (Kandasamy & Greene, 2020).

Importantly, emerging research suggests that genetic predispositions and epigenetic mechanisms may play a role in how individuals respond to these psychosocial factors (Braga et al., 2021).

While these treatments are important, they highlight the need to consider genetic and epigenetic factors in TMD, as these may play a key role in the progression and persistence of the disorder (Gui & Barbosa, 2015).

2.2. Methodology

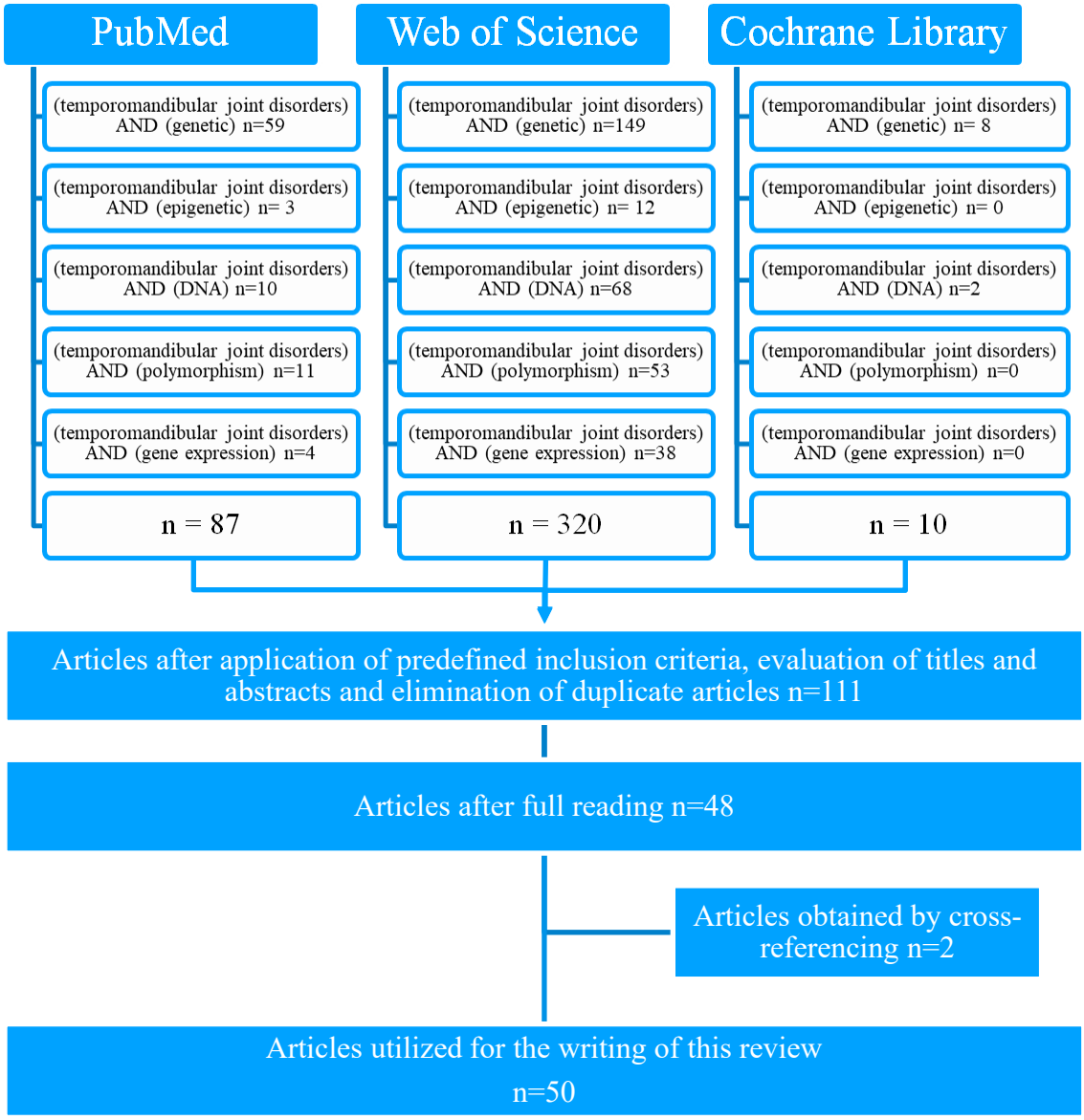
The research strategy of this work is illustrated in the flow chart below (cf. Figure 1).

In this critical literature review, the bibliographic research process was conducted using the databases PubMed, Cochrane Library, and Web of Science, operating with the following keywords: “temporomandibular joint disorders,” “genetic,” “epigenetic,” “DNA/genome,” “polymorphism,” “gene expression,” combined in different ways using the Boolean operators "AND" and "OR." The search was performed in September 2024 and included articles published between January 2014 and September 2024, written in English, French, and Portuguese that align with the objectives of the study. The selected publications included reviews, systematic reviews, clinical trials, comparative studies, and meta-analyses.

The exclusion criteria applied were as follows: articles that do not mention genetic polymorphisms, mutations, or epigenetic factors and focusing solely other TMD etiologies, studies unrelated to TMD or focused on other pathologies, and descriptive studies without a functional or critical perspective of topic.

Figure 1

Flow chart of the research strategy



Based on predefined inclusion criteria, after evaluating titles and abstracts and eliminating duplicate articles, 111 articles were selected for full reading as they aligned with the objectives of the study. The temporal organization of the publications was as follows: 20 articles from 2024, 8 articles from 2023, 11 articles from 2022, 14 articles from 2021, 11 articles from 2020, 8 articles from 2019, 10 articles from 2018, 6 articles from 2017, 12 articles from 2016, 6 articles from 2015, 5 articles from 2014.

After full reading of these 111 articles, *48 articles* directly addressed the topic of this review, so they were incorporated in this work.

Moreover, due to their importance in the development, 2 articles were selected outside the time frame, which were obtained by cross-referencing.

In total, 50 articles were utilized for the writing of this critical review.

2.3. Literature review

2.3.1. Genes associated with temporomandibular disorders

2.3.1.1. Influence of genetics and epigenetics on temporomandibular disorders pathophysiology

Genetic and epigenetic changes significantly influence TMD onset and progression (Alshahrani et al., 2024). Genetics involves irreversible gene variations, while epigenetics refers to reversible gene expression changes without DNA sequence alteration. Epigenetic changes are affected by stress, lifestyle, and environmental factors impacting organism development and survival. (Toghill et al, 2015; Murray & Sessle, 2024).

Both genetics and epigenetics, along with environmental influences, significantly affect the structure and function of the musculoskeletal components of the sensorimotor system. Many genes, genetic variations, and epigenetic factors contribute to bone structure, metabolic processes, and muscle function, influencing the progression of disease (Murray & Sessle, 2024). These factors contribute to unique neuroanatomy, which plays a crucial role in an individual's sensitivity to pain. Furthermore, genetic differences can affect the chronic nature of disease, making some individuals more prone to temporary neuroplastic changes (Polonowita et al., 2024). In TMDs, genetic variability between individuals in the trigeminal pain expression and in the modulation pathways, has been identified (Korczeniewska et al., 2023). Additionally, genetic and epigenetic factors interact with

psychosocial elements, which can modulate pain perception and sensorimotor behavior (Murray & Sessle, 2024). In the case of TMDs, genetic differences may influence how psychosocial factors, such as stress, affect the development and progression of the disorder (Slade et al., 2011). The biopsychosocial model described a dynamic and interactive perspective on human disease experience, recognizing the complex relationship between the mind and body. The model emphasizes how biological, psychological, and social factors interact and influence one another in shaping a person's overall health and well-being. These factors, including genetic and epigenetic influences, are interconnected in a continuous loop, where each factor influences the others, highlighting the complex, bidirectional nature of human experiences and interactions (Polonowita et al., 2024).

Understanding these genetic influences and their clinical implications requires a thorough identification of the key genes involved, alongside a review of the research developments leading to these critical findings (Alshahrani et al., 2024).

2.3.1.2. Genetic factors related to inflammatory and structural changes in temporomandibular joints

Considering the inflammatory and degenerative nature of TMD, seven studies that identified different genes in this context are presented (cf. Annex A).

The ANKH gene is a key genetic factor in the pathophysiology of structural changes in TMD. It encodes a protein essential for skeletal development and bone remodeling, regulating osteoblasts, osteoclasts, and pyrophosphate transport, a molecule that controls bone mineralization and prevents excessive tissue calcification. Histological examination of TMJ in ank mutant mice showed joint space narrowing, a hallmark of fibrous ankylosis. In a cohort of 55 TMD patients, the ANKH-OR polymorphism was strongly associated with TMJ closed lock, especially in homozygotes for this variant. This polymorphism was also more frequent in older patients, suggesting that ANKH variations may affect not only TMD onset but also its progression and severity. These findings highlight ANKH’s crucial role in joint integrity and cartilage metabolism, central to TMD-related inflammation (Huang et al., 2011).

Yamaguchi et al. (2014) were the first to use a genome-wide association study (GWAS) to investigate the genetic factors involved in TMJ bone changes. GWAS is a powerful method for identifying genes linked to common diseases. The researchers used an Illumina Human Omni Express Bead Chip to study the genomes of 146 patients with TMJ

degenerative bone changes, such as osteophytes, erosion, and/or flattening, or temporomandibular joint osteoarthritis (TMJOA), and 374 healthy controls. All individuals were diagnosed based on signs of degenerative changes observed on panoramic radiographs, magnetic resonance imaging (MRI), and/or computed tomography (CT) scans of one or both mandibular condyles. After inspection, the study analyzed around 550,000 single nucleotide polymorphisms (SNPs) to find any connections to TMJ bone changes. This led to the identification of 41 SNPs located in 22 different regions of the genome, providing valuable candidates for future research into the genetic causes associated with degenerative bone changes in the TMJ. While no significant associations were found with known TMD-related genes, the study highlighted new loci, such as TSPAN9, APOL3, and MRC2, which may play a role TMJOA and other degenerative bone changes in the TMJ (Yamaguchi et al., 2014).

Xiao et al. (2015) conducted a case-control study (n=240) to examine the association between specific genetic polymorphisms and TMJOA in Han Chinese women. The study analyzed five SNPs in GDF5, SMAD3, RUNX2, TGFβ1, and CHST11 genes involved in the Transforming Growth Factor Beta (TGFβ) signaling pathway and critical for cartilage formation, bone remodeling, and joint function. Significant associations were identified for SNPs in GDF5 and SMAD3, with a weaker correlation for RUNX2. Triple combinational analysis revealed a fivefold increase in TMJ OA risk among individuals carrying five or six risk alleles, suggesting that SNPs of genes related to TGFβ signaling pathway family might contribute to the risk of TMJ OA in that population (Xiao et al., 2015).

Hattori et al. (2015) investigated the role of interleukin-17A (IL-17A), a pro-inflammatory cytokine primarily produced by Th17 cells, in the inflammatory response of the TMJ. In this study, synovial fibroblasts were isolated from the TMJ of three TMD patients (n=3), who underwent either arthroscopy for internal derangement or open joint surgery for TMJOA. The researchers demonstrated that IL-17A receptors are expressed on synovial fibroblasts. When exposed to 10 ng/mL of IL-17A, these cells showed a significant increase in the expression of pro-inflammatory mediators, particularly IL-6 and the chemokines CXCL1, IL-8, and CCL20, as confirmed through DNA microarray analysis and real-time PCR. At the protein level, IL-6 secretion increased in a dose- and time-dependent manner, validating the inflammatory response. Additionally, pathway analysis revealed that IL-17A-induced inflammation is mediated through the activation

of NF-κB and the PI3K/Akt signaling pathway. These findings suggest that genetic polymorphisms in IL-17A play a critical role in the chronic inflammation observed in TMD, by amplifying the production of pro-inflammatory cytokines and chemokines in the synovial environment, thereby contributing to tissue degradation and pain sensitization (Hattori et al., 2015).

Bonato et al. (2016) conducted a descriptive, cross-sectional, randomized study (n=260) to investigate the association between genetic polymorphisms and chronic systemic arthralgia in TMD patients. Participants were divided into four groups based on TMD and systemic arthralgia status: articular TMD with systemic arthralgia (n=85), no articular TMD with systemic arthralgia (n=82), articular TMD without systemic arthralgia (n=21), and controls without either condition (n=72). The study analyzed 14 SNPs in the Osteoprotegerin (OPG), Receptor Activator of Nuclear Factor Kappa-B (RANK), and Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) genes, a key regulators of bone remodeling and joint homeostasis, with OPG inhibiting and RANK/RANKL promoting bone resorption. Altered bone metabolism is implicated in TMJOA, where subchondral bone damage, driven by changes in osteoblast and osteoclast activity, contributes to cartilage degeneration. Previous studies reported decreased osteoprotegerin in TMJOA synovial fluid, suggesting its role in pathophysiology. Bonato et al. (2016) identified significant associations between OPG, RANK, and RANKL genetic variants and articular TMD. Specifically, OPG polymorphisms were linked to increased TMJ degeneration and pain, while a RANK polymorphism was associated with a lower TMD risk. These findings suggest that variations in the OPG/RANK/RANKL pathway may drive synovitis onset, cartilage degradation, and subchondral bone remodeling in TMD patients (Bonato et al., 2016).

Other metabolic regulators may also contribute to the pathophysiology of TMD. Among these, micronutrients such as vitamins have drawn attention for their role in maintaining bone and cartilage integrity. In relation to the degenerative nature of TMD pathophysiology, researchers first focused on Vitamin D (VD). Yilmaz et al. (2018) conducted a case-control study (n=119) to investigate the association between genetic variations in the Vitamin D receptor (VDR) gene and the risk of developing TMJOA. The study was conducted on a Turkish population, including 49 patients with TMJOA and 70 healthy controls. The patients were divided into two subgroups: those with anterior disc displacement with reduction (n=24) and those without reduction (n=25). By analyzing

two specific genetic markers in the VDR gene (Apa1 and Taq1), the researchers observed a slight increase in the risk of TMJOA in certain genetic profiles. However, these associations were not statistically significant, likely due to the small sample size. Despite these limitations, the study highlighted the potential involvement of Vitamin D-related genetic factors in TMJ pathophysiology and suggested the need for larger studies to confirm these findings (Yilmaz et al., 2018).

Tang et al. (2024) conducted an animal study to investigate the role of the Vitamin D Receptor (VDR) in the inflammatory response and degeneration of the TMJ. The study used mice with a genetic modification that removed the VDR gene and subjected them to abnormal mechanical stress. The results showed that the absence of VDR increased inflammation, promoted bone resorption due to elevated osteoclast activity, and inhibited chondrocyte proliferation. These findings suggest that VDR plays a critical role in the pathological changes of the TMJ, particularly under mechanical stress. (Tang et al., 2024).

Regarding other metabolic regulators two reviews referred that genetic variations in folate metabolism have also been shown to influence inflammatory processes involved in the development of TMD (Melis & Di Giosia, 2016; Alshahrani et al., 2024). The reviews of Melis and Di Giosia (2016) and Alshahrani et al. (2024) suggested that genetic polymorphisms in folate-related genes, including SHMT1, MTHFD1, and MTRR genes, can disrupt folate metabolism, affecting nucleotide synthesis and DNA methylation, both essential for maintaining tissue integrity and modulating inflammatory responses. So, these genetic variations were found to significantly increase the risk of developing TMD, with a clear association between these alterations and symptoms such as myofascial pain, chronic pain, and disc displacement (Alshahrani et al., 2024).

2.3.1.3. Genes influencing pain onset, chronicity, and intensity in painful temporomandibular disorders

In addition to genes influencing bone and cartilage metabolism, genetic factors contribute to the development of persistent pain conditions by influencing key processes such as nociceptive sensitivity, psychological well-being, inflammation, and autonomic responses. Seven studies examining genetic factors involved in the onset, severity, and chronicity of painful TMDs are summarized in Annex A.

The OPPERA study investigates the genetic foundations of temporomandibular disorders (TMD) by identifying variants that may increase susceptibility to chronic TMD and pain. Based on established chronic pain mechanisms, Smith et al. (2011) hypothesized that TMD pathogenesis involves disruptions in catecholamine, serotonin, opioid, and cytokine pathways. The study initially examined 23 candidate genes, later expanding to over 350 pain-related genes, uncovering novel genetic factors linked to TMD and persistent pain. By comparing 166 individuals with chronic TMD and 1,442 healthy controls, along with an additional cohort of 182 TMD cases and 170 controls, the researchers ensured rigorous classification using the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). Chronic TMD was defined as pain lasting at least six months, present on 15 or more days in the past month, and elicited by palpation of at least three masticatory muscles or one TMJ. A DNA microarray, the Pain Research Panel, examined 3,295 genetic variations. Results confirmed associations with the serotonin receptor 2A gene (HTR2A) and the catechol-O-methyltransferase gene (COMT), and suggested potential risk genes including the nuclear receptor subfamily 3 group C member 1 gene (NR3C1), the calcium/calmodulin-dependent protein kinase IV gene (CAMK4), the cholinergic receptor muscarinic 2 gene (CHRM2), the interferon-related developmental regulator 1 gene (IFRD1), and the G protein-coupled receptor kinase 5 gene (GRK5). These genes are implicated in pain pathways: NR3C1 encodes the glucocorticoid receptor involved in hypothalamic-pituitary-adrenal (HPA) axis regulation; CHRM2 regulates cholinergic signaling affecting pain perception; CAMK4 influences neuronal gene expression and opioid tolerance; IFRD1 controls inflammation and tissue regeneration; and GRK5 modulates receptor desensitization linked to chronic pain. The study by Smith et al. (2011) provided deeper insight into the genetic basis of TMD than prior research focused on single markers, representing the first large-scale candidate gene analysis of chronic TMD (Smith et al., 2011).

The pathophysiology of TMD also involves complex mechanisms such as TMJ inflammation, nerve sensitization, autonomic nervous system dysfunction, and impaired pain regulation. Chronic TMD is often classified as a functional somatic pain syndrome, similar to conditions as irritable bowel syndrome and fibromyalgia, which are characterized by persistent pain without an identifiable organic cause. These disorders may share a common genetic overlapping that amplifies pain perception and disrupts brain, immune, and neuroendocrine functions. A key player in these processes is tumor

necrosis factor-alpha (TNF-α), a major inflammatory cytokine that modulates pain and is implicated in various chronic pain syndromes, including TMD. Elevated levels of TNF-α have been observed in the synovial fluid of TMD patients, highlighting its central role in the inflammatory and pain pathways of the disorder (Wang et al., 2021).

Furquim et al. (2016) investigated the role of genetic variations in TNF-α in TMD susceptibility and pain sensitivity. The study focused on the TNFA-308 (rs1800629) single nucleotide polymorphism and its association with TMD risk and pressure pain thresholds. It included 152 TMD patients and 91 healthy controls, with genotypic and allelic frequencies assessed via real-time PCR. Pain sensitivity was measured using an algometer at multiple sites, including the TMJ, temporal muscle, and masseter muscle. Results showed a significant association between the TNFA-308 polymorphism and TMD; carriers of the GA genotype were 2.87 times more likely to develop TMD than controls. Additionally, A-allele homozygotes exhibited lower pain sensitivity in the TMJ and anterior temporal muscle compared to G allele carriers. These findings suggest TNF-α genetic variations affect both TMD risk and pain perception, offering insights into the disorder's genetic mechanisms (Furquim et al, 2016).

Further investigations into the genetic factors contributing to TMD continue to refine the understanding of the disorder's underlying mechanisms. Oporto et al. (2018) explored for the first time the contribution of genetic variants in dopaminergic pathways to bruxism development. Dopamine plays a crucial role in motor control, reward processing, and circadian regulation, and its dysfunction may contribute to both bruxism and the onset or chronicity of painful TMD by affecting pain perception and movement control. Their case-control study evaluated the frequency of SNPs in six dopamine-related genes: dopamine receptor D1 (DRD1), dopamine receptor D2 (DRD2), dopamine receptor D3 (DRD3), dopamine receptor D4 (DRD4), dopamine receptor D5 (DRD5), and monoamine oxidase B (MAOB). A total of 130 patients undergoing bruxism treatment were classified into three groups: awake bruxism (n = 61), sleep bruxism (n = 26), and awake-sleep bruxism (n = 43), and compared to a control group (n = 59). Their findings revealed that specific genetic variants were associated with different manifestations of bruxism: a variant in DRD2 was linked to a lower risk of awake-sleep bruxism, a variant in DRD3 increased the risk of sleep bruxism, and a variant in DRD5 reduced the risk of awake bruxism. These results highlight the interplay between dopamine, movement regulation, and pain perception, reinforcing the hypothesis that genetic variations in

dopaminergic pathways may not only contribute to bruxism but also influence pain onset, pain intensity, and the chronicity of painful TMD (Oporto et al., 2018).

Brancher et al. (2019) further explored the role of genetic polymorphisms in COMT and their association with TMD and anxiety in adolescents. As highlighted by the OPPERA study, COMT encodes an enzyme responsible for degrading catecholamines such as dopamine, epinephrine, and norepinephrine, which are critical in pain modulation. Variations in COMT activity can affect pain perception, positioning it as a key gene in TMD pathophysiology. The study analyzed a representative sample of 298 adolescents aged 10 to 14 years, including 149 individuals diagnosed with TMD and 149 matched controls. TMD diagnosis was established according to RDC/TMD criteria and included subtypes such as myofascial pain, disc displacement, arthralgia, and painful TMD. Anxiety was assessed using the State-Trait Anxiety Inventory. Genotyping of COMT polymorphisms was performed using TaqMan chemistry, and associations were evaluated through multivariate logistic regression analysis. The results revealed significant associations between COMT variants rs4818 and rs6269 and the presence of myofascial pain and disc displacement, suggesting a genetic predisposition to specific TMD subtypes. Additionally, these polymorphisms were linked to elevated anxiety levels, indicating a potential interaction between genetic influences on pain modulation and emotional regulation. (Brancher et al., 2019). Later, a new advancement in understanding the genetic factors influencing TMD and pain sensitivity emerged. Fiamengui et al. (2020) conducted the first study to evaluate four genetic polymorphisms related to inflammatory and catecholaminergic pathways in TMD patients, specifically assessing their impact on pressure pain threshold (PPT). The study included 268 participants, divided into 131 TMD patients and 137 healthy controls, all selected based on the RDC/TMD. Participants underwent PPT testing for the TMJ, anterior temporalis, and masseter muscles, and the genetic polymorphisms assessed were TNFA-308 (a variation in the TNF-α gene), IL6−174 (a variation in the IL-6 gene), and Val158Met (a polymorphism in the COMT gene). The results revealed that TMD patients exhibited significantly lower PPT values compared to controls, with certain SNPs influencing not only TMD susceptibility but also pain sensitivity. Specifically, SNPs IL6−174 and Val158Met were found to contribute to increased pain sensitivity in the TMJ and masseter muscles. This pivotal discovery supports the hypothesis that a genetic predisposition to an abnormal inflammatory response may act as a trigger for TMD. Furthermore, it

underscores the role of genetic variations in shaping individual pain perception, reinforcing the idea that heightened pain sensitivity could be a key factor in the chronicity and severity of TMD symptoms (Fiamengui et al., 2020).

Lim et al. (2021) investigated brain signal variability (BOLDSV) alterations in patients with chronic myofascial TMD to better understand pain sensitivity mechanisms. The study included 12 chronic myofascial TMD patients and 24 healthy controls who underwent resting-state functional magnetic resonance imaging (fMRI) to assess BOLDSV in specific brain regions. Results demonstrated that reduced BOLDSV in motor and sensory cortical areas correlated with increased pain intensity and heightened sensitivity of the masseter muscle. Notably, TMD patients exhibited significantly lower BOLDSV in the dorsolateral prefrontal cortex (DLPFC) compared to controls, with this reduction associated with greater pain frequency, suggesting impaired cognitive control over pain may contribute to TMD chronicity. Furthermore, diminished BOLDSV in orofacial motor and sensory regions correlated with both increased pain intensity and masseter sensitivity. The study also explored the impact of the COMT Val158Met polymorphism, revealing that carriers of the 158Met allele showed decreased BOLDSV in the DLPFC and increased BOLDSV in the temporal pole, underscoring the modulatory role of genetic factors on pain perception. These findings highlight that altered brain signal variability reflects both sensory-discriminative and cognitive-emotional dimensions of pain, contributing to the persistence and severity of TMD symptoms. (Lim et al., 2021).

Campello et al. (2023) conducted a case-control study investigating the association of polymorphisms in TNF-α, IL-6, and IL-10 with chronic TMD pain in elderly females (N = 34). The study included 17 patients aged 60–74 with chronic TMD pain and 17 age-matched controls treated for other dental issues. DNA was extracted via the Salting Out method, and PCR analyzed the –308G/A TNF-α, –174G/C IL-6, and –1082A/G IL-10 polymorphisms. No significant correlation was found between IL-6 polymorphism and TMD pain. However, the IL-10 –1082A/G polymorphism was associated with pain, with the AA genotype linked to reduced IL-10 secretion, disrupting immune balance. This imbalance between pro-inflammatory cytokines (TNF-α, IL-6) and anti-inflammatory IL-10 may promote TMJ inflammation and chronic pain. The –308G/A TNF-α polymorphism showed significant association with chronic TMD pain. These findings suggest IL-10 genetic variations, particularly the AA genotype, contribute to chronic

TMD pain pathogenesis by fostering an inflammatory environment that enhances pain sensitization and tissue degradation, leading to peripheral and central sensitization, impaired tissue repair, and joint dysfunction (Campello et al., 2023).

In 2024, Zlendic et al. investigated the association between SNPs in the C-X-C motif chemokine ligand 8 (CXCL8), also known as interleukin-8, and TNF genes with TMD presence, pain intensity (low/high), and the presence of chronic arthralgia/myalgia in patients with pain-related TMD. This case-control study included 85 TMD patients and 85 controls, with genetic analysis performed using buccal mucosa swabs and TaqMan assays to genotype the relevant SNPs. The study found that specific genetic variations in the CXCL8 and TNF genes were linked to higher pain intensity in TMD patients. These findings highlight the importance of genetic factors, particularly CXCL8 and TNF, in understanding the pathophysiology of TMD and its clinical manifestations. Specifically, IL-8 contributes to the recruitment of immune cells to the site of inflammation, further intensifying pain and inflammation in the TMJ (Zlendic et al., 2024a)

However, various researchers have analyzed the literature through systematic reviews and meta-analyses to gain a more comprehensive interpretation of the genetic factors influencing pain onset, chronicity, and intensity in painful TMDs (cf. Annex B).

Visscher and Lobbezoo (2015) conducted a systematic review to investigate the role of heritability in TMD pain. The review included 21 studies, comprising five familial aggregation studies and 16 genetic association studies, selected from a total of 473 publications. The analysis provided modest evidence supporting the genetic contribution to TMD pain, particularly focusing on candidate genes from the serotonergic and catecholaminergic systems. However, the authors ’suggested that genetic alterations in the serotonin system may play a significant role in both TMD pain and related conditions like depression, revealing a complex interaction between pain sensitivity and mood regulation (Visscher & Lobbezoo, 2015).

Melis and Di Giosia (2016) conducted a systematic review to assess the role of genetic factors in the etiology of TMD. A total of 1999 articles were initially identified through a PubMed search, which was narrowed down to 24 relevant studies, with two additional papers found through references. The review found that while co-occurrence of TMD signs and symptoms within families was not evident, several gene polymorphisms were associated with a higher or lower risk of developing TMD. The genes identified were mainly related to serotonin activity and metabolism but also reaffirmed the role of the T-

cell receptor pathway, particularly through evidence of T-cell infiltration and activation in the dorsal horn of the spinal cord, which plays a crucial role in the development of pain after peripheral nerve injury. as it was mentioned an association between eight SNPs in the T-cell receptor pathway and an increased risk of TMD with widespread pain (Melis & Di Giosia, 2016).

Campello et al. (2021) conducted a systematic review (n=672) to investigate the association between genetic variations in serotonin receptor genes and bruxism. The review included four eligible studies that evaluated individuals with sleep bruxism (n=187), awake bruxism (n=105), both sleep and awake bruxism (n=89), and controls (n=291). The review focused on variations in the 5-HT2A et Serotonin 1A receptor gene (5-HT1A), which are involved in serotonin signaling and have been implicated in pain regulation and muscle activity. The authors suggested that serotonin receptor gene variations, particularly in the 5-HT2A receptor gene, may contribute to the pathogenesis of bruxism, which in turn could play a significant role in the development of TMD (Campello et al. 2021).

A systematic review and meta-analysis by Brancher et al. (2021) explored the association between genetic polymorphisms in COMT and TMD. After screening 1,903 articles, 10 studies were included in the analysis, with 3 meeting meta-analysis criteria. The studies assessed the presence or absence of TMD and categorized the condition into subgroups, including myofascial pain (with or without limited opening), disk displacement (with or without reduction), and arthralgia. The analysis revealed that specific polymorphisms, such as rs6269 and rs9332377, were significantly associated with myofascial pain and painful TMD. Data analysis supported the idea that COMT plays a significant role in pain regulation and could serve as a critical genetic marker for understanding the biological mechanisms underlying TMD, particularly in myofascial pain and painful TMD (Brancher et al., 2021).

A systematic review and meta-analysis by Alshahrani et al. (2024) examined the association between genetic polymorphisms and TMD. After screening 851 articles, 19 studies involving human subjects with various TMD subtypes were included. These studies assessed the relationship between genetic factors and clinical features such as myofascial pain, disc displacement, and chronic pain. Several studies reported significant associations between serotonin transporter gene (5-HTT) polymorphisms and TMD symptoms. Given the role of 5-HTT in serotonin reuptake, mood regulation, and pain

perception, these polymorphisms may influence pain sensitivity and contribute to TMD onset and maintenance. The review also highlighted the potential involvement of the dopaminergic system, with DRD4 gene polymorphisms linked to altered pain processing and TMD symptom persistence. These findings suggest that genetic variations in dopaminergic pathways may modulate pain perception in TMD. Additionally, the review noted interactions between genetic susceptibility and environmental stressors. Several studies examined COMT polymorphisms, especially Val158Met. Low-activity COMT haplotypes were associated with increased pain sensitivity and elevated stress hormone levels, indicating that carriers may have heightened vulnerability to stress-induced pain and chronic TMD risk. (Alshahrani et al., 2024).

2.3.1.4. Genes influencing etiologic factors or individual adaptability to temporomandibular disorders

Finally, some genes induce changes in individual adaptability to TMDs. Five studies that identified different genes or genetic variants in this context are presented (cf. Annex A and Annex B).

A crucial aspect in understanding the genetic factors underlying TMD is the role of estrogen, a hormone that significantly influences TMJ function through its effects on bone metabolism, cartilage integrity, and inflammation. In this context, Bonato et al. (2016) conducted a cross-sectional study to investigate the association between polymorphisms in the estrogen-related receptor beta gene (ESRRB) and TMD risk. The sample included individuals with TMD (n = 13), individuals with rotator cuff disease (RCD) but without TMD (n = 16), individuals with both conditions (n = 49), and controls with neither condition (n = 30). A total of eight SNPs in the ESRRB gene were analyzed. Estradiol levels were measured using chemiluminescent immunoassay, and surface electromyography was used to assess head and cervical muscle activity. The authors found a significant association between the rs6574293 polymorphism in ESRRB and increased susceptibility to TMD. Additionally, ESRRB variants were linked to central sensitization, suggesting that altered estrogen signaling may contribute to both joint dysfunction and heightened pain perception in TMD especially in woman (Bonato et al., 2016).

Chung et al. (2017) conducted a case-control study to examine whether specific

polymorphisms contribute to facial asymmetry and TMD in patients. The study analyzed five genes, actinin alpha 3 (ACTN3), ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), estrogen receptor alpha (ESR1), paired-like homeodomain transcription factor 1 (PITX1), and paired-like homeodomain transcription factor 2 (PITX2). The researchers assessed a cohort of 174 patients, divided into symmetric and asymmetric groups based on postero-anterior cephalometric measurements. TMD symptoms were assessed using jaw pain and function questionnaires. A significant association was identified between two SNPs in the ESR1gene and craniofacial asymmetry linked to TMD (Chung et al., 2017).

DNA methylation has been identified as a key epigenetic modification that regulates gene expression. Oporto and Salazar (2018) conducted a case-control study (n=158) to investigate DNA methylation levels in individuals with bruxism. Participants were categorized into sleep bruxism (n=32), awake bruxism (n=42), combined bruxism (n=42), and control (n=42) groups. DNA methylation was assessed using a colorimetric assay. The results reveal significantly lower levels of methylation in all bruxism groups compared to the controls. Since bruxism is a known risk factor for TMDs, the observed epigenetic alterations may contribute to TMDs development (Oporto & Salazar, 2018).

In a case-control study (n=948), Ao et al. (2024) explored the impact of DNA methylation on the progression of TMD. Blood samples were collected from 496 patients with chronic TMD and 452 healthy controls, all participants in the OPPERA study. Genome-wide methylation profiling was performed to detect differentially methylated regions (DMRs) associated with TMD. The analysis identified six DMRs located near genes involved in inflammation and neuronal function, including fibromodulin (FMOD), palmitoyl-protein thioesterase 1 (PM20D1), zinc finger protein 718 (ZNF718), zinc finger protein 57 (ZFP57), and ring finger protein 39 (RNF39). Differences in methylation profiles between chronic TMD patients and controls show a potential role for epigenetic regulation in pain persistence (Ao et al., 2024).

Wu et al. (2024) conducted a Mendelian Randomization study to explore the causal relationship between mental health disorders, specifically depression, and TMD. The study used large-scale Genome-Wide Association Study (GWAS) data for major depression, bipolar disorder, and schizophrenia, and included n=211,023 individuals of European descent in the TMD dataset (5,668 TMD patients and 205,355 controls). The main approach utilized was Inverse Variance Weighting to evaluate the causal association

between the three mental disorders and TMD. The results revealed a significant genetic association between major depression and an increased risk of developing TMD, while no significant relationship was observed between bipolar disorder, schizophrenia, and TMD. These findings suggest that depression may play a role in the development of TMD (Wu et al., 2024).

Recently, a systematic review by Leucuța et al. (2024) explored the association between hormonal factors and the physiopathology of TMD, as well as individual adaptability to the TMDs. The review included 22 studies published between 2008 and 2023, found through PubMed/MEDLINE, Scopus, Embase, and Web of Science. The analysis revealed that polymorphisms in the ESR1 gene were significantly more prevalent in women with TMD. Furthermore, the review highlighted that women with polycystic ovary syndrome experienced a higher incidence of TMD, emphasizing the contribution of hormonal imbalances to TMD pathogenesis. In premenopausal women, beta-estradiol levels were significantly associated with the onset and progression of TMD (Leucuța et al., 2024).

2.3.2. Genetic mutations and temporomandibular disorders expression

2.3.2.1. Pathophysiologic mechanisms of TMJ inflammatory and structural alterations associated with genetic mutations

Genetic alterations contribute to TMJ structural changes by disrupting bone mineralization and homeostasis. The ANKH gene encodes a transmembrane protein that exports inorganic pyrophosphate (PPi), an inhibitor of ectopic mineralization, maintaining osteoblast and osteoclast balance. Mutations in ANKH impair PPi transport, lowering extracellular PPi levels and promoting inappropriate calcification in the joint. Huang et al. (2011) showed in ank/ank mutant mice that such mutations cause ectopic mineral deposits, fibrous ankylosis, partial joint fusion, and narrowing of the TMJ space. These findings indicate that ANKH dysfunction disrupts mineral homeostasis, leading to degenerative joint changes characteristic of TMD. The resulting fibrous ankylosis restricts joint mobility and contributes to chronic pain and dysfunction, key features of advanced TMD. (Huang et al., 2011).

The OPG/RANKL/RANK pathway regulates osteoclast differentiation and bone resorption to maintain TMJ integrity. OPG acts as a decoy receptor for RANKL, blocking

its interaction with RANK on osteoclast precursors and inhibiting osteoclastogenesis. Polymorphisms in the OPG gene, such as rs11573875, reduce OPG expression, leading to excessive osteoclast activation and subchondral bone resorption, which weakens joint structure and contributes to TMJ arthralgia. Conversely, certain RANK haplotypes, like AGTGC, may enhance osteoclast regulation, preserving bone homeostasis. These variants influence bone remodeling, synovial inflammation, and chondrocyte survival, exacerbating TMJOA degeneration and pain. (Bonato et al., 2016).

Beyond bone and cartilage genetics, inflammation and immune responses also contribute to TMJ degeneration. The SNP rs878962 in TSPAN9, encoding a tetraspanin protein involved in cell signaling, may influence TMJ degeneration by affecting inflammatory pathways and tissue remodeling, as platelets play a role in inflammatory arthritis. Though TMJOA is typically non-inflammatory, secondary inflammation can worsen damage. Additionally, SNPs rs80575 in APOL3 and rs2460300 in MRC2 may alter gene expression by impacting protein-DNA interactions. MRC2, expressed in mesenchymal cells like chondrocytes and osteoblasts, regulates extracellular matrix remodeling; its altered expression could disrupt bone homeostasis, promoting TMJOA-related degeneration. (Yamaguchi et al., 2014).

At the molecular level, IL-17A is a key pro-inflammatory cytokine in TMJ disorders. Synovial fibroblasts express all IL-17 receptors, including IL-17RC splice variants influencing signaling. IL-17A stimulation upregulates inflammatory genes such as CXCL1, IL-8, and CCL20; notably, CCL20 recruits Th17 cells, amplifying local IL-17A production and inflammation. IL-17A also promotes IL-6 secretion, linking it to tissue destruction, osteoclast activation, and pain. Main pathways include NF-κB and PI3K/Akt; IL-17A shares downstream effectors with IL-1β and TNF-α. Inhibiting TAK1 blocks NF-κB activation, while PI3K/Akt sustains inflammatory mediator production and cell survival, perpetuating leukocyte recruitment, cytokine release, and joint damage in TMJ degeneration. (Hattori et al., 2015).

Xiao et al. (2015) confirmed that genetic variations in key genes of the TGFβ pathway contribute to TMJOA development. The SNP rs143383 in the promoter region of the GDF5 gene reduces its expression by about 27% by altering the binding of transcription factors such as Sp1 and DEAF-1. Reduced GDF5 levels may impair cartilage maintenance and promote degradation driven by inflammatory molecules like IL-1. SMAD3, a downstream signaling protein of TGFβ receptors, supports cartilage integrity

by enhancing type II collagen production and suppressing MMP-13, a cartilage-degrading enzyme. The rs12901499 SNP in SMAD3 is associated with TMJOA, likely by influencing pathway activity rather than SMAD3 protein levels directly. RUNX2, a transcription factor regulating late-stage chondrocyte differentiation and bone formation, contains the intronic SNP rs1406846, which shows a weak link to TMJOA, though its exact role remains unclear. Notably, the presence of multiple risk alleles in these genes may increase susceptibility to TMJOA by aggravating cartilage degradation and abnormal TMJ remodeling. (Xiao et al., 2015).

Moreover, the VDR gene, which encodes the vitamin D receptor, plays a pivotal role in regulating bone and cartilage metabolism through the vitamin D-VDR signaling pathway. A functional VDR is essential for calcium homeostasis and suppression of inflammatory mediators in joint tissues. Polymorphisms including Apa1 (rs7975232) and Taq1 (rs731236) have been linked to increased susceptibility to TMJOA and internal derangement. Notably, the heterozygous Aa genotype of Apa1 is associated with degeneration of the articular disc and cartilage loss. These effects may result from impaired VDR-mediated repression of particularly MMP3, which degrades the cartilage extracellular matrix (Yilmaz et al., 2018). Additionally, as demonstrated by Tang et al., (2024), experimental deletion of the VDR gene in mice leads to altered chondrocyte gene expression, upregulation of pro-inflammatory cytokines such as IL-1β and IL-6, and abnormal cartilage thickening and subchondral bone damage (Tang et al., 2024).

2.3.2.2. Pathophysiological mechanisms related to the onset, chronicity and intensity of pain in painful temporomandibular disorders associated with genetic mutations

Painful TMDs are influenced by genetic mutations affecting pain onset, persistence, and intensity. Several variants impact neurotransmitter systems involved in pain regulation. The COMT gene encodes catechol-O-methyltransferase, which degrades catecholamines (dopamine, norepinephrine, epinephrine) essential for modulating pain and stress (Slade et al., 2011). Polymorphisms such as rs174697, rs4818, and rs6269 reduce COMT activity, raising catecholamine levels. This increase may overstimulate beta-adrenergic receptors, heightening pain sensitivity and contributing to pain initiation and chronicity in TMD patients. These COMT variants are also linked to greater anxiety, potentially intensifying muscle tension and parafunctional behaviors like jaw clenching, which aggravate TMD symptoms (Brancher et al., 2019). Lastly, the COMT Val158Met

polymorphism also highlights this mechanism. The Met allele results in reduced COMT activity and increased pain sensitivity, especially in the masseter muscle, suggesting its involvement in TMD pain persistence and intensity. These genetic variations influence both physiological and psychological pathways involved in pain modulation, suggesting that COMT mutations play a significant role in the development, chronicity, and severity of painful TMD (Fiamengui et al., 2020).

Additional polymorphisms in genes such as NR3C1, CHRM2, CAMK4, IFRD1, and GRK5 may contribute to TMD by disrupting glucocorticoid receptor function, cholinergic transmission, neuronal transcription, and GPCR (G protein-coupled receptor) signaling. These dysfunctions can lead to increased pain sensitivity, prolonged pain duration, and impaired ability to regulate inflammation, thereby contributing to the onset, chronicity, and intensity of pain in TMD patients (Smith et al., 2011).

Polymorphisms in dopamine receptor genes also affect TMD pain onset. Variants in DRD2 (rs1800497) and DRD3 (rs6280) modify dopaminergic signaling in the central nervous system, influencing motor control and pain perception. The DRD2 rs1800497 G allele is associated with increased receptor density and activity, potentially offering protection against bruxism, whereas the A allele reduces dopaminergic function and may worsen symptoms. In contrast, the DRD3 rs6280 C allele, linked to heightened dopamine release during stress and reward responses, may increase vulnerability to bruxism and muscle hyperactivity, facilitating pain initiation and chronification. These genetic variants disrupt neural circuits controlling jaw muscle activity, resulting in abnormal contractions, microtrauma, and inflammation, central contributors to painful TMD development. (Oporto et al., 2018).

Moreover, a significant SNP in the HTR2A gene (rs9316233) modulates serotonin signaling. The minor G allele reduces pain sensitivity by altering serotonin pathways, which lowers the nervous system's tendency to develop chronic pain, thus offering a protective effect against TMD (Slade et al., 2011).

The TNFA-308 polymorphism, a G-to-A substitution, is a recognized risk factor. A-allele carriers exhibit increased TNF-α expression, a pro-inflammatory cytokine that promotes TMJ and muscle inflammation. TNF-α may also contribute to pain chronicity by sensitizing CNS structures like the hippocampus, amygdala, and hypothalamus, potentially altering pain perception and causing neurodegenerative changes. These effects may underlie comorbidities such as sleep disturbances and cognitive dysfunction in TMD.

Notably, A-allele carriers sometimes display higher pressure pain thresholds, indicating a complex link between inflammation and pain modulation. Overall, TNFA-308 illustrates how genetic variants influence immune, inflammatory, and neural pathways shaping TMD pain. (Furquim et al., 2016; Fiamengui et al., 2020).

Polymorphisms in cytokine genes such as IL6−174 and IL-10 –1082A/G also influence TMD pain by altering cytokine production. The IL6−174 variant increases IL-6 levels, promoting inflammation and joint degradation, and is linked to lower pressure pain thresholds in the TMJ and temporalis muscle (Fiamengui et al., 2020). Similarly, the IL-10 –1082A/G AA genotype, associated with reduced IL-10 secretion, is more common in chronic TMD pain patients. As IL-10 downregulates pro-inflammatory cytokines like TNF-α and IL-6, its deficiency favors persistent inflammation, likely contributing to TMD pain onset and severity. (Campello et al., 2023).

The CXCL8 gene SNP rs2227307, associated with IL-8 production, correlates with increased pain intensity in TMD patients. Zlendic et al. (2024a) reported a higher frequency of the minor G allele in individuals with TMD pain compared to controls, particularly among those with severe pain, indicating a genetic predisposition to amplified pain sensitivity. IL-8, a pro-inflammatory cytokine encoded by CXCL8, is elevated in chronic TMD and contributes to local inflammation. This heightened cytokine expression promotes neuroimmune interactions that sensitize nociceptive pathways, thereby intensifying and sustaining pain. Such genetic-inflammation interplay likely underpins both the onset and chronicity of TMD-related pain. (Zlendic et al., 2024a).

Genetic mutations can also influence neural circuits responsible for pain modulation and cognitive-emotional regulation. For example, the COMT Val158Met polymorphism reduces enzyme activity and affects brain regions such as the dorsolateral prefrontal cortex (dlPFC) and temporal pole. The study by Lim et al. (2021), using blood-oxygen-level-dependent signal variability (BOLDSV) show that chronic TMD patients with more Met alleles have decreased dlPFC variability and increased variability in the temporal pole, a region implicated in emotional pain processing. Since COMT modulates dopamine and indirectly affects μ-opioid neurotransmission, these genetic variations likely influence both the cognitive and emotional aspects of pain perception. These findings suggest that COMT polymorphism contributes to individual differences in pain experience by altering brain signal variability and the efficiency of pain modulatory circuits. Overall, the mechanisms by which mutations like COMT Val158Met affect

neural function may underlie the onset, persistence, and severity of pain in TMD patients (Lim et al., 2021).

2.3.2.3. Pathophysiologic mechanisms associated with genetic mutations influencing etiologic factors or individual adaptability to temporomandibular disorders

Estrogen plays a critical role in TMJ function, with mutations in the ESRRB gene predisposing individuals to more severe TMD symptoms. Bonato et al. (2016) identified ESRRB polymorphisms linked to both TMD and rotator cuff disease (RCD), suggesting shared genetic susceptibility. Specifically, rs1676303 and rs6574293 were associated with RCD and TMD, respectively, while rs4903399 was found in both. ESRRB regulates steroid hormone activity and estrogen receptor function; its polymorphisms may alter gene expression involved in inflammation, pain sensitivity, and tissue damage, increasing TMJ vulnerability to stress and hypoxia. This may explain why some individuals develop TMD more readily or suffer greater pain. Clinically, TMD and RCD patients often show reduced jaw and neck muscle activity, possibly as protection against chronic pain or from impaired neuromuscular control. Altered pain processing, hormonal imbalance, and disrupted estrogen signaling, partly driven by ESRRB variants, may also contribute to TMD pain persistence. (Bonato et al., 2016).

Polymorphisms in the ESR1 gene are linked to increased TMD risk, likely through effects on TMJ inflammation. The ESR1 rs1643821 variant correlates with a higher frequency of anterior disc displacement without reduction. Estrogen may exacerbate inflammation in TMD by activating immune cells such as monocytes, which amplify central pain signaling. Hormonal disorders like polycystic ovary syndrome, marked by hormonal imbalance and inflammation, are also associated with higher TMD prevalence. These findings suggest that variations in hormone receptor genes influence TMD etiology, pain sensitivity, and disease susceptibility. (Leucuța et al., 2024).

Mutations affecting estrogen signaling may also relate to TMD risk factors like malocclusion, promoting TMD development. ESR1 polymorphisms influence bone density and are linked to certain malocclusion patterns and TMJOA. These genetic factors alter craniofacial growth and bone remodeling, causing structural imbalances that trigger pain and contribute to TMD chronicity and severity. Variants in the ENPP1 gene also affect bone growth, mineral density, and muscle composition, contributing to skeletal

asymmetry and TMD symptoms. For example, SNPs rs6569759 and rs858339 in ENPP1 are associated with altered mineralization and insulin signaling in skeletal muscles, possibly explaining muscle pain (such as myalgia) and joint issues like arthralgia and disc displacement in TMD. (Chung et al., 2017).

Beyond genetic mutations, epigenetic mechanisms also shape TMD susceptibility and symptoms. Oporto et al. (2018) found DNA hypomethylation in bruxism patients, potentially causing abnormal gene activation. These changes affect genes tied to neurotransmission, muscle function, stress response, and circadian rhythms. Polymorphisms in serotoninergic pathway genes, which regulate muscle tone and stress adaptation, are linked to bruxism, a TMD risk factor. Environmental influences like stress, anxiety, smoking, caffeine, and alcohol may worsen these epigenetic shifts. Enzymes such as TET dioxygenases modulate gene expression via methylation in differentiated cells. Together, genetic and epigenetic factors likely shape adaptability to etiological triggers and the severity or persistence of TMD symptoms. (Oporto et al., 2018).

Ao et al. (2024) highlight the role of epigenetics in chronic TMD, identifying hypomethylation in genes like ZFP57, RNF39, ZNF718, and PM20D1. ZFP57, a transcriptional repressor that maintains DNA methylation patterns, may lose this function when hypomethylated, triggering epigenetic changes that enhance inflammation and stress responses, contributing to persistent pain. Similarly, RNF39, involved in immune function and brain plasticity, shows altered methylation linked to chronic pain regulation, suggesting immune and neural changes sustain pain signals. ZNF718 and PM20D1 display biphasic methylation patterns, reflecting complex timing in inflammatory control that may affect TMD pain duration. Environmental factors like trauma and stress further modify these epigenetic marks, shaping gene expression and individual vulnerability to chronic TMD (Ao et al., 2024).

2.3.3. Genetic and epigenetic contributions to temporomandibular disorders diagnosis and treatment approaches

The multifactorial nature of TMD supports a biopsychosocial model for management, prioritizing conservative, reversible treatments for symptom relief and reserving invasive procedures for severe or refractory cases. (Kandasamy & Greene, 2020; Ferrillo et al.,

2022). As knowledge of TMD etiology advances, genetic and epigenetic factors are now acknowledged as critical elements for refining diagnostic accuracy and guiding personalized treatment strategies (Gui & Barbosa, 2015).

Six studies examining the genetic contribution to TMDs diagnosis and treatment approaches are presented in Annex A and Annex B.

Genetic variations substantially influence TMD clinical presentation, progression, and treatment response. Polymorphisms in genes such as COMT and the matrix metalloproteinase (MMP) family are linked to pain sensitivity, joint degeneration, and therapeutic outcomes (Braga et al., 2021; Zlendic et al., 2024b). The COMT gene, regulating catecholamine metabolism, plays a pivotal role in pain modulation. Individuals with the Val158Met GG genotype generally report lower pain sensitivity, potentially explaining why some patients exhibit minimal pain despite clear TMD pathology. Such genetic insights can guide clinicians in predicting pain severity and customizing management strategies. (Braga et al., 2021).

These findings signify a crucial shift in TMD diagnostics and therapeutics. Zlendic et al. (2024b) demonstrated that minor alleles of rs4646310 and rs4680 in the COMT gene correlate with poorer responses to standard TMD treatments. Patients carrying these variants exhibited reduced pain relief and symptom improvement after six months of therapy. The diminished efficacy may stem from altered neurotransmitter metabolism and opioid receptor function, increasing pain sensitivity. This evidence supports stratifying patients by genetic profiles to enable earlier, personalized interventions for those at higher risk of treatment failure. (Zlendic et al., 2024b).

Although genetic effects on TMD are generally modest, integrating them into diagnostic frameworks offers potential to improve patient outcomes through personalized treatments. For example, patients harboring pain-associated polymorphisms might benefit from tailored therapies such as NSAIDs to reduce inflammation, occlusal splints to alleviate joint overload from behaviors like sleep bruxism, and adjunctive supportive interventions. While supplements like glucosamine and chondroitin have been proposed for cartilage protection, current evidence remains inconclusive. A pilot study by Planello et al. (2011) investigated COMT variants as predictors of treatment response in TMD, revealing that patients with low COMT activity responded favorably to propranolol, a nonselective β-adrenergic antagonist. Conversely, individuals with high COMT activity, as noted by Braga et al. (2021), showed no benefit. These preliminary data suggest COMT

polymorphisms may modulate treatment efficacy, highlighting the need for further research in larger cohorts. (Braga et al., 2021).

Besides COMT, genes like ESR1 are linked to TMD susceptibility and could guide personalized treatments. Although study methods and populations vary, these markers offer insights into TMD biology and may be incorporated into clinical diagnostics. (Jayaseelan & Arumugam, 2022).

Genetic factors are potential therapeutic targets in TMD etiologies like bruxism. Bruxism has a strong genetic basis involving complex genetic, neurological, and psychological interactions. Although tools like electromyography and heart rate monitoring aid diagnosis, treatment outcomes vary. This clinical heterogeneity calls for integrated diagnostics and personalized management. Current treatments show mixed results, highlighting the need for more research on genetic predispositions and treatment effects. (Heyat et al., 2020).

Li et al. (2023) highlighted the importance of genetic variants in understanding TMD pain mechanisms. Genetic insights could enhance diagnosis by identifying patients predisposed to specific pain patterns and support personalized treatment selection. While many variant functions remain unclear, GWAS advances offer promising paths for targeted therapies. Currently, no TMD treatments are tailored to genetic profiles, but these findings pave the way for personalized pain management (Li et al., 2023).

Concerning the role of epigenetic factors in the onset of TMD, the study by Ao et al. (2024) demonstrated that differential DNA methylation at specific genetic loci correlates with the onset and progression of TMD-related pain. Importantly, their findings suggest dynamic regulation of these epigenetic markers throughout the disorder's development. While current clinical applications remain limited, the authors emphasize that these epigenetic changes may serve as targets for future therapeutic strategies aimed at interrupting pathological pathways during critical phases of TMD progression (Ao et al., 2024).

2.4. Discussion

This critical review highlights both the promising insights and persistent challenges in translating genetic and epigenetic findings into meaningful clinical applications for TMDs.

The studies included in this review have explored multiple genetic polymorphisms related to pain modulation, inflammation, and hormonal regulation, especially within the COMT, ESR1, and TNF genes (Huang et al., 2011; Hattori et al., 2015; Bonato et al., 2016; Lim et al., 2021; Campello et al., 2023). These variants appear to contribute to the susceptibility and severity of TMD, particularly in relation to individual differences in pain perception.

Despite these findings, the clinical applicability of such genetic markers remains limited. The majority of studies involved relatively small sample sizes, generally under 100 participants (Huang et al., 2011; Hattori et al., 2015; Bonato et al., 2016; Lim et al., 2021; Campello et al., 2023), which diminishes statistical power and raises concerns regarding reproducibility. Additionally, the demographic homogeneity of cohorts and the lack of ethnic diversity restrict the generalizability of results to broader populations. From my perspective, overcoming these limitations through large-scale, multicentric studies that include diverse ethnic and clinical profiles is essential to confirm genetic associations and their functional consequences.

Moreover, TMDs are most likely polygenic and multifactorial, resulting from the cumulative effect of numerous low-impact genetic variants in interaction with environmental factors such as chronic stress, trauma, and parafunctional habits. This genetic complexity challenges the identification of clinically useful single-gene biomarkers and highlights the need for integrative disease models that combine genetic, epigenetic, environmental, and psychosocial factors.

Another critical issue is the heterogeneity in diagnostic criteria and phenotyping of TMD across studies, which complicates cross-study comparisons and meta-analyses. The use of standardized and validated diagnostic protocols, such as the DC/TMD, should be prioritized in future research to ensure consistency. Furthermore, many studies targeted narrowly defined subgroups, such as adolescents (Brancher et al., 2019) or elderly women (Campello et al., 2023), which, while providing valuable insights, limit the overall applicability of conclusions. Expanding inclusion criteria to encompass a wide range of ages, sexes, and clinical subtypes would improve understanding of gene–environment interactions in TMD.

Importantly, the presence of publication bias in this field must also be considered. Positive results demonstrating genetic associations are more likely to be published, while studies reporting no association are often underrepresented. This skew may contribute to an

overestimated perception of genetic influence, calling for more transparent reporting and inclusion of negative findings in future literature.

Even though these genetic markers usually have a small impact individually, they highlight the potential usefulness of incorporating genetic information into the diagnosis and treatment of TMDs. This personalized approach could be particularly beneficial for patients harboring pain-related gene variants. However, to date and from a clinical standpoint, the cost-effectiveness and practical integration of genetic testing remain limited. Notably, current diagnostic protocols for TMD, including the widely used DC/TMD criteria, do not incorporate genetic or epigenetic factors, reflecting a gap between emerging research and routine clinical practice.

The specificity of identified genetic polymorphisms also presents a challenge. Many of these variants have been implicated in a variety of pain-related and inflammatory disorders beyond TMD, including depression (Wu et al., 2024), rotator cuff pathology (Bonato et al., 2016), and craniofacial asymmetries (Chung et al., 2017). This suggests these genes may be markers of a general pain susceptibility rather than TMD-specific risk factors. Clinically, this non-specificity diminishes their value as stand-alone diagnostic or prognostic biomarkers. Thus, genetic testing should be integrated with comprehensive clinical and psychosocial assessments to avoid overdiagnosis and to tailor treatment plans appropriately.

In terms of methodology, the studies employed various molecular techniques, including polymerase chain reaction (Huang et al., 2011; Hattori et al., 2015; Furquim et al., 2016; Yilmaz et al., 2018; Campello et al., 2023), TaqMan SNP genotyping (Oporto et al., 2018; Brancher et al., 2019; Fiamengui et al., 2020; Zlendic et al., 2024b), and restriction fragment length polymorphism analysis (Yilmaz et al., 2018; Campello et al., 2023). They remain costly and require specialized laboratory infrastructure. This restricts their routine use in clinical settings. Bridging the gap between research and practice will require technological advancements that reduce costs and simplify testing procedures. Additionally, clinicians will need training in genetic counseling and interpretation to meaningfully incorporate these data into patient management.

Recent advances in artificial intelligence and machine learning may offer innovative solutions to address the complexity of gene-environment interactions in TMD. These computational tools could help identify high-risk phenotypes, uncover latent patterns across large datasets, and develop predictive models to guide targeted interventions.

Genetic factors contribute not only to the etiology of TMD but also hold promise as potential therapeutic targets, especially in conditions such as bruxism. Bruxism, increasingly recognized as having a significant genetic basis, results from complex interactions among genetic, neurological, and psychological influences. While diagnostic tools like electromyography and heart rate monitoring have enhanced detection capabilities, treatment outcomes for bruxism remain highly variable. This clinical heterogeneity underscores the urgent need for integrated diagnostic tools and individualized management strategies. Current pharmacological and non-pharmacological treatments produce inconsistent results, emphasizing the necessity for more targeted research on genetic predispositions and their role in treatment response (Heyat et al., 2020). With advances in genetic databases and analytical methods, there is considerable potential to develop precise diagnostic markers and personalized therapies that more effectively address bruxism.

Regarding epigenetic factors, the study by Ao et al. (2024) demonstrated that differential DNA methylation at specific genetic loci correlates with the onset and progression of TMD-related pain. Importantly, their findings suggest dynamic regulation of these epigenetic markers throughout disease development. However, studying such epigenetic phenomena remains particularly challenging due to their temporal variability and sensitivity to external influences such as stress and chronic inflammation. Longitudinal study designs and advanced epigenomic tools will be required to better understand the role of these modifications across disease trajectories.

Although clinical applications remain limited, these epigenetic changes may serve as promising targets for future therapies aimed at interrupting pathological pathways during critical stages of TMD progression. In fact, pharmacological manipulation of epigenetic mechanisms, such as using drugs that inhibit DNA methyltransferases, has already been explored in cancer research to reverse abnormal gene silencing. In the context of TMD, such approaches could help modulate pathological DNA methylation patterns that contribute to chronic pain and inflammation. However, these strategies remain experimental and require further validation before being applied in clinical settings.

Regarding therapeutic implications, although no current treatments are based specifically on genetic profiles, the identification of pain-related gene variants offers potential for personalized medicine. Pharmacogenomics (the study of how genetic variations influence drug response) may allow for more personalized TMD treatment. For instance,

individuals with COMT polymorphisms linked to heightened pain sensitivity could benefit from tailored pharmacological or behavioral interventions targeting central pain pathways.

In my opinion, ideal future studies would be prospective, randomized controlled trials assessing the efficacy of genotype-guided therapies. These might include pharmacogenomics-informed drug prescriptions, epigenetic therapies, or combined psychosocial and biological interventions. Additionally, the development of accessible genetic testing panels for TMD risk and pain modulation genes would facilitate clinical implementation. Such precision medicine approaches could revolutionize TMD management by enabling earlier diagnosis, more effective treatments, and improved patient quality of life.

However, the ethical, legal, and social implications of genetic testing must not be overlooked. Safeguards surrounding informed consent, data confidentiality, and equitable access to these technologies will be essential to ensure that advancements in genetic science do not exacerbate existing health disparities. Furthermore, dental professionals must be adequately trained to interpret and apply genetic findings responsibly within their clinical practice.

In conclusion, while genetic and epigenetic research has enhanced our understanding of TMD pathophysiology, substantial challenges remain before these insights can be translated into routine clinical care. Future efforts should focus on methodological rigor, large and diverse cohorts, integration of multidimensional data, and development of personalized therapeutic protocols. The promise of genetics-driven personalized treatment for TMD is compelling but requires a coordinated, multidisciplinary research agenda to become a clinical reality. Continued collaboration between researchers, clinicians, and patients will be key to unlocking the full potential of genetic and epigenetic discoveries in transforming TMD diagnosis and treatment.

3. CONCLUSION

Current evidence identifies several key genes implicated in TMD susceptibility. For instance, COMT influences pain perception by modulating neurotransmitter levels, while ESR1 is involved in hormonal regulation, affecting joint and muscle function. Moreover, TNF-α encodes a pro-inflammatory cytokine that contributes to inflammation and tissue degradation, and 5-HT2A plays a role in psychological traits and pain modulation pathways. These genes interact within complex biological pathways regulating inflammation, pain sensitivity, hormonal balance, and psychological stress responses, all of which contribute to the multifactorial nature of TMD.

In addition, epigenetic modifications such as DNA methylation of genes like ZFP57 and PM20D1 play a role in modulating gene expression in response to environmental and psychosocial factors, influencing the persistence of pain and central sensitization mechanisms. Together, these genetic and epigenetic factors provide a biological basis for the clinical variability observed in TMD patients and underscore the importance of adopting an integrated biopsychogenetic model.

Despite the limitations of the reviewed studies, the evidence clearly indicates that genetic and epigenetic factors play a significant—and possibly crucial—role in the pathogenesis, classification, and individual variability of TMD. These findings support a shift from viewing TMD solely as a mechanical or psychosocial condition toward a more comprehensive biopsychogenetic perspective.

Nevertheless, current evidence is not yet sufficient to warrant the routine use of genetic or epigenetic markers in clinical diagnosis or treatment. Major limitations include the small number of high-quality studies, heterogeneity in diagnostic criteria, and the lack of functional validation for many of the identified variants. More longitudinal, large-scale, and interdisciplinary research is essential to confirm these associations, elucidate underlying mechanisms, and develop clinically applicable biomarkers.As precision medicine continues to progress in various medical fields, an important question remains: could genetics and epigenetics become the key to a more predictive, preventive, and personalized approach to TMD management in the future?

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ANNEXES

Annex A. Observational and original studies identifying genes associated with temporomandibular disorders

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Huang et al., 2011	Case-Control study	ANKH	n= 55 TMD patients	- identify ANKH gene polymorphisms associated with different types of TMD, particularly TMJ closed lock	- Standardized clinical examination (mandibular range of motion, joint pain, joint sounds) - T-MRI for diagnosis of disc displacement with or without reduction	- Genomic DNA extracted from peripheral blood leukocytes - PCR and sequencing - Statistical analysis: univariate and multivariate logistic regression	- Homozygosity for the ANKH-OR variant significantly associated with TMJ closed lock and in older patients - ANKH-OR may be a potential genetic marker for TMJ closed lock
Yamaguchi et al., 2014	Case-Control study (Genome-Wide Association Study)	TSPAN9, APOL3, MRC2	n= 520 participants (146 patients with TMJ degenerative bone changes, 374 healthy controls)	- Identify SNPs associated with TMJ degenerative bone changes using a genome-wide approach	Radiographic signs (panoramic, MRI, and/or CT) of osteophytes, erosion, and/or condylar flattening	Genotyping using Illumina Human OmniExpress Bead Chip; analysis of 550,000 SNPs; statistical comparison between cases and controls	41 SNPs across 22 loci associated with degenerative TMJ changes, top SNP rs878962 in TSPAN9 gene
Xiao et al., 2015	Case-control study	GDF5, SMAD3, RUNX2, TGFβ1, CHST11	n= 240 participants (114 female patients with TMJOA, 126 healthy female controls)	- Investigate the association between SNPs in genes related to the TGFβ signaling pathway and TMJOA risk in Han Chinese women	RDC/TMD	- Sequencing of genomic DNA to examine SNPs; logistic regression model to analyze allelic	- Significant associations for SNPs in GDF5 and SMAD3 with TMJOA - weaker correlation for RUNX2; fivefold increase in TMJOA risk in patients with five or six risk alleles

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Hattori et al., 2015	Case-control study	IL-17A	n= 3 TMD patients (underwent arthroscopy or open joint surgery for TMJOA)	Investigate the role of IL-17A in the inflammatory response of the TMJ	Diagnosis based on clinical procedures and surgical indications (internal derangement, TMJOA)	- Synovial fibroblasts isolated from the TMJ, treated with IL-17A, - DNA microarray analysis, - real-time PCR, - protein secretion measurement	-IL-17A increased the expression of IL-6, CXCL1, IL-8, and CCL20; -significant role of IL-17A in chronic inflammation and tissue degradation in TMD
Bonato et al., 2016	Case-control study	OPG, RANK, RANKL	n= 260 participants, divided into 4 groups: articular TMD with systemic arthralgia (n=85), no articular TMD with systemic arthralgia (n=82), articular TMD without systemic arthralgia (n=21), and controls with neither condition (n=72)	- investigate the potential genetic associations between RANK, RANKL, OPG polymorphisms and the development of TMD and chronic systemic arthralgia.	RDC/TMD diagnostic criteria	Three stages: 1) Clinical TMD diagnosis using RDC/TMD, 2) Assessment of arthralgia with a questionnaire, - Genotyping (saliva samples for SNP analysis)	- OPG gene polymorphisms associated with increased TMJ degeneration and pain in patients with chronic arthralgia and TMD - RANK gene polymorphisms linked to lower risk of chronic joint pain in TMD patients - Genetic variations in the OPG/RANK/RANKL signaling pathway contribute to chronic joint pain in individuals with and without TMD, influencing synovitis onset and cartilage degradation

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Yilmaz et al., 2018	Case-control study	Vitamin D Receptor (VDR)	n= 119 individuals (49 with TMJOA, 70 healthy controls)	- investigate the association between genetic variations in the VDR gene and the risk of developing TMJOA.	TMJOA, subgroups with anterior disc displacement with reduction and without reduction	- Blood samples analyzed for genetic markers (Apa1 and Taq1) via PCR-based restriction fragment length polymorphism (RFLP) assay. - Genomic DNA extracted via proteinase K and phenol/chloroform method. Gel electrophoresis for visualization.	- No statistically significant associations were found between VDR polymorphisms (Apa1 and Taq1) and TMJOA risk. - slight increase in risk observed for certain genotypes.
Tang et al., 2024	Case-control study	Vitamin D Receptor (VDR)	Mices with VDR gene ablation and rescue diet	- explore the anti-inflammatory effect of VD-VDR signaling in the pathological degeneration of TMJ.	Unilateral anterior crossbite induced TMJ disorders	- Mice subjected to mechanical stress, histological staining, immunohistochemistry, and micro-CT analysis were performed. - - In vitro experiments on healthy and Vdr−/− chondrocytes were conducted.	Vdr−/− mice showed decreased TMJ cartilage thickness, increased osteoclasts, inhibited chondrocyte proliferation, and elevated inflammatory gene expression compared to the controlled

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Smith et al., 2011	Case-control study	HTR2A, COMT, NR3C1, CAMK4, CHRM2, IFRD1, GRK5	Chronic TMD cases: n = 166 + 182 (total 348) Healthy controls: n = 1,442 + 170 (total 1,612)	-Investigate the genetic foundations of TMD and identify genetic variants that may increase the likelihood of developing chronic TMD and pain	RDC/TMD criteria, including a history of pain lasting at least 6 months, pain reported at least 15 days in the past month, and pain upon palpation of at least 3 masticatory muscles or one TMJ	Comparison of genetic differences between chronic TMD cases and healthy controls using a gene chip (Pain Research Panel)	- HTR2A and COMT associated with TMD. - Other risk genes: NR3C1, CAMK4, CHRM2, IFRD1, GRK5.
Furquim et al., 2016	Case-control study	TNF-α	TMD cases: n = 152 Healthy controls: n = 91	- Explore the association between TNF-α genetic variations (TNFA-308 SNP) and TMD susceptibility and pain sensitivity	TMD diagnosis confirmed by clinical examination, pain sensitivity measured using an algometer at TMJ, temporal muscle, and masseter muscle	-Real-time polymerase chain reaction (PCR) to assess genotypic and allelic frequencies, -pressure pain threshold evaluation using an algometer	- TNFA-308 SNP associated with TMD. - The GA genotype was 2.87 times more likely to develop TMD compared to controls. - A-allele homozygotes with lower pain sensitivity compared to individuals with the ancestral G allele.
Oporto et al., 2018	Case-control study	DRD1, DRD2, DRD3, DRD4, DRD5, MAOB	Bruxism patients: n = 130 (awake bruxism = 61, sleep bruxism = 26, awake-sleep bruxism = 43) Healthy controls: n = 59	- Investigate the contribution of genetic variants in dopaminergic pathways to bruxism development and its association with TMD pain	- clinical criteria, using questionnaires and clinical exams to categorize patients into three groups	genetic analysis of SNPs in six dopamine-related genes using PCR and TaqMan SNP genotyping assays	Specific genetic variants associated with bruxism forms: -DRD2 variant : lower risk of awake-sleep bruxism -DRD3 variant : increased risk of sleep bruxism -DRD5 variant : reduced risk of awake bruxism -Dopaminergic genetic variations influence both bruxism and TMD pain perception.

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Brancher et al., 2019	Case-control study	COMT (rs4818, rs6269)	N= 298 Adolescents diagnosed with TMD: n = 149 (aged 10–14 years) Healthy controls: n = 149	- Investigate the association between genetic polymorphisms in COMT and the presence of TMD and anxiety in adolescents.	Diagnosis based on RDC/TMD criteria, categorizing participants into myofascial pain, disc displacement, arthralgia, and painful TMD.	- DNA extraction and genotyping of COMT via TaqMan chemistry and endpoint analysis. - Anxiety levels assessed with the State-Trait Anxiety Inventory. Logistic multivariate regression used for statistical analysis	- Significant association between COMT polymorphisms and myofascial pain and disc displacement subtypes of TMD. - COMT polymorphisms linked to higher anxiety levels, suggesting a connection between pain modulation and emotional regulation in adolescents
Fiamengui et al., 2020	Case-control study	TNFA-308 (TNF-α), IL6−174 (IL-6), Val158Met (COMT)	n= 268 (131 TMD patients, 137 healthy controls)	- Evaluate the impact of selected SNPs on pressure pain threshold (PPT) in TMD patients versus healthy controls	RDC/TMD	-PPT measurement (TMJ, masseter, anterior temporalis) using digital algometer -Genotyping via Real-Time PCR (TaqMan assays) on saliva DNA samples - -Statistical analysis: Student's t-test, Pearson chi-square, multiple linear regression, bivariate analysis; SPSS 25.0	- TMD patientswithlower PPTs; I - L6−174 and Val158Met associated with increased pain sensitivity and TMD susceptibility

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Lim et al., 2021	Case-control study	COMT Val158Met polymorphism (rs4680)	n = 36 (12 chronic myofascial TMD patients, 24 healthy controls)	-Investigate alterations in brain signal variability (BOLDSV) in TMD patients E-xplore the relationship between BOLDSV, pain intensity/sensitivity, and the COMT Val158Met polymorphism	Chronic myofascial TMD diagnosis	-Resting-state functional MRI to measure BOLDSV - Genotyping for COMT Val158Met polymorphism	Chronic myofascial TMD patients with reduced BOLDSV in motor, sensory, and dorsolateral prefrontal regions. -Lower BOLDSV associated with higher pain intensity, greater masseter sensitivity, and higher pain frequency. -COMT 158Met allele carriers with lower BOLDSV in the dorsolateral prefrontal cortex and higher BOLDSV in the temporal pole. - -Altered BOLDSV may contribute to the persistence and severity of TMD symptoms.
Campello et al. (2023)	Case-control study	TNF-α (–308G/A), IL-6 (–174G/C), IL-10 (–1082A/G)	n= 34 elderly females (17 with chronic TMD pain, 17 healthy controls)	To investigate the relationship between polymorphisms in TNF-α, IL-6, and IL-10 and the development of chronic TMD pain in elderly females.	DC/TMD	- DNA extraction from blood samples via the Salting Out method. - DNA quantification with NanoDrop spectrophotometer - TNF-α –308G/A analyzed by PCR–restriction fragment length polymorphism technique -IL-6 –174G/C analyzed by PCR -IL-10 –1082A/G analyzed by PCR–allele-specific amplification -Data analyzed with BioEstat 5.3 software	-No significant association between IL-6 –174G/C polymorphism and chronic TMD pain - Significant association between TNF-α –308G/A polymorphism and chronic TMD pain - IL-10 –1082A/G polymorphism associated with chronic TMD pain; AA genotype linked to reduced IL-10 secretion and immune imbalance promoting inflammation

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Zlendic et al., 2024	Case-control study	CXCL8 (rs2227306, rs2227307) and TNF (rs1800629)	n = 170 participants (85 TMD patients 85 healthy controls)	-To evaluate the association between CXCL8 and TNF gene polymorphisms and TMD presence, pain intensity, and the presence of chronic arthralgia/myalgia	Diagnosed using a validated protocol for pain-related TMDs	-DNA extracted from buccal mucosa swabs -Genotyping performed using TaqMan SNP assays	High pain intensity was associated with carrying the minor alleles "G" and "T" -Minor allele "G" was more prevalent in TMD patients with arthralgia compared to controls -Logistic regression showed that minor allele "G" increasing age, and female sex were significant predictors of high pain intensity
Bonato et al., 2016	Case-Control Study	ESRRB (Estrogen-related receptor beta)	Subjects with TMD (n=13), subjects with rotator cuff disease (RCD) but without TMD (n=16), subjects with both conditions (n=49), and a control group with neither condition (n=30)	investigate the association between ESRRB polymorphisms and TMD risk	RDC/TMD	-Chemiluminescent immunoassay for estradiol; -surface EMG for head/cervical muscle activity; -analysis of 8 SNPs	-ESRRB associated with higher TMD risk; E SRRB polymorphisms may affect inflammation, tissue repair, and pain sensitivity; estrogen fluctuations may amplify TMD symptoms, especially in women

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Chung et al., 2017	Case-Control Study	ACTN3, ENPP1, ESR1, PITX1, PITX2	n=174 patients with dentofacial deformities, grouped by facial symmetry	Examine gene polymorphisms in relation to facial asymmetry and TMD before/after ortho-gnathic treatment	-Jaw pain/function questionnaire; -cephalometric analysis	-SNP genotyping; -analysis of jaw function and asymmetry	-ESR1 linked to C-shaped asymmetry; -ESR1 linked to principal component variation; -supports estrogen signaling's role in TMD via structural and pain mechanisms
Oporto & Salazar, 2018	Case-Control Study	epigenetics - DNA methylation	n=158 patients (32 sleep bruxism, 42 awake, 42 both, 42 controls)	-Investigate DNA methylation levels in bruxism patients as epigenetic contributors to TMD	-5-item bruxism questionnaire, -patient interview, -assessment of clinical signs like muscle pain and tooth wear.	-Colorimetric assay for DNA methylation; -comparative analysis of bruxism subgroups and control group	-Hypomethylation in bruxism -epigenetic alteration may contribute to TMDs development
Wu et al., 2024	Mendelian Randomization	Genetic variants associated with major depression, bipolar disorder, and schizophrenia.	n= 211,023 European individuals (5,668 cases and 205,355 controls).	-Assess genetic causal links between psychiatric disorders (depression, bipolar, schizophrenia) and TMD	standardized semi-structured interviews, where diagnoses of cases were made in accordance with international consensus standards.	-Mendelian randomization using large GWAS datasets for psychiatric traits and TMD : - Five sensitivity analyses including MR-Egger, Maximum Likelihood, Weighted median, MR. RAPS and MR-PRESSO used as supplements. - heterogeneity tests and pleiotropic tests to ensure the robustness.	- Major depression with significant genetic association with TMD; - bipolar disorder and schizophrenia did not; highlights depression's role in TMD pathophysiology

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Ao et al., 2024	Case-Control Study	epigenetics - DNA methylation; FMOD, PM20D1, ZNF718, ZFP57, RNF39	n= 948 (496 chronic painful TMD cases; 452 controls)	Examine genome-wide DNA methylation patterns associated with chronic TMD and aid diagnosis and guide therapies	OPPERA study TMD diagnosis criteria	-Genome-wide methylation analysis of blood samples; -identification of differentially methylated regions (DMRs)	-6 DMRs linked to genes involved in inflammation and neuronal response; - methylation patterns may influence chronicity or resolution of TMD pain - Found dynamic DNA methylation patterns at specific loci associated with TMD onset and progression; potential for early diagnostic biomarkers and future epigenetic-targeted treatments
Zlendic et al., 2024	Prospective cohort	COMT (rs4646310, rs6269, rs4818, rs4680)	n = 60 TMD pain patients	To investigate how COMT genotypes influence treatment response in TMD pain patients	DC/TMD criteria	-Genotyping via buccal swabs and TaqMan assays; - 6-month standardized treatment; statistical analysis of genotype-treatment response associations	-Minor allele carriers of COMT showed poorer treatment outcomes . -Genetic profile may guide personalized treatment plans.
Braga et al., 2021	Case-control study	COMT Val158Met, MMP1-1607 TNFα-308	n=1 female	Discuss multifactorial etiology of TMD, including genetic predisposition, and personalized treatment approach.	RDC/TMD: arthralgia, myofascial pain, disc displacement without reduction, TMJ degenerative disease	Clinical exam, MRI, CBCT, pressure algometer for pain threshold, g enetic SNP analysis, anamnesis (sleep bruxism, anxiety, migraine), personalized multimodal treatment	-Personalized treatment led to long-term stabilization of TMJ degeneration. Genetic profiling useful for prognosis and therapy guidance.

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Jayaseelan & Arumugam, 2022	observational study	IL1B, VEGFA, ESR1, CCL2, IL6	In silico (DisGeNET datasets)	To identify genetic biomarkers for early diagnosis and to distinguish TMD from related conditions like osteoarthritis	N/A	-Integration of three gene datasets related to TMD phenotypes; -identification of shared genes; - gene ontology pathway analysis	-Pathways such as ESR1 gene may represent therapeutic targets. - Requires experimental validation.

Annex B. Systematic and meta-analyses identifying genes related to temporomandibular disorders.

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Visscher & Lobbezoo, 2015	Systematic review	5-HT2A, COMT, T-cell receptor pathway	21 studies (5 familial aggregation studies + 16 genetic association studies) from 473 publications	- review the role of heritability in TMD pain, including both familial aggregation and genetic association studies	studies focusing on TMD pain, including myalgia and arthralgia; variable diagnostic criteria across studies	systematic literature search in PubMed and Embase following PRISMA guidelines	Modest evidence for genetic contribution to TMD pain; variations in serotonin receptors linked to increased pain sensitivity and connection with depression; mutations in the T-cell receptor pathway associated with widespread pain in TMD patients
Melis & Di Giosia (2016)	Systematic Review	5-HT2A, T-cell receptor pathway, catecholamine activity, estrogen metabolism, folate metabolism, glutathione activity, ANKH, OPPERA genes, cytokine activity *SHMT1*, *MTHFD1*, *MTRR*, genes in serotonin,,	26 clinical studies (24 + 2 additional)	To review literature on genetic factors in the etiology of TMDs	Co-occurrence of TMD signs and symptoms in families, risk of widespread pain	- PubMed search, -References from included studies and review articles also examined	-Genetic polymorphisms associated with TMD risk, mainly related to serotonin, T-cell receptor pathway, folate-related genes, and cathecholamine genes -No co-occurrence of TMD in families, but certain genes linked to higher or lower TMD risk -Insights into potential targeted treatments based on genetic risk factors

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Campello et al. (2021)	systematic review	5-HT2A, 5-HT1A	4 studies	-investigate the association between genetic variations in serotonin receptor genes (5-HT2A and 5-HT1A) and bruxism etiology	Bruxism (sleep and awake) leading to jaw pain, limited jaw movement, and headaches	- Systematic review registered in PROSPERO - Literature search in multiple databases (Web of Science, Scopus, Embase, PubMed/MEDLINE, ProQuest) for articles published before May 2021 -Case-control, cohort, and cross-sectional studies included with a control group -NOS and JBI used to assess methodological quality of included studie	-SNP of 5-HT2A receptor gene associated with sleep bruxism -No significant association found for SNPs in awake bruxism or combined sleep and awake bruxism groups -Variability in results could be explained by genetic differences in different ethnic populations
Brancher et al. (2021)	Systematic Review and Meta-Analysis	COMT	1,903 articles screened, 10 studies included, 3 studies for meta-analysis	-explore the association between genetic polymorphisms in the COMT gene and TMDs	TMD subgroups: myofascial pain (with or without limited opening), disk displacement (with or without reduction), and arthralgia	- Systematic review	-Polymorphisms in the COMT gene significantly associated with myofascial pain and painful TMD -role of COMT in pain regulation and its potential as a genetic marker for understanding the biological mechanisms behind TMD

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Leucuta et al., 2024	Systematic Review	ESR1	22 studies	-Summarize estrogen and genetic predisposition's influence on TMD, especially in women	RDC/TMD criteria.	-comprehensive literature search via PubMed/MEDLINE, Scopus, Embase, and Web of Science databases	-ESR1 polymorphisms more prevalent in TMD women; -polycystic ovary syndrome linked to higher TMD rates; -beta-estradiol levels associated with TMD onset and progression
Alshahrani et al. (2024)	Systematic Review	5-HTT, DRD4, COMT, SHMT1, MTHFD1, MTRR	851 articles screened, 19 studies included	-investigate the correlation between genetic factors and the onset and progression of TMDs	Myofascial pain Chronic pain Disc displacement	-comprehensive search in databases including ScienceDirect, PubMed, Cochrane Library, Dimensions, and Emerald. - Studies selection via modified PICOS criteria - methodological quality of the studies assessed using the *Joanna Briggs Institute Critical Appraisal Checklist for Non-randomized Experimental Investigation*	-Genetic polymorphisms significantly influenced the development of TMD symptoms (myofascial pain, chronic pain, disc displacement) -Variations in *SHMT1*, *MTHFD1*, and *MTRR* genes were significantly associated with increased risk of TMD. - Variations in the 5-HTT gene associated with TMD progression, particularly in mood regulation and pain perception -Dopaminergic pathways (DRD4 gene) influenced pain signal processing and the severity of TMD symptoms -COMT Val158Met variant linked to heightened pain sensitivity and increased TMD chronicity

Author / Year	Study type	Gene	Sample	Objectives	TMD diagnostic criteria	Methodology	Results
Heyat et al., 2020	Literature Review	11 genes related to bruxism : DRD2, DRD3, DRD5, KLF7, HTR2A, TMEM37, FRRS1L, CSRP1, CDKL5, SLC6A4, MECP2.	Approximately n=115,077 subjects across 75 research articles on bruxism detection, treatment, children and adolescents	Highlight critical issues and future research directions in bruxism and its association with TMD	study includes TMD in context of bruxism research (Electromyogram; Electroencephalogram; Questionar)	-Systematic mapping process -Network visualization -Literature review - software tools: VOSviewer, MATLAB, MEGA-X	-Genetic factors play a key role in bruxism, a TMD subtype -Clinical variability calls for integrative diagnostics and personalized therapies based on genetic profiles. -Current treatments with mixed results
Li et al., 2023	Systematic review	COMT and more than 30 locis	n=57 GWAS studies;	To explore genetic variants involved in pain perception and how they could improve diagnosis and treatment of TMD	N/A	Review of 57 GWAS studies founded via MEDLINE and Embase	-Identified pain-related genes involved in inflammation and neural pathways; - data could help identify patients genetically predisposed to TMD pain and guide future personalized treatments