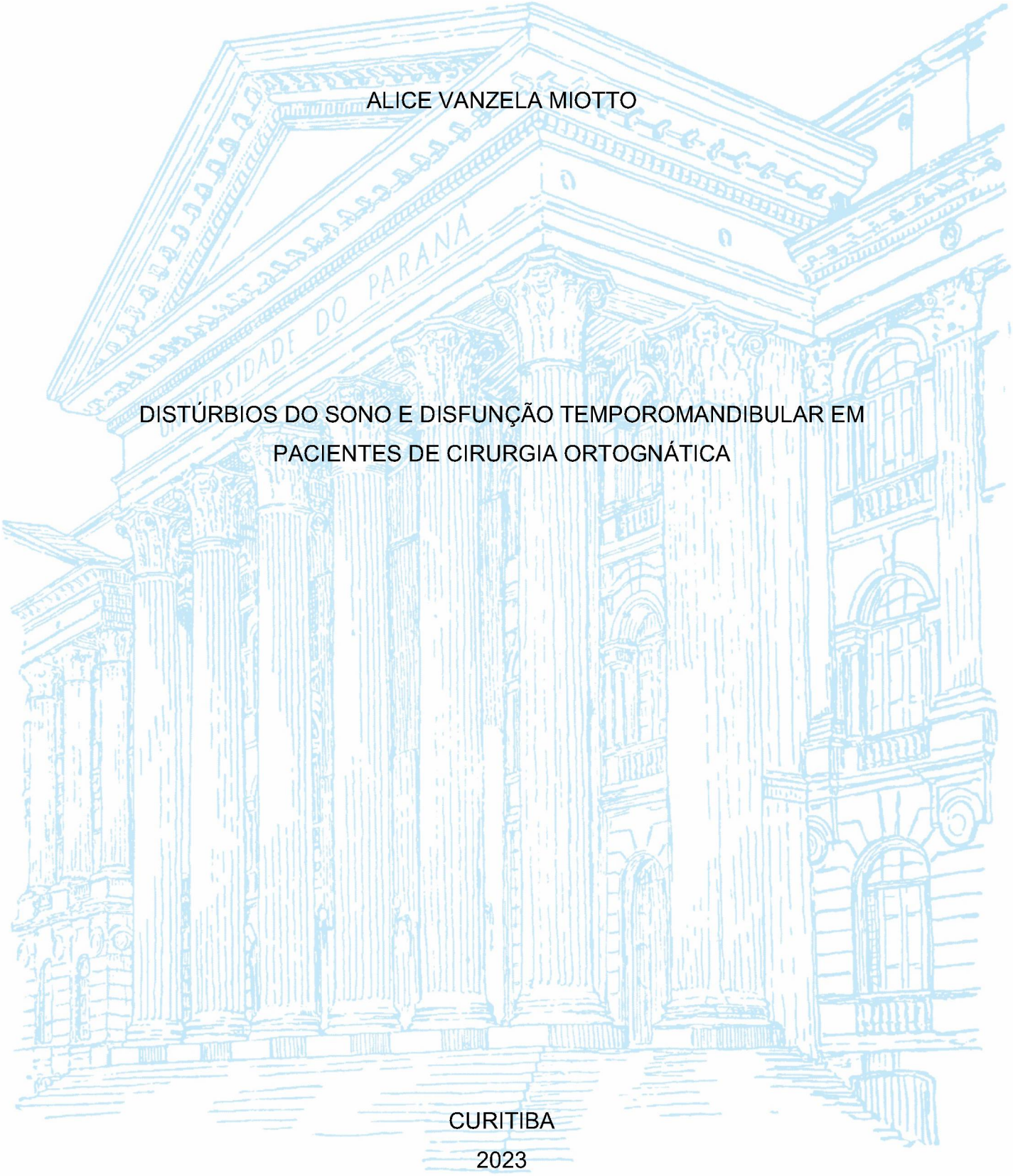
UNIVERSIDADE FEDERAL DO PARANÁ

ALICE VANZELA MIOTTO



DISTÚRBIOS DO SONO E DISFUNÇÃO TEMPOROMANDIBULAR EM PACIENTES DE CIRURGIA ORTOGNÁTICA

CURITIBA

2023

ALICE VANZELA MIOTTO

DISTÚRBIOS DO SONO E DISFUNÇÃO TEMPOROMANDIBULAR EM PACIENTES DE CIRURGIA ORTOGNÁTICA

Dissertação apresentada ao curso de Pós-Graduação em Odontologia, Setor de Ciências da Saúde, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestrado em Odontologia.

Orientadora: Profa. Dra. Rafaela Scariot
Coorientador(a): Profa. Dra. Aline Sebastiani

CURITIBA

2023

Miotto, Alice Vanzela
Distúrbios do sono e disfunção temporomandibular em pacientes de cirurgia ortognática [recurso eletrônico] / Alice Vanzela Miotto – Curitiba, 2023.
1 recurso online: PDF.

Dissertação (mestrado) – Programa de Pós-Graduação em Odontologia. Setor de Ciências da Saúde, Universidade Federal do Paraná, 2023.

Orientador: Profa. Dra. Rafaela Scariot
Coorientador: Profa. Dra. Aline Sebastiani

1. Deformidades dentofaciais. 2. Cirurgia ortognática. 3. Qualidade do sono. 4. Bruxismo do sono. I. Scariot, Rafaela. II. Sebastiani, Aline. III. Universidade Federal do Paraná. IV. Título.

CDD 617.643

Maria da Conceição Kury da Silva CRB 9/1275

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SETOR DE CIÊNCIAS DA SAÚDE
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ODONTOLOGIA - 40001016065P8

ATA Nº175

ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRA EM ODONTOLOGIA

No dia vinte e sete de março de dois mil e vinte e tres às 09:00 horas, na sala Plataforma teams, Online - plataforma teams, foram instaladas as atividades pertinentes ao rito de defesa de dissertação da mestranda **ALICE VANZELA MIOTTO**, intitulada**: Distúrbios do Sono e DisfunçãoTemporomandibular em Pacientes de Cirurgia Ortognática**, sob orientação da Profa. Dra. RAFAELA SCARIOT. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação ODONTOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: RAFAELA SCARIOT (UNIVERSIDADE FEDERAL DO PARANÁ), JULIANA FELTRIN DE SOUZA CAPARROZ (UNIVERSIDADE FEDERAL DO PARANÁ), DANIEL BONOTTO (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestra está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, RAFAELA SCARIOT, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 27 de Março de 2023.

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RAFAELA SCARIOT
Presidente da Banca Examinadora

Assinatura Eletrônica
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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ODONTOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **ALICE VANZELA MIOTTO** intitulada: **Distúrbios do Sono e DisfunçãoTemporomandibular em Pacientes de Cirurgia Ortognática**, sob orientação da Profa. Dra. RAFAELA SCARIOT, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

Pacientes com deformidades dentofaciais (DDF) podem apresentar risco aumentado para alguns desfechos em saúde como disfunções temporomandibulares (DTM), bruxismo e distúrbios do sono (DS). Os objetivos desta pesquisa foram 1) analisar a relação/associação entre DTM e DDF por meio de um estudo transversal com grupo de comparação, comparar pacientes com deformidade dentofacial em preparo ortodôntico para a cirurgia ortognática com indivíduos sem deformidade dentofacial sobre a presença de DTM 2) bem como, analisar a associação entre polimorfismos no gene da Melatonina denominados *Melatonin Receptor Type 1 (MTNR1A) com DS e BS.* Para isto, (1) foram selecionados randomicamente 40 individuos que foram submetidos a CO no serviço de Cirurgia e Traumatologia Bucomaxilofacial da Universidade Federal do Paraná (UFPR) e 40 individuos sem DDF pareados por sexo, idade e raça auto reportada que fizeram outros tratamentos na clínicas odontológicas da UFPR. Os criterios de inclusão adotados para o grupo com DDF foram: pacientes com deformidades dentofaciais com necessidade de tratamento através da cirurgia ortognática, maiores de 18 anos, que aceitaram participaram da pesquisa e assinaram o TCLE. Os critérios de inclusão para o grupo sem DDF foram ser maior de 18 anos, assinar o TCLE e não apresentar deformidade dentofacial. Os critérios de exclusão para ambos os grupos foram: fissura lábio-palatina ou síndrome associada, cirurgias prévias na região cervicofacial, alterações cognitivas e neurológicas, mais de 4 elementos dentários ausentes, diagnóstico de artitre e artrose em outras articulações e pacientes que fizeram uso de anti-inflamatórios, analgésicos ou ciclobenzaprina na semana da avaliação. O diagnóstico de DTM foi realizado através do *Diagnostic Criteria for Temporomandibular Disorder (DC-TMD).* Os fatores biopsicossociais foram avaliados através dos questionários GAD-7 e PHQ-15 (Eixo II DC/TMD). O bruxismo do sono e em vigilia foram avaliados através do *Oral Behaviors Checklist (OBC).* O diagnóstico da Apneia Obstrutiva do Sono (AOS) foi realizado de forma subjetiva através do questionário de *STOP-Bang* e de forma objetiva através da polissonografia tipo IV (Biologix®). A qualidade do sono foi investigada pelo *Pittsburgh Sleep Quality Index (PSQI)*. O DNA dos participantes foi coletado apartir de células epiteliais da mucosa bucal para avaliação através de testes genéticos de polimorfismos no *gene MTNR1A (rs6553010, rs13140012 e rs6847693).* Todos os dados coletados foram submetidos à análise estatística através do *software* SPSS versão 21.0. Resultados: (1) a artralgia foi mais prevalente no grupo com DDF com um total de 45%, comparado a 10% nos indivíduos sem DDF (P=0.010). O bruxismo do sono foi associado com a ocorrência de DTM articular nestes pacientes (p=0.046) (2) Observou-se uma associação significativa entre os polimorfismos do gene *MTNR1A* com bruxismo do sono, latência do sono, presença de distúrbios do sono e uso de medicamentos para dormir.

Palavras-chave: deformidades dentofaciais; cirurgia-ortognática; síndrome da disfunção da articulação Temporomandibular; qualidade do sono; bruxismo do sono.

ABSTRACT

Patients with dentofacial deformities (DFD) may be at increased risk for some health outcomes like temporomandibular disorders (TMD), bruxism, and sleep disorders (SD). The objectives of this research were 1) to analyze the relationship/association between TMD and DFD through a cross-sectional study with a comparison group, comparing patients with dentofacial deformity undergoing orthodontic preparation for orthognathic surgery with individuals without dentofacial deformity regarding TMD diagnoses, and 2) to analyze the association between polymorphisms in the Melatonin gene called Melatonin Receptor Type 1 (*MTNR1A*) with DS and BS. For this, (1) a total of 40 individuals were randomly selected from the ones referred to OC at the Oral and Maxillofacial Surgery and Traumatology service at the Federal University of Paraná (UFPR), and 40 individuals without DFD matched by sex, age, and self-reported race who underwent other treatments at the UFPR dental clinics. The inclusion criteria adopted for the group with DFD were patients with dentofacial deformities needing treatment through orthognathic surgery, over 18 years of age, who agreed to participate in the research and accepted the informed consent form (ICF). Inclusion criteria for the group without DFD were patients without dentofacial deformities, over 18 years of age, who agreed to participate and signed the informed consent form. Exclusion criteria for both groups were cleft lip and palate or associated syndrome, previous surgeries in the cervicofacial region, cognitive and neurological alterations, more than four missing dental elements, arthritis and arthrosis diagnosed in other joints, and patients who used anti-inflammatories, analgesics, or cyclobenzaprine in the evaluation week. The TMD was identified using the Diagnostic Criteria for Temporomandibular Disorder (DC-TMD). The experimental GAD-7 and PHQ-15 (Axis II DC/TMD) evaluated the biopsychosocial factors. The Oral Behaviors Checklist (OBC) assessed sleep and wake bruxism. Obstructive Sleep Apnea (OSA) diagnosis was made subjectively through STOP-Bang sessions and objectively through type IV polysomnography (*Biologix*®). Sleep quality was investigated by the Pittsburgh Sleep Quality Index (PSQI). Participants' DNA was collected from oral mucosal epithelial cells for evaluation through genetic testing of polymorphisms in the *MTNR1A* gene (*rs6553010*, *rs13140012*, and *rs6847693*). All collected data were submitted for statistical analysis using SPSS software version 21.0. Results: (1) Arthralgia was identified in 45% of patients with DFD undergoing emergency orthodontic treatment, compared to 10% of individuals without DFD ($p = 0.010$). Sleep bruxism was associated with the occurrence of joint TMD in these patients ($p = 0.046$). (2) A significant association was observed between *MTNR1A* gene polymorphisms and sleep bruxism, sleep latency, presence of sleep disorders, and use of sleep medication.

Keywords: dentofacial deformities; orthognathic surgery; temporomandibular joint disorders; sleep quality; sleep bruxism.

AGRADECIMENTOS

Agradeço à Deus, pela dádiva da vida, pela fé que sempre manteve meu coração firme nesse propósito.

Aos meus pais, que nunca mediram esforços para me proporcionar o melhor em educação e que sempre me incentivaram a estudar e a crescer e aos meus irmãos, que sempre estão ao meu lado apoiando e incentivando todas as escolhas da minha vida.

Agradeço a Universidade Federal do Paraná por me proporcionar um ensino público de qualidade na graduação e na pós-graduação e por ser minha casa durante tantos anos.

Agradeço a todos os pacientes que aceitaram participar dessa pesquisa.

Agradeço ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Paraná pela oportunidade em realizar o meu tão almejado mestrado, em uma instituição tão renomada e especial.

À todos os Professores do Programa de pós-graduação em Odontologia da Universidades Federal do Paraná de cada disciplina ofertada por todos os ensinamentos transmitidos, em especial ao Prof. Dr. Delson João da Costa por tornar as clínicas de coleta de dados leves e produtivas.

Agradeço a professora Dra. Rafaela Scariot por ter me orientado e transmitido de maneira única seus conhecimentos, hoje sinto enorme admiração e respeito pela professora que é e que foi para mim ao longo desses dois anos.

Agradeço a professora Dra. Aline Sebastiani por ter aberto as portas para a pesquisa em sono e dor e por ter proporcionado a melhor coorientação que eu poderia ter.

Por fim, ao meu amor José Felipe Serpa, que esteve ao meu lado nestes anos de estudo e dedicação intensos no curso de mestrado em Odontologia da UFPR e a nossa futura filha que ainda tem algumas semanas e já transforma tanto em mim.

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LISTA DE ABREVIATURAS OU SIGLAS

AASM – *American Academy of Sleep Medicine*

AMM – Cirurgia de avanço maxilar e mandibular

AOS – Apneia Obstrutiva do Sono

CO – Cirurgia Ortognática

CPAP – *Continuous positive airway pressure*

DTM – Disfunção Temporomandibular

DFF – Deformidade dentofacial

GAD 7 – Questionário de Saúde do Paciente- *Patient Health Questionnaire*

MLT - Melatonina

IDO – Índice de Dessaturação de Oxigênio

PSQI – *Pittsburg Sleep Quality Index*

PHQ – 15 – Desordem de ansiedade generalizada - *Generalized Anxiety Disorder*

PSQI – Pittsburg Sleep Quality Indez

TCLE – Termo de Consentimento Livre e Esclarecido

SDS – Dodecilsulfato de Sódio

Tris-HCl – Hidrocloridrato de Tris

mM – Milimole

SDS – Dodecilsulfato de Sódio

EDTA – Ethylenediaminetetraacetic Acid

RPM – Rotação por minuto

SNP – Single nucleotide polimorphism

LISTA DE SÍMBOLOS

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SUMÁRIO

1 INTRODUÇÃO E REVISÃO DE LITERATURA

1.1 DEFORMIDADES DENTOFACIAIS

1.1.1 ASPECTOS FÍSICOS

Deformidades dentofaciais (DDF) são definidas como sérios problemas de má oclusão dentária associada a alterações esqueléticas que requerem um tratamento combinado entre ortodontia e cirurgia ortognática (FERRAZ *et al.,* 2011). Tais deformidades podem ser mínimas como uma leve projeção do mento, ou extrema, como um excesso maxilar vertical severo ou uma microssomia hemifacial. O acometimento pode estar em uma ou duas bases ósseas, nos planos vertical, horizontal e transversal, tanto de maneira isolada como combinada, acarretando diferentes tipos de deformidades. As principais DDF são: classe II esquelética, classe III esquelética, biprotrusão esquelética, mordida aberta anterior esquelética e mordida cruzada posterior bilateral esquelética (FISH *et al.*, 1993).

A presença de DDF pode afetar a função mastigatória, respiratória e articular desses indivíduos, bem como sua fonética e estética facial (Sadek *et al.*, 2007; Almasri *et al.*, 2014). Também, as DDF parecem relacionadas à limitação funcional, à dor e ao comprometimento na interação social desses indivíduos (Choi *et al.*, 2015), desencadeando maior índice de depressão e problemas psicológicos (Kim *et al.*, 2009; Baherimoghaddam *et al.,* 2016). Desta forma, pacientes com DDF podem ter risco aumentado para alguns desfechos negativos em saúde, como disfunções temporomandibulares (DTM) e distúrbios do sono (FERRAZ *et al.,* 2011; SEBASTIANI *et al.*, 2018; SEBASTIANI *et al.,* 2016).

Um estudo realizado no Departamento de Cirurgia Bucomaxilofacial, do Hospital da Universidade de Malmö, Suécia, incluiu 121 pacientes de DDF com idade média 22,5 (+-7,4 anos) 70 mulheres e 51 homens que iriam se submeter a cirurgia ortognática no grupo caso e 56 pacientes no grupo controle (sem DDF), idade média 23,4 (+-7,4 anos) 33 mulheres e 23 homens, no exame inicial, o grupo caso teve maior frequência de dor miofascial (P = 0,035) e artralgia (P = 0,040) do que o grupo controle (C. ABRAHAMSSON, *et al.,* 2013).

Uma recente revisão de literatura, avaliou 34 artigos referente a presença de DTM em pacientes em diferentes morfologias faciais, e apesar da heterogeneidade dos desenhos de estudos e dos achados nos artigos revisados, parece razoável sugerir que os perfis esqueléticos de classe II e o padrão de crescimento hiperdivergente estão provavelmente associados a um aumento da frequência de deslocamento do disco da ATM e distúrbios degenerativos (MANDREFINI *el al.,* 2016).

Outro estudo, avaliou a prevalência de DTM em 100 pacientes de DDF previamente à

cirurgia ortognática, antes da cirurgia 35% dos pacientes apresentavam pelo menos um sinal ou sintoma de DTM, como: 27 pacientes (27%) apresentavam estalido, 8 (8%) dor, 4 (4%) crepitação e 7 (7%) pacientes tinham indicações de DTM na ressonância magnética. (AMJAD M. ALWARAWREH *et al.,* 2018).

Achado semelhante a uma revisão sistemática, que incluiu um total de 542 pacientes em 6 estudos incluídos na análise em que a prevalência de DTMs para pacientes de cirurgia ortognática no pré-operatório foi de 32,5% (IC 95% = 26,7% a 38,9%). Os resultados deste estudo mostram que os pacientes que vão ter uma correção de sua má oclusão por ortodontia e cirurgia ortognática têm uma incidência significativa de DTM quando comparados a uma população controle, mas que após o tratamento, a incidência de DTM não difere de uma população de controle. (AL-MORAISSI *et al.,* 2017).

Ainda as DDF podem impactar o espaço das vias áreas, sendo reduzido e favorecendo o colapso e dificultando a passagem de ar, caracterizando a Apneia Obstrutiva do Sono (AOS) (TRENCH, 2015). Essa resistência respiratória frequentemente é acompanhada de micro despertares, pode influenciar na qualidade do sono e ser um fator causal para bruxismo do sono secundário ao fator respiratório, quando e episódio de bruxismo ocorre ao final do episódio da parada respiratória. (MANFREDINI *et al.*, 2015).

Um estudo coorte retrospectivo avaliou 260 pacientes com DDF. Destes, 23% (60 de 262) tinham AOS confirmada por PSG (grupo II). A idade média dos indivíduos foi de 34 anos (variação de 13 a 63) e incluiu 25 mulheres (42%). Vinte por cento dos indivíduos (12/60) tinham <18 anos de idade e 37% dos indivíduos (22/60) tinham >40 anos de idade. Pacientes com deficiência mandibular primária e DDF de face curta eram mais propensos a ter AOS (P < 0,001 e P = 0,001, respectivamente) (JEFFREY *et al.,* 2018).

1.1.2 ASPECTOS PSICOLÓGICOS

Existem vários relatos sobre associações entre doença clínica/sintomas somáticos e distúrbios psicológicos, como a depressão, em indivíduos com DDF. Como as doenças geralmente têm fatores etiológicos físicos e/ou emocionais, pode ser significativo avaliar aspectos psicológicos e mudanças de comportamento nos indivíduos com DDF (KIM *et al.*, 2009).

A depressão é um distúrbio complexo que se manifesta de muitas maneiras diferentes (ANTHES, 2014) e está entre os transtornos mais incapacitantes em todo o mundo, acarretando profundas consequências sociais e econômicas (ZHANG *et al.* 2015).

Estudos clínicos revelaram que a dor crônica e o estresse crônico, frequentemente induzem a depressão (Slavich *et al.*, 2014; Agbaje *et al.*, 2018). Nesse grupo podem ser incluídos os pacientes com má-oclusão severa, uma vez que dentre as suas queixas, estão a

dor orofacial (AGBAJE *et al.*, 2018).

Os fatores psicossociais, atividade social e intensidade da dor podem também afetar a qualidade subjetiva do sono em pacientes com DTM dolorosa. (POLUHA RL, *et al.,* 2023)

1.2 DISFUNÇÃO TEMPOROMANDIBULAR

Segundo a Academia Americana de Dor Orofacial, o conceito de Dor Orofacial inclui toda a dor associada a tecidos moles e mineralizados (pele, vasos sanguíneos, ossos, dentes, glândulas ou músculos) da cavidade oral e da face. Usualmente, essa dor pode ser referida na região da cabeça e/ou pescoço ou mesmo estar associada a cervicalgias, cefaleias primárias e doenças reumáticas como fibromialgia e artrite reumatoide, a DTM é um tipo de Dor Orofacial, definida como um conjunto de distúrbios que envolvem os músculos mastigatórios, a articulação temporomandibular (ATM) e estruturas associadas. A principal manifestação clínica é a dor, acompanhada de limitação e/ou não coordenação dos movimentos mandibulares e ruídos articulares e impactando diretamente na qualidade de vida, já que atinge funções básicas como mastigação e fala.

Estudos epidemiológicos estimam que 40% a 75% da população apresentem ao menos um sinal ou sintoma de DTM, como ruídos na ATM e 33% pelo menos um sintoma, como dor na face ou na ATM (LEEUW, 2010). No Brasil estima-se que 37,5% da população apresente ao menos um sintoma de DTM (GONÇALVES *et al.*, 2009*)*.

A etiologia das DTM mais aceita atualmente é o modelo biopsicossocial, que envolve uma combinação de fatores biológicos, psicológicos e sociais, todos relacionados a componentes genéticos. Segundo essa perspectiva, entende-se que um problema biológico pode ter antecedentes psicológicos, assim como consequências comportamentais.

A sobrecarga oclusal e hábitos parafuncionais (BS) são frequentemente citados como fatores biomecânicos; aumento dos níveis de hormônios de estrogênio são considerados fatores biológicos e entre os fatores biopsicossociais destaca-se o estresse, ansiedade ou depressão (SLADE *et al.,* 2013). Considerando todos esses fatores, nota-se que o sono também participa desse complexo quadro etiológico. A relação de causa-efeito entre dor e sono ruim ainda não é completamente compreendida, aparentemente ambas sustentam um quadro bidirecional. A literatura aponta que a dor tem um grande impacto na qualidade do sono dos pacientes com DTM e ressalta a importância do manejo integrado desse paciente, além de melhorar a qualidade do sono, reflete na maior eficácia dos tratamentos para a DTM e outras desordens idiopáticas da dor (VEIGA *et al.,* 2013; DREWECK *et al.,* 2020).

Após anos de debate sobre o papel das características oclusais como fatores causais ou de risco para DTM, atualmente atribui-se uma baixa relevância para a oclusão dentária e a

relação inter-arcadas (MANFREDINI *et al.,* 2016). Entretanto, a literatura aponta um alto índice de sinais e sintomas de DTM nos indivíduos na fase de tratamento ortodôntico pré-cirúrgico quando comparado ao pós-cirúrgico (SEBASTIANI *et al.,* 2016), porém alguns autores não atribuem essa melhora a correção da DDF (DERVIS; TUNCER, 2002). Em uma revisão sistemática recente (AL-MORAISSI *et al.,* 2017; DE CLERCQ *et al.,* 1998), um total de 542 pacientes foram incluídos através de 6 estudos que compararam a frequência de DTMs entre os pacientes que se submeteriam a cirurgia ortognática (n = 321) e a população sem deformidades dentofaciais (n = 221). Houve uma diferença significativa entre os 2 grupos. O risco relativo para pacientes com deformidades dentofaciais antes da cirurgia ortognática, em comparação com um grupo controle, foi de 1,63. Os resultados deste estudo mostram que os pacientes que vão ter correção de sua má oclusão por ortodontia e cirurgia ortognática têm uma incidência significativa de DTMs quando comparados a uma população controle, mas que, após o tratamento, a incidência de DTMs não difere daquela de uma população de controle. Os autores deste trabalho comentam ainda que as razões para esses achados não são claras, evidenciando as lacunas sobre o conhecimento desse tema. Alguns autores sugerem que os perfis esqueléticos de classe II e o padrão de crescimento hiperdivergente estão provavelmente associados a um aumento da frequência de deslocamento do disco da ATM e distúrbios degenerativos (MANFREDINI *et al.,* 2015).

Os Critérios Diagnósticos para Disfunção Temporomandibular (DC/TMD) é o instrumento mais aceito atualmente para o diagnóstico de DTM. Recentemente foi traduzido e validado para a língua portuguesa do Brasil. É constituído por 2 eixos. O Eixo I é composto pelos achados do exame físico (ANEXO 2) e o Eixo II engloba os aspectos emocionais (SCHIFFMAN *et al*., 2014).

1.3 DISTÚRBIOS DO SONO

1.3.1 AOS

A Apneia Obstrutiva do Sono (AOS) é caracterizada por episódios recorrentes de obstrução parcial ou total das vias aéreas superiores durante o sono, micro despertando o indivíduo diversas vezes. Essa condição traz inúmeros prejuízos para as funções cognitivas, como perda de memória e dificuldade de concentração. Além de aumentar o risco de acidentes de trabalho e automobilísticos, prejuízos nas relações familiares, no estudo e no trabalho. Os pacientes com AOS têm risco aumentado de diversas cardiopatias, como aumento da pressão arterial, infarto agudo do miocárdio e acidente vascular encefálico (JAVAHERI *et al.,* 2017). A AOS atinge 9% a 49% da população, porém muitos pacientes

não são diagnosticados (SENARATNA *et al.,* 2017). A gravidade da AOS é descrita pelo índice de apneia-hipopneia (IAH) que se baseia na quantidade desses episódios de obstrução das vias aéreas superiores por hora de sono. É considerada AOS leve quando o paciente apresenta de 5 a 15 eventos por hora de sono, moderada entre 15 a 30 eventos e severa mais de 30 eventos por hora de sono. Essa fragmentação do sono traz inúmeros prejuízos a saúde e é altamente associada à morbidade e mortalidade, sendo uma questão de saúde pública (YOUNG *et al.*, 2008; ALESSANDRI-BONETTI *et al.,* 2019). Cerca de 25% dos pacientes com AOS apresentam sonolência diurna, sono não reparador e fadiga. O relato do parceiro de ronco excessivo e engasgos ou falta de ar também pode acontecer (LARATTA *et al;* 2017). Existem muitos fatores de risco subjacentes, condições de predisposição e comorbidades associadas para AOS como obesidade, circunferência do pescoço aumentada e deformidades dentofaciais como: micrognatia, retrognatia, hipertrofia tonsilar, macroglossia ou depósito de gordura (FERRAZ *et al.,* 2011). Dados de um estudo feito com estimativa de prevalência global demonstrou que a AOS afeta aproximadamente 1 bilhão de adultos (30-69 anos), sendo que cerca de 425 milhões precisariam de tratamento por apresentar apneia moderada e severa (BENJAFIELD *et al.*, 2019). No Brasil dados de prevalência chegam a 32,9% em estudos que utilizaram critérios validados para o diagnóstico (TUFIK *et al.,* 2010, SATEIA; 2014).

O estudo polissonográfico de noite inteira realizado em laboratório especializado é o método padrão ouro para o diagnóstico da AOS e outros distúrbios do sono. Esse exame demanda estrutura física e técnicos qualificados para a coleta de sete ou mais canais de dados, incluindo eletroencefalograma e eletro-oculograma para estadiamento do sono, eletromiograma, eletrocardiograma e canais respiratórios (LARATTA *et al.,* 2017). Por se tratar de um exame de alto custo e com acesso limitado, outros métodos de diagnóstico vêm sendo propostos para uma triagem dos indivíduos de risco. Entre esses métodos, o uso de questionários validados com acurácia diagnóstica testada em comparação com a polissonografia é útil e amplamente utilizado em pesquisas (CHIU *et al.*, 2017; BERTOLAZI *et al.*, 2011). Equipamentos portáteis também são utilizados para melhorar o diagnóstico da AOS quando a polissonografia não é possível. A oximetria de pulso, é um bom exemplo, por ser um método não invasivo e de baixo custo para a mensuração da dessaturação de oxigênio. O aprimoramento das leituras e armazenamento dos dados por meio de aplicativos de smartphone vem se mostrado uma alternativa promissora e de acessibilidade para esse tipo de exame (PENZEL; SCHÖBEL; FIETZ, 2018).

Os tratamentos indicados para AOS dependem da sua gravidade iniciando por orientações e medidas comportamentais como perda de peso e mudança na posição de dormir, passando por aparelhos intra orais de avanço mandibular e aparelhos de pressão de ar positiva (CPAP- *continuous positive airway pressure*) até procedimentos cirúrgicos, como

a cirurgia ortognática (LARATTA *et al.*, 2017). Segundo recomendações da *American Academy of Sleep Medicine (*AASM), a cirurgia de avanço maxilar e mandibular (AMM) está indicada no tratamento de AOS moderada e grave em pacientes com falta de adesão ou relutantes ao uso do CPAP e em pacientes com falta de adesão ao uso do AIO (am) na AOS leve e moderada. A cirurgia concomitante de AMM além de preservar as relações maxilomandibulares aumentam o espaço faríngeo pela expansão da estrutura esquelética, nos quais os tecidos moles da faringe e língua estão aderidos, resultando na diminuição de colapso da faringe (FERRAZ *et al.,* 2011).

A relação entre DDF e AOS é bastante complexa e ainda desconhecida em alguns aspectos. A literatura demonstra que os indivíduos com AOS apresentam uma redução do espaço faríngeo e algumas anormalidades anatômicas clinicamente evidentes, como micrognatia e retrognatia, ou achados radiográficos sutis, como posicionamento inferior do osso hióide e comprimento mandibular e maxilar mais curtos em comparação com indivíduos controle (NEELAPU *et al.,* 2017). Contudo, há trabalhos que apesar de encontrarem relação entre DDF e AOS não encontraram associação estatística entre os diferentes padrões esqueléticos e as características de AOS devido à grande variação individual (KIM *et al.,* 2020). Como dito anteriormente, a cirurgia ortognática é uma das modalidades de tratamento para AOS, principalmente a cirurgia de AMM (ZAGHI *et al.,* 2016). Entretanto outras modificações maxilo mandibulares realizadas por meio de cirurgias ortognática sobre AOS ainda são pouco estudadas. Neste trabalho, para analisar o risco de presença de AOS, o instrumento STOP BANG foi utilizado (ANEXO 6). É uma ferramenta autoaplicável, que consiste em oito perguntas, cujas respostas são apenas sim ou não. A presença de 3 ou mais respostas afirmativas indica um alto risco para AOS *(*BOYNTON *et al.,* 2013*;* FONSECA *et al.,* 2016).

1.3.2 OUTROS DISTÚRBIOS DO SONO

Os distúrbios relacionados ao sono compreendem diversas condições como insônias, distúrbios respiratórios do sono, transtornos centrais da hipersonolência, transtornos do sono-vigília do ritmo circadiano, parassonias e distúrbios de movimento relacionados ao sono (AMERICAN ACADEMY OF SLEEP MEDICINE, 2014). A insônia primária (IP) é uma perturbação do sono ligada ao SNC. É caracterizada pela dificuldade em iniciar e/ou manter o sono e pela ausência de sono reparador durante um período não inferior a um mês. Acomete mais os adultos jovens, e as mulheres e pode apresentar uma manifestação crônica. A IP é observada de 12,5% a 22,2% dos pacientes com insônia crônica. A insônia primária crônica deve se diferenciar da insônia vinculada a uma inadequada higiene do sono, síndrome depressiva ou ao transtorno de ansiedade generalizado (MONTI; 2000).

A etiologia e a fisiopatologia da insônia envolvem fatores genéticos, ambientais, comportamentais e fisiológicos que culminam em uma hiperexcitação prejudicando a qualidade do sono. A insônia é um fator de risco para o desenvolvimento de outras condições sistêmicas e emocionais, como manifestações dolorosas. Essa qualidade do sono ruim gera um aumento da hiperalgesia relacionado à sensibilização central e pode desempenhar um papel etiológico nos distúrbios da dor idiopática, por exemplo as DTM. Um estudo demonstrou que em pacientes como mialgia crônica, a insônia foi o distúrbio do sono mais prevalente acometendo 38% dos sujeitos e foi associada com redução dos limiares de dor mecânica e térmica em pontos faciais e corporais (SMITH *et al.,* 2009).

O diagnóstico de insônia é estabelecido por uma história completa de comportamentos de sono, problemas médicos e psiquiátricos e medicamentos, complementada por um registro prospectivo dos padrões de sono (diário do sono) (BUYSSE; 2013). Neste trabalho foi utilizado o Índice de Qualidade de Sono de Pittsburgh – *Pittsburhg Sleep Quality Index (PSQI)* (ANEXO 7), um instrumento constituído por 19 questões em autorrelato e 5 questões direcionadas ao acompanhante de quarto. As 19 questões são categorizadas em 7 componentes, graduados em escores de zero (nenhuma dificuldade) a três (dificuldade grave). Os componentes do PSQI são: C1 qualidade subjetiva do sono, C2 latência do sono, C3 duração do sono, C4 eficiência habitual do sono, C5 alterações do sono, C6 uso de medicamentos para dormir C7 disfunção diurna do sono. A soma dos valores atribuídos aos 7 componentes varia de 0 a 21 escore total do questionário indicando que quanto maior o escore total pior é a qualidade do sono. Um escore total maior que 5 indica que o indivíduo está apresentando grandes disfunções em pelo menos dois componentes, ou disfunção moderada em pelo menos três componentes (BUYSSE *et al.,* 1989; BERTOLAZI *et al.,* 2011).

O tratamento para índice de qualidade de sono ruim são: higiene adequada do sono, terapia cognitiva e de conduta e uso de fármacos hipnóticos. Revisões quantitativas da literatura (meta-análises) apoiam a eficácia das intervenções comportamentais, cognitivas e farmacológicas para a insônia. Os tratamentos comportamentais devem ser usados sempre que possível, e os medicamentos devem ser limitados à menor dose necessária e à menor duração necessária.

1.4 ANÁLISE DE POLIMORFISMOS GENÉTICOS

Diversos fatores podem alterar e afetar a qualidade e a quantidade de sono impactando decisivamente na qualidade devida de indivíduos (Huysmans *et al.*, 2017), contribuindo assim para o desenvolvimento de sintomas de ansiedade, estresse e depressão (Steiger et al., 2019). Embora a percepção do distúrbio e o impacto que o mesmo cause sobre cada indivíduo

varie muito entre indivíduos, alguns fatores tais como o relato de dor, uso de medicamento para dormir, desvios emocionais, psíquicos e sociais podem ser comuns e as características clinicas apresentadas pelos mesmos assemelham-se (Chang 2015; Steiger *et al.*, 2019). Esses fatores, em conjunto, são designados como fatores ambientais que quando somados ao background genético individual resultam em diversos desfechos em saúde. O avanço tecnológico e o interesse crescente pela área têm permitido a investigação de mudanças na sequência de DNA como mutações e polimorfismos de base única, ou interrogar diferentes níveis de expressão gênica de determinadas moléculas (WANG *et al.,* 2012).

Polimorfismos genéticos são variações observadas na sequência de nucleotídeos que compõe o DNA. É importante destacar que essas variações na sequência de nucleotídeos que compõe o DNA ocorrem naturalmente em cerca de 1% da população em geral, conferindo assim a biodiversidade genética. Entretanto, eventualmente, essas variações podem alterar a expressão gênica e predispor a doença (Alcazar *et al*, 2010).

Uma molécula de especial interesse é a melatonina (MLT), já que apresenta papel central na fisiologia do sono e características antioxidantes. A melatonina é o principal hormônio produzido pela glândula pineal e influencia diretamente o ritmo circadiano, transmitindo informações sobre o ciclo diário de luz e contribuindo para a regulação do sono. Além disso, a melatonina tem recebido atenção especial no tratamento da dor por se mostrar eficaz e apresentar poucos efeitos adversos (WANG *et al.,* 2012).

A MLT é sintetizada a partir da serotonina através de duas etapas enzimáticas. Primeiramente, a serotonina é acetilada pela N-acetiltransferase (NAT) para produzir N-acetilserotonina (NAS). A segunda etapa envolve a transferência de um grupo metilo de adenosilmetionina (S) para o grupo 5-hidroxi N-acetilserotonina , através da enzima hidroxiindole-O-metiltransferase (HIOMT) formando assim a MLT (Dubocovich *et al*. 2010).

A produção e a secreção de MLT são mediadas em grande parte pelas fibras nervosas retinianas que passam pelo trato retinohipotalâmico até o núcleo supraquiasmático, seguindo então para o gânglio cervical superior e finalmente para a glândula pineal. Este sistema neuronal é ativado pela escuridão e inibido pela luz (Engl 1997) (Comai *et al.* 2019).

Existem dois receptores que possuem alta afinidade com este hormônio: o Receptor da Melatonina Tipo 1A (*MTNR1A*) e o Tipo 1B (*MTNR1A*). Quanto à localização desses receptores na cadeia de DNA (ácidodesoxirribonucleico), o *MTNR1A* está na posição 4q35.2 mostra a figura (GeneCards®: The HumanGene Database).

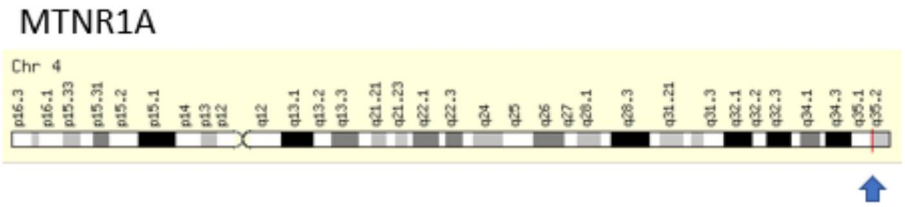

Figura 1: posição do *MTNR1A* na cadeia do DNA

O gene que codifica a expressão da melatonina é o *Melatonin Receptor Type 1 (MTNR1A)* e localiza-se no cromossomo 4, posição 4q35.2. Desequilíbrios na expressão desse gene, em especial polimorfismos genéticos, parecem afetar diretamente a produção de melatonina e poderiam desencadear alterações metabólicas importantes levando o indivíduo a quadros de estresse e depressão, alterações no estado de vigília e hiperalgesia mecânica (BONNEFOND *et al.,* 2012).

Este importante papel que a MLT desempenha na regulação do ciclo circadiano também deixa claro que o desequilíbrio desse sistema pode levar à uma alteração do sono, aumento de estresse, alterações de humor e até mesmo depressão, fazendo com que a qualidade de vida do indivíduo fique seriamente comprometida (Daut e Fonken, 2019).

2 OBJETIVOS

2.1.1 OBJETIVO GERAL

1. Avaliar a associação sobre a presença de sinais e sintomas de Disfunção Temporomandibular (DTM) em pacientes com DDF que irão se submeter à cirurgia ortognática e indivíduos sem DDF
2. Avaliar a associação dos polimorfismos do gene da melatonina *MTNR1A (rs6553010, rs13140012 e rs6847693)* no bruxismo do sono e na qualidade do sono em uma população brasileira adulta.

2.1.2 OBJETIVOS ESPECÍFICOS

- Comparar os grupos sobre a presença de DTM Muscular, DTM Articular e Cefaleia secundária à DTM em indivíduos com e sem DDF.
- Comparar os grupos com e sem DDF para sintomas físicos, ansiedade, dor na face e dor além da face.
- Avaliar a influência dos *polimorfismos rs13140012, rs6553010 e rs6847693* do gene *MTNR1A* nos componentes do *PSQI* sendo eles: C1 qualidade subjetiva do sono, C2 latência do sono, C3 duração do sono, C4 eficiência habitual do sono, C5 alterações do sono, C6 uso de medicamentos para dormir C7 disfunção diurna do sono.

3 ARTIGOS

3.1 TEMPOROMANDIBULAR DISORDERS IN PATIENTS WITH DENTOFACIAL DEFORMITY

ABSTRACT

Individuals seeking orthodontic treatment combined with orthognathic surgery have a high prevalence of temporomandibular disorders (TMD), but the relationship between TMD diagnoses and dentofacial deformities (DFD) is still controversial. Therefore, this cross-sectional study with a comparison group aimed to compare patients with dentofacial deformities in orthodontic preparation for orthognathic surgery to individuals without dentofacial deformities on TMD diagnoses. **METHODOLOGY:** Eighty patients were randomly selected from the stomatology department of the Federal University of Paraná between July 2021 and July 2022. Forty composed the group with DFD, and 40 formed the group without DFD. The groups were matched for sex, age, and self-reported race. The Diagnostic Criteria for TMD (DC/TMD) was used to diagnose TMD based on the Axis I criteria. The psychosocial aspects, oral behaviors in wakefulness, and sleep bruxism were evaluated through Axis II. **Results:** The statistical analysis for comorbidities, habits, psychosocial variables, and most TMD diagnoses showed similarity between the two groups ($p > 0.05$), except for arthralgia, which was more prevalent in the group with DFD ($p = 0.01$). Arthralgia in the DFD group was associated with the variable sleep bruxism ($p = 0.046$). **Conclusion:** The prevalence of arthralgia is higher in patients with DFD undergoing surgical orthodontic treatment when compared to individuals without DFD. Sleep bruxism is associated with the occurrence of joint TMD in these patients.

Key words: temporomandibular disorders; dentofacial deformity; sleep bruxism.

INTRODUCTION

Dentofacial deformities (DFD) correspond to dental malocclusion associated with skeletal pattern alterations that require a combined treatment between orthodontics and orthognathic surgery.[1] Such deformities can be minimal, as a slight projection of the chin, and extreme, as a severe vertical maxillary excess or a hemifacial microsomia. The involvement may be in one or two bases of the bone, in the vertical, horizontal, and transverse planes, both in isolation and in combination, causing different types of deformities. The main DFDs are skeletal class II, skeletal class III, skeletal biprotrusion, skeletal anterior open bite, and skeletal bilateral posterior crossbite.[2]

Among the reasons for performing orthognathic surgery, aesthetic purposes are frequently reported, as well as functional improvement, including complaints related to temporomandibular dysfunction.[3,4,5] Although current concepts no longer consider occlusion as a central role in the occurrence of TMD, when it comes to dentofacial deformities, several studies[6,7] suggest a high prevalence of this condition in these individuals, especially in groups seeking surgical treatment. It is also important to note that many of these patients often have emotional problems, including anxiety and depression, due to the negative impact of the deformity.[8,9]

Temporomandibular disorders (TMD) comprehend heterogeneous conditions involving the masticatory muscles, the temporomandibular joint (TMJ), or both, and their associated structures. Several studies have investigated TMD in ortho-surgical patients[10,11]; however, the way the deformity impacts specific TMD diagnoses still needs to be determined. There is controversy among the studies and a scarcity of papers that evaluate comparison groups using valid tools. The RDC/TMD[12] instrument followed the biopsychosocial model of TMD assessment and classification, consisting of Axis I (physical diagnoses) and Axis II (psychosocial aspects). It was updated in 2014 for the DC/TMD and recently validated for the Portuguese language in Brazil.[13]

This study aimed to compare patients with dentofacial deformity in orthodontic preparation for orthognathic surgery with individuals without dentofacial deformity on TMD diagnoses.

MATERIAL AND METHODS

This is an observational cross-sectional study with a comparison group, which was developed in the Oral and Maxillofacial Surgery and Traumatology Service (CTBMF) facilities

and the dental clinics of the Department of Stomatology of the Federal University of Paraná (UFPR), Jardim Botânico campus, located at Av. Pref. Lothário Meissner, 632, Jardim Botânico, Curitiba, Paraná. The study went on for 36 months.

ETHICAL ASPECTS

The research project was sent to the Research Ethics Committee of the Health Sciences Sector of UFPR for consideration. The research was only initiated after its final approval, with CAAE number: 52207821.9.0000.0102. In addition, the study followed all the recommendations of the Declaration of Helsinki[14] regarding research with humans. Individuals were invited to participate in the study and received information about the objectives and justifications for the research through the Informed Consent Form (ICF). They received information about the benefits and risks to which they would be exposed. They were also aware that the treatment would continue, regardless of the refusal to participate in the research, and about their freedom to discontinue participation at any time. Individuals who consented to participate in the study signed the ICF and were included. Only the researchers had access to the questionnaires to ensure the confidentiality of the data.

SAMPLE

The study included a total of 80 participants. The Open Epi Software calculated the sample size. The calculation was based on a previous study in which a prevalence of 31% was found in the general adult population. For the control group, a prevalence of 63.8% was found in patients with DFD who would undergo orthognathic surgery.[10] Thus, the calculation was performed with a bilateral confidence interval of 95% and power of 80%, with the ratio of controls to cases of 1:1. Among the 80 selected participants, 40 formed the group with DFD, and 40 formed the group without DFD. The sample selection was performed as follows: for the group with DFD, all individuals who would undergo orthognathic surgery by the CTBMF Service of UFPR and who met the inclusion criteria were invited to participate in the study. The participants were recruited when they went through the stage of the clinical examination before orthognathic surgery. Patients in the group without DFD were approached in the first evaluation in other dental clinics of UFPR. The individuals who agreed to participate in the research and met the inclusion and exclusion criteria were selected.

According to the inclusion criteria, the group with DFD consisted of patients with dentofacial deformities, except cleft lip and palate or associated syndrome, requiring treatment through orthognathic surgery, over 18 years of age, who accepted to participate in the research

and signed the ICF. The inclusion criteria for the group without DFD comprised patients over 18 years of age, who signed the ICF and did not present dentofacial deformity. An experienced professional conducted a dentofacial deformity analysis. The exclusion criteria for both groups were the presence of previous surgeries in the cervicofacial region, cognitive and neurological alterations, diagnosis of arthritis and arthrosis in other joints, and patients who used cyclobenzaprine in the week of the evaluation.

The group without DFD was selected with matching for sex, age, and race with the case group to maintain a more homogeneous sample and eliminate potential biases.

DATA COLLECTION

Demographic data were collected from all survey participants, such as sex, age, and self-reported race, the last divided into whites and non-whites due to the n and low number of other races. In addition, data were collected on drug use, the presence of comorbidities (fibromyalgia, arthritis/arthrosis, and gastroesophageal reflux), and patients' habits such as smoking (smoker and non-smoker) and the amount of coffee intake (less than 3 cups and more than 3 cups per day).

For the analysis of DFD types, an experienced surgeon evaluated the patient's profile and occlusion and classified the face profiles into three categories: I, II, or III. Profile I is a straight profile, and profile II presents a negative step between the maxilla and mandible, corresponding to patients with mandibular retrognathism. Profile III presents a positive step between the maxilla and mandible, indicating anteroposterior maxillary deficiency, mandibular prognathism, or both.[16] They were also evaluated considering mandibular asymmetry (higher than 4 mm) and vertical facial patterns: an anterior open bite, vertical maxillary excess, and vertical maxillary deficiency.

All participants were diagnosed with TMD through the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). The tool was used by trained and calibrated examiners using inter-examiner Kappa (0.95) as nominal or ordinal variables. This tool consists of two axes: Axis I, which includes the information collected on the physical examination, and Axis II, with the emotional aspects. Both axes were used in this research.[12]

Axis I consists of three questionnaires: TMD pain screening, DC/TMD symptom questionnaire, and TMD physical examination. To diagnose TMD in patients, the DC/TMD procedures involve gathering the patient's medical history, performing a physical exam that includes muscle palpation (applying 1.0 kg pressure) and joint palpation (applying 0.5 kg pressure), assessing the presence of symptoms during mandibular function, and measuring the maximum opening of the jaw both with and without pain. Based on the collected findings, the tool identifies two main categories of physical diagnoses (muscular and joint). It generates

a decision flowchart and a table of diagnostic criteria to assist in the diagnostic process. Painful TMD conditions in muscles are classified as myalgia, local myalgia, myofascial pain, myofascial pain with spreading, and myofascial pain with referred pain. Regarding joint TMDs, they can be structural or joint pain per se (arthralgia), and both diagnoses are performed independently on each side of the TMJ. Structural disorders are classified as disc displacement with reduction, disc displacement with reduction and intermittent locking, disc displacement without reduction and without limitation of opening, disc displacement without reduction and with limitation of opening, degenerative joint disease, and subluxation. As for arthralgia, it is considered present or absent joint pain. Axis I also classifies headaches attributed to TMD as present or absent. The degenerative joint disease was diagnosed through the symptom of crackling during mandibular function without complementary imaging.

Axis II incorporates behavioral instruments regarding pain, psychological state, and psychosocial and behavioral functioning. For axis II, the tools used were the *Patient Health Questionnaire* (PHQ-15), the *Generalized Anxiety Disorder* (GAD-7), the human body pain drawing to identify pain points in and beyond the face, and the *Oral Behaviors Checklist (OBC),* which was used to diagnose awake and sleep bruxism.

The OBC is a self-evaluation tool with 21 questions in which the patient answers the weekly and monthly frequency of oral and parafunctional behaviors. According to the sum of the scores, the result is classified as no oral behaviors for scores up to four points, mild from four to 12 points, moderate from 13 to 19 points, and severe with more than 20 points. Based on the OBC data, combined with the clinical evaluation, the awake bruxism was classified as absent, infrequent, frequent, or very frequent according to the answers to the OBC questionnaire of the DC/TMD. Sleep bruxism was classified as absent, possible, and probable. It was considered possible when it had a positive response in the *OBC* questionnaire and probable when, in addition to a positive response in the *OBC*, it presented at least one of the clinical signs: dental wear, marks on soft tissue such as jugal mucus and tongue and/or muscle fatigue upon awakening; pain in masseter and temporal muscle palpation; masseter hypertrophy.[17]

The *PHQ-15* comprises 15 questions about physical symptoms like nausea, dizziness, and body aches. The overall health conditions and physical symptoms are classified according to severity level, medium, and severe. The instrument called *GAD-7* consists of seven items to evaluate the symptoms of generalized anxiety disorder. The maximum total score is 21, where zero means the absence of anxiety, 5-9 means a mild degree, 10-14 indicates a moderate degree, and 15-21 means a severe degree of anxiety. Pain drawings are a self-applied tool represented by a drawing of the whole body and face in which the patient makes

markings where they identify the pain. The score is the sum of the number of markings.

A pilot study to verify the methodology and applicability of the questionnaires was conducted with 10 patients with DFD who met the inclusion and exclusion criteria of the present study and who would undergo orthognathic surgery at the Oral and Maxillofacial Surgery and Traumatology Service (CTBMF) of UFPR Paraná, Brazil.

DATA ANALYSIS

Calibration within the examiners was performed using the *kappa* coefficient and, when applicable, the intraclass correlation coefficient.

Because of the sample size of this study, we chose to dichotomize several variables. When examining the variables of Axis I, specifically myalgias, it was only considered whether the diagnosis was present or absent. If present, that meant all sub-diagnoses were included. Similarly, structural TMJ disorders were grouped into absent or present, considering the sides of the TMJ (right and left) individually. Our study considered the presence of arthralgia, regardless of the affected side. As for the variables of Axis II, GAD-7 was grouped as follows: the subject could be classified as 1- without anxiety and mild anxiety or 2- moderate and severe anxiety. Also, the subject could be classified as 1- without symptoms and mild symptoms or 2- moderate and severe symptoms at the PHQ-15. Regarding the CBO, parafunctional habits were dichotomized according to 1-absent and minor or 2-present and very present. Wake bruxism was also dichotomized into 1-frequent and infrequent or 2-frequent and very frequent.

The results obtained were submitted to descriptive and inferential statistical analysis. The categorical variables of DC/TMD between the groups were compared using the Chi-square test or Fisher's exact test. For the numerical variables, the normality condition was evaluated by the Kolmogorov-Smirnov test. The comparison of numerical variables with non-normal distribution between groups was performed using the Mann-Whitney test. Statistical significance was defined as $p < 0.05$. Data were analyzed using the *IBM SPSS Statistics* v. 24 computer program.

RESULTS

The sample was primarily composed of women, with 52 of them (65%) and 28 men (35%) equally distributed between groups. The median age was 30 (19-61). Regarding race, 65 patients (81.25%) were white, and 15 were non-white (18.75%). The non-white patients were represented by 12 reported as brown and three as black. The groups were matched for sex ($p = 1.0$), age ($p = 0.823$), and self-reported race ($p = 1.0$).

Table 1 shows the homogeneity between the groups for the comorbidities and habits evaluated. Within the DFD group, seven patients presented facial profile I, 12 had facial profile II, and 21 had facial profile III. A total of 10 patients presented asymmetry. Regarding the vertical alteration patterns of the face, six patients presented vertical excess, six presented vertical deficiency, and nine presented open bite.

TABLE 01: RESULT OF THE ASSOCIATION OF HABITS AND COMORBIDITIES BETWEEN THE GROUPS WITH DFD AND WITHOUT DFD.

Results		**Group with DFD n (%)**	**Group without DFD n (%)**	***p-value***
Medication Antidepressants	No	37 (92.5)	36 (90.0)	1.000
	Yes	3 (7.5)	4 (10.0)	
Comorbidity	No	37 (92.5)	36 (90.0)	0.899
	Fibromyalgia	1 (2.5)	1 (2.5)	
	Gastroesophageal reflux	2 (5.0)	3 (7.5)	
Smokers	No	34 (85.0)	39 (97.5)	0.108
	Yes	6 (15.0)	1 (2.5)	
Coffee intake	No	24 (60.0)	29 (72.5)	0.344
	Yes	16 (40.0)	11 (27.5)	

Note: Chi-square test independent samples with a significance value of 0.05. Bold values indicate statistical significance.

Table 2 compares the groups based on the diagnoses of Axis I, which were determined using the DC/TMD instrument. One can observe no significant differences between the groups regarding the presence of most TMD diagnoses, as indicated by p-values above 0.05. However, the only exception was arthralgia, where the group with DFD had a significantly higher prevalence, with 18 individuals (45%), compared to the group without DFD, which had four individuals (10%) with arthralgia ($p = 0.01$). Out of the 18 participants who experienced joint pain (arthralgia) in the DFD group, 13 (72%) presented bilateral arthralgia, while five (28%) presented unilateral arthralgia. Also, 12 individuals (67%) presented disc displacement with reduction, with four of them unilaterally, and in eight of them, it was present on both sides of the TMJ.

In terms of diagnosing disc displacement, our analysis found that despite presenting similar results, the occurrence was common in both groups. Out of the 40 participants in the DFD group, 22 (55%) had DDwR, with 11 of them only on one side (unilateral DDwR) and 11 on both sides. Additionally, 12 had painful symptoms related to their disc displacement, while 10 did not experience pain. In the group without DFD, the four patients with arthralgia had no other joint disorders. Degenerative diseases in TMJ were identified in one patient from the case group and two from the control group.

TABLE 02: RESULT OF TMD ASSOCIATION BETWEEN THE GROUP WITH DFD AND THE GROUP WITHOUT DFD

Results		**Group with DFD** n (%)	**Group without DFD** n (%)	***p-value***
Myalgia	Absent	18 (45.0)	16 (40.0)	0.821
	Present	22 (55.0)	24 (60.0)	
Left DDwR	Absent	27 (67.5)	28 (70.0)	1.000
	Disc displacement with reduction	13 (32.5)	12 (30.0)	
Right DDwR	Absent	20 (50.0)	29 (72.5%)	0.066
	Disc displacement with reduction	20 (50.0)	11 (27.5%)	
Arthralgia	Absent	22 (55.0)	36 (90.0)	**0.010**
	Present	18 (45.0)	4 (10.0%)	
TMD	Absent	30 (75.0)	29 (72.5%)	1.000
Headache	Present	10 (25.0)	11 (27.5%)	

Note: Chi-square test independent samples with a significance value of 0.05. Bold values indicate statistical significance.
Acronym: DD: disk offset; LEFT: left; RIGHT: right. DDwR disc displacement with reduction.

There was no difference in maximum mouth opening between groups with and without DFD. The median pain-free mouth opening value in the DFD group was 43 mm (22-63 mm), and in the group without DFD, it was 45 mm (20-77 mm) ($p = 1.0$). The median mouth opening with pain was 48 mm (33-65 mm) in the group with DFD and 45 mm (30-78 mm) in the group without DFD ($p = 0.117$).

Regarding the variables of Axis II of the DC/TMD, there was no difference between the groups ($p > 0.05$) in anxiety levels (GAD-7), in physical symptoms (PHQ-15), and pain in and beyond the face. Table 3 shows this relationship between the groups. When considering the score for the pain points beyond the face on the pain drawing, there was also no association in the numerical variable between the groups. In the group with DFD, the median score was 2 (0-19), and in the group without DFD, it was 2 (0-12) ($p = 0.262$).

As the only differing diagnosis between the two groups was arthralgia, the other variables were compared in the DFD group between patients with and without arthralgia.

TABLE 03: RESULT OF THE ASSOCIATION OF THE INSTRUMENTS AXIS II DC/TMD BETWEEN THE GROUP WITH DFD AND THE GROUP WITHOUT DFD.

Results		**Group with DFD n (%)**	**Group without DFD n (%)**	***p-value***
Physical symptoms	Absent and Low Severity	15 (37.5)	19 (47.5)	0.498
	Medium and High Severity	25 (62.5)	21 (52.5)	
Anxiety	Absent and mild	22 (55.0)	29 (72.5)	0.162
	Moderate and Severe	18 (45.0)	11 (27.5)	
Pain in the face	Absent	15 (37.5)	19 (47.5)	0.176
	Present	25 (62.5)	19 (47.5)	
Pain Beyond the Face	Absent	16 (40.0)	21 (47.5)	0.370
	Present	24 (60.0)	19 (47.5)	

Note: Chi-square test independent samples with a significance value of 0.05. Bold values indicate statistical significance.

The results showed that the subjects' self-reported race, age, facial profile, and asymmetry variables were unrelated to arthralgia within the DFD group ($p > 0.05$). Regarding sex, women had a higher prevalence of joint pain ($p > 0.001$).

Table 4 compares participants with and without arthralgia within the group with DFD to the other variables of axis I and II of DC/TMD and the parafunctional habits. The only variable that presented a significant association with arthralgia was sleep bruxism. The prevalence of probable sleep bruxism was 72% in individuals with joint pain compared to 59% in the group without joint pain ($p = 0.046$).

TABLE 4: COMPARISON BETWEEN PATIENTS WITH AND WITHOUT JOINT PAIN WITHIN THE GROUP WITH DFD.

Variable		**No arthralgia 22n (%)**	**With arthralgia 18n (%)**	***p-value***
Myalgia	No	13 (59.0)	5 (27.0)	0.062
	Yes	9 (41.0)	13 (72.0)	
Articular Disorder Left	No DDwR	18 (81.0)	9 (50.0)	0.341
	With DDwR	4 (18.0)	9 (50.0)	
Articular Disorder Right	No DDwR	13 (59.0)	7 (38.0)	0.046
	With DDwR	9 (41.0)	11 (61.0)	
Pain Beyond the Face	No	9 (41.0)	7 (38.0)	1,000
	Yes	13 (59.0)	11 (61.0)	
PHQ-15	No - Low	11 (50.0)	4 (22%)	0.104
	Moderate - severe	11 (50.0)	14 (77%)	
GAD-7	None/light	14 (63.0)	8 (44%)	0.225
	Moderate to severe	8 (36.0)	10 (55.5%)	
OBC	None/light	5 (22.0)	3 (16%)	0.339
	Moderate - severe	17 (77.0)	15 (83%)	
Sleep Bruxism	Absent	3 (13%)	5 (27%)	**0.046**
	Possible	6 (27%)	0 (0%)	
	Probable	13 (59%)	13 (72%)	
Wake Bruxism	Absent; uncommon	13 (59%)	8 (44%)	0.525
	Common; very common	9 (41%)	10 (55.5%)	
	Yes	4 (18%)	2 (11%)	
Coffee intake	0 to 3 cups	13 (59%)	11 (61%)	1.00
	> 3 cups	9 (40%)	7 (38%)	

Note: Chi-square test independent samples with a significance value of 0.05. Bold values indicate statistical significance.

DISCUSSION

Understanding temporomandibular dysfunctions in individuals with dentofacial deformity is essential for the proper management and care of the patients. Although there is a higher emphasis on correcting the occlusion and even the face of these individuals, it is necessary to understand all the factors that affect their quality of life and promote patient well-being through multidisciplinary treatment.

To our knowledge, no studies are comparing the diagnoses of DC/TMD among patients with and without DFD in the Brazilian population to this date. The instrument DC/TMD is currently the most accepted one for diagnosing TMD.

It is important to consider that studies involving the diagnosis of TMD are complex, mainly due to their different diagnoses and their multifactorial etiology. Regarding the occlusal factor, after years of debate about the role of occlusal characteristics as causal or risk factors for TMD, a low relevance for dental occlusion and the inter-arcaded relationship is currently attributed[18]. However, regarding DFD, several studies suggest a high prevalence of TMD in this population,[19,] and a higher prevalence of pain and depression was also reported compared to patients without DFD.[20]

A systematic review suggests that class II skeletal profiles and hyper-divergent growth patterns are likely associated with an increased frequency of TMJ disc displacement and degenerative disorders. Another study that used cone beam tomography and RDC/TMD to diagnose TMD found more prevalent bone changes in patients with skeletal malocclusion class II.[21] On the other hand, a study comparing the prevalence of TMD in patients with dentofacial deformities associated with class III malocclusion found that it was similar to patients without dentofacial deformities.[22] Nevertheless, a recent systematic review showed that patients undergoing orthodontic-surgical treatment have a higher incidence of TMD when compared to a control population.[23] In this context, it is necessary to be aware that orthognathic surgery is not the treatment of choice for TMD control. Studies investigating the role of OS in TMD show that some patient profiles may improve from specific symptoms after surgery[10,24], whereas others may even worsen[25], and there could be no changes at all[26]. Clinical studies show that the effects of orthognathic surgery on TMD vary and are difficult to predict, so orthognathic surgery cannot be considered a reliable therapy for joint dysfunctions.[27]

Regardless, understanding the profile of these patients and their functional problems and conducting research for TMD before orthognathic surgery can positively influence the outcome of ortho-surgical treatment, which is still a wildly neglected factor by surgeons. The prior identification of patients with TMD, parafunction, or both, should imply a treatment plan incorporating cognitive-behavioral approaches to help patients understand their need to maintain relaxed masticatory muscles and control TMD prior to orthognathic surgery.

In this study, we found that surgical patients with DFD present a higher prevalence of arthralgia compared to a control population, corroborating other studies.[20] This fact emphasizes the need for TMD monitoring and control before the surgical procedure. Arthralgia is a type of TMD associated with peripheral etiological factors such as parafunction and joint overload (which can occur during sleep or wakefulness).[28] TMD control encompasses a combination of non-invasive therapies, including patient education, self-care, cognitive behavioral therapy, physiotherapy, pharmacotherapy, and occlusal devices. Minimally invasive therapies such as physiotherapy with intraarticular injection (IAI) of hyaluronic acid (HA) or corticosteroid (CS), arthrocentesis with or without HA, CS, and platelet-rich plasma (PRP), arthroscopy with or without HA, and PRP, or invasive therapies such as open joint surgery.[30]

The possible relationship between bruxism and symptoms of temporomandibular disorders is still controversial in the literature due to the complexity of the etiology and diagnosis of both conditions.[31,32] In the present study, a significant difference was found concerning the probable sleep bruxism, which was more prevalent in the group with joint pain. Thus, we suggest that the joint overload caused by this condition contributes to the development of TMD.

Ortho-surgical patients who present sleep bruxism and TMD before orthognathic surgery can benefit from the therapies mentioned above, such as counseling therapy; manual therapy; laser therapy; dry needling; intramuscular injection of local anesthesia (LA) or botulinum toxin-A (BTX-A) (insufficient evidence); muscle relaxants; hypnosis/relaxation therapy; oxidative ozone therapy (insufficient evidence), especially when unable to use the interocclusal device because they are in orthodontic treatment. Some studies suggest inadequate evidence for certain therapies, and it is important to prioritize treatments with stronger scientific evidence as the first choice in the hierarchy of treatment options.[33]

From our perspective, a patient with important pain complaints before surgery should not undergo a surgical procedure before being adequately treated. It is important to remember that orthognathic surgery is an aggressive procedure and that the entire musculature must adapt to the new positioning of the bone basis. During mandibular surgeries, an extensive detachment is performed in the region of the masseter muscle and medial pterygoid. Besides that, during osteotomies of the mandibular branches, the TMJ is significantly impacted during chiseling to separate the segments. The fixation of the mandibular segments can also cause condylar torque and cause more joint alterations.[34] In the first postoperative weeks after orthognathic surgery, patients' main complaints include pain or discomfort in temporomandibular joints and difficulty opening the mouth.

Thus, a patient already undergoing surgery with pain will have more challenges in dealing with the postoperative period in functional and psychological terms. Long-lasting pain leads to changes in the central nervous system causing central sensitization and increased

pain sensitivity, which may be associated with depression.[35]

Temporomandibular disorders are complex conditions, and their interrelationships should be seen through pain models, inserting the biopsychosocial perspective in the evaluations. This study investigated psychosocial variables between groups and their relationship with arthralgia. Although we found no association in this study, we must remember that we worked with a small and restricted sample. A previous study comparing patients with and without DFD found that patients with DFD had a higher prevalence of pain and depression.[20] Thus, these variables should continue to be investigated in other studies in patients with DFD.

Regarding the study's limitations, we believe the most important is the sample size (case group = 40 and control group = 40), seeing that important associations could be found with a larger n. The sample size also made it impossible to segment the sample according to the type of deformity that influences the preoperative diagnosis of TMD. Another limitation was that bruxism was identified without polysomnography, which makes it impossible to deliver a definite diagnosis according to the international consensus on bruxism.[17] Finally, while the clinic holds the authority for TMD diagnosis as per DC/TMD guidelines, and the signs and symptoms show high accuracy for specific diagnoses, it is possible that disc displacement without reduction (DDwoR) without limited opening went undiagnosed in this study due to the absence of complementary exams such as magnetic resonance imaging (MRI) of the TMJ. In addition, the diagnosis of DD had to be grouped in our study for inferential analysis due to the sample size.

Further studies should be conducted with a higher sample size to confirm our findings. We recommend other studies to investigate the factors that contribute to TMD in this population, to target treatment approaches for these patients.

CONCLUSION

The prevalence of arthralgia is higher in ortho-surgical patients with dentofacial deformity when compared to individuals without dentofacial deformity. Sleep bruxism is associated with the occurrence of joint TMD in these patients.

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3.2 THE ROLE OF POLYMORPHISMS OF THE GENES *MTNR1A RS6553010, RS13140012*, AND *RS6847693* IN SLEEP BRUXISM AND SLEEP QUALITY.

ABSTRACT

This study aimed to evaluate the role of the genetic polymorphisms of *MTNR1A rs6553010*, *rs13140012*, and *rs6847693* in sleep quality and sleep bruxism. The sample consisted of 80 adult patients from the stomatology department of the Federal University of Paraná. Sleep quality was investigated through the *Pittsburgh Sleep Quality Index (PSQI)*. The DC/TMD Axis II OBC questionnaire was used to diagnose sleep bruxism and awake bruxism, along with the physical examination. Obstructive sleep apnea (OSA) was diagnosed through the ODI (oxygen desaturation) index using type 4 polysomnography (*Biologix*®) to rule out cases of bruxism secondary to respiratory factors. Body Mass Index (BMI) was calculated by the formula BMI = weight(kg)/height(m)^2. The participants' DNA was collected from epithelial cells of the buccal mucosa to evaluate the polymorphisms *rs6553010*, *rs13140012*, and *rs6847693* associated with the Melatonin gene called *Melatonin Receptor Type 1 (MTNR1A)*. All collected data were subjected to statistical analysis using SPSS *software* version 21.0, with a significance level of $p < 0.05 \leq$ Results: There was no association between obstructive sleep apnea risk and the assessed polymorphisms. The ODI found in the group was a total of 74 individuals (92.5%) had normal ODI values. Four individuals presented a mild degree (5%), two showed a moderate degree (2.5%), and none showed a severe degree. The associations found were between the polymorphisms of the genes: *MTNR1A rs6847693* dominant with sleep bruxism ($p = 0.05$); *MTNR1A rs13140012* additive ($p = 0.015$) and recessive ($p = 0.09$) with BMI; *MTNR1A Rs6847693* dominant ($p = 0.044$) with sleep latency; *MTNR1A rs6553010* recessive ($p = 0.039$) with the presence of sleep disorders; and *MTNR1A rs6553010* additive ($p = 0.046$) with use of sleep medications. Conclusion: A significant association was found between the polymorphisms of the *MTNR1A* gene with sleep bruxism, BMI, sleep latency, sleep disorders, and the use of sleep medications.

Keywords: genetic polymorphisms, sleep bruxism, sleep disorders.

INTRODUCTION

Bruxism was consensually defined as repetitive masticatory muscle behavior characterized by clenching or grinding of the teeth; bracing or thrusting of the mandible; or both and specified as either sleep bruxism or awake bruxism. Sleep and awake bruxism are generally considered different behaviors observed during sleep and wakefulness. Therefore, it is recommended to replace the single definition of bruxism with two distinct definitions. 1- Sleep bruxism is a masticatory muscle activity during sleep that is characterized as rhythmic (phasic) or non-rhythmic (tonic), and it is not a movement disorder or a sleep disorder in otherwise healthy individuals, and 2- Awake bruxism is a masticatory muscle activity during wakefulness characterized by repetitive or sustained tooth contact; bracing and thrusting of the mandible; or both. It is also not a movement disorder in otherwise healthy individuals.[1]

The etiology of bruxism is multifactorial, including the use of cigarettes, alcohol, medications, and caffeine[2] as factors associated with increased muscle activity during sleep. It also occurs in OSA, where dental contact may happen at the end of the apnea episode, characterizing bruxism secondary to the respiratory factor. When bruxism is a primary factor, the literature describes that bruxism is mediated by neurotransmitters that act in the Central Nervous System (CNS), mainly through dopaminergic pathways (Lobbezoo e Naeije, 2001). More recently, the genetic *background* of the individual has come to be considered as a factor that can contribute to the development of this condition (Oporto et al., 2018) once several studies indicate that single nucleotide polymorphisms (SNPs) are associated with bruxism.[3, 4, 5, 6]

Sleep bruxism should not be considered a sleep disorder but rather a behavior that can be a protective factor, a risk for some clinical consequences, or both. These consequences include dental wear, cracks, fractures, and TMD pain.[1]

Sleep-related disorders include insomnia, sleep breathing disorders, central hypersomnolence disorders, circadian rhythm sleep-wake disorders, parasomnia, and sleep-related movement disorders. Primary insomnia (PI) is a sleep disturbance linked to the CNS. It is characterized by difficulty initiating sleep, maintaining it, or both, and non-restorative sleep for at least one month. It affects mainly young adults and women and can present a chronic manifestation. Primary insomnia is observed in 12.5% to 22.2% of patients with chronic insomnia.[7]

In the context of sleep, melatonin plays a role in regulating the sleep-wake cycle and circadian rhythm. Nonetheless, more research is needed to thoroughly investigate its effects on the human body. Melatonin is produced mainly in the intestines, reaching concentrations 400 times greater than in the pineal gland and up to 100 times greater than in the blood.[8,9,]

Initially, it was described as a sleep hormone, as it is secreted in the dark and induces sleep. However, it is now broadly appreciated that it presents a wide array of activities encompassing antioxidant, anti-inflammatory, anti-apoptotic, antisympathetic nerve activation, endothelial cell preservation, neuroprotection, hepatoprotection, immunomodulation, thermoregulation, mood, and sexual behavior modulation.[10,11] Stress and inflammatory processes change the metabolic pathway of tryptophan towards the kynurenic pathway. The lack of melatonin decreases serotonin levels, with all the possible consequences associated with depression, increased stress[12], sleep bruxism[13], dysregulation of the circadian rhythm, and the increase of other sleep disorders.

Although the perception of sleep disorders and the impact they cause on each individual varies significantly, some shared factors, such as pain reporting, use of sleep medication, and emotional, psychic, and social deviations may be present in these disorders.[14,15,16] These are designated as environmental factors and could result in several health outcomes when added to the individual genetic background.[16] Two receptors have a high affinity for the melatonin hormone: the Melatonin Receptor Type 1A (*MTNR1A*) and Melatonin Receptor Type 1B (*MTNR1B*). Imbalances in *MTNR1A* gene expression, especially genetic polymorphisms, seem to affect melatonin production directly and could trigger important metabolic changes leading to stress and depression, changes in wakefulness, and mechanical hyperalgesia.[17]

Considering the need for further investigations into the relationship between sleep bruxism and sleep disorders, and the role of melatonin in these alterations, this study aimed to evaluate the role of the *MTNR1A* rs6553010, *rs13140012*, and *rs6847693* polymorphisms in sleep quality and sleep bruxism in a Brazilian population.

METHODOLOGY

The sample consisted of 80 patients from the stomatology department of the Federal University of Paraná, Brazil, they were invited to participate in the study during their visits to dental clinics. Data collection took place from July 2021 to July 2022. Inclusion criteria were being over 18 years of age, accepting to participate in the research by signing an informed consent form (ICF), and not being under treatment for TMD or sleep disorders. Exclusion criteria were being under 18 years of age, presenting cognitive or intellectual difficulty interpreting self-administered questionnaires, and being under treatment for TMD or sleep disorders.

The diagnosis of bruxism was separated into two groups (sleep and awake). It was based on the patient's self-reporting or reporting by a roommate if applicable, and it was

classified as absent, possible, or probable. In addition, the diagnosis considered the presence of at least one clinical sign or symptom, such as dental wear, marks on the inner cheek, fatigue in the jaw muscles upon waking, and/or an increase in the masseter muscle size. However, a definitive diagnosis of sleep bruxism was not made because a polysomnography test was not performed to confirm its presence.

To verify oral behaviors, the instrument *"Oral Behaviors Checklist (OBC)"* was used as a tool with 21 questions in which the patient self-evaluates and responds to the weekly and monthly frequency of oral and parafunctional behaviors. The result is according to the sum of the scores: no oral behaviors sum up to four points, mild from four to 12 points, moderate 13 to 19 points, and severe with more than 20 points. Based on the OBC data, combined with the clinical evaluation, the awake bruxism was classified as absent, infrequent, frequent, or very frequent according to the answers to the OBC questionnaire of the DC/TMD. Sleep bruxism was classified as absent, possible, and probable. It was considered possible when it had a positive response in the *OBC* questionnaire and probable when, in addition to a positive response in the *OBC*, it presented at least one of the clinical signs: dental wear, marks on soft tissue such as jugal mucus and tongue and/or muscle fatigue upon awakening; pain in masseter and temporal muscle palpation; masseter hypertrophy.[1]

The Pittsburgh Sleep Quality Index (PSQI) was used to assess the subjective sleep quality, consisting of 19 self-report questions and five questions directed to the roommate. The 19 questions are categorized into seven components, graded in scores from zero (no difficulty to sleep) to three (severe difficulty to sleep). The components of the *PSQI* are C1 subjective sleep quality, C2 sleep latency, C3 sleep duration, C4 habitual sleep efficiency, C5 sleep disorders, C6 use of sleep medications, and C7 daytime sleep dysfunction. The subjective quality of sleep is classified as very good, good, or very bad. For statistical analysis, we dichotomize it into a) very good and good; b) bad and very bad. Sleep latency is classified into scores: 0, 1, 2, and 3, which are established according to the time to start sleeping (more than 15 minutes to sleep, between 16 and 30, from 31 to 60, and more than 60 minutes) and the frequency in the week (no time, less than once a week, between once and twice in the week and two to three times in the week). These scores were dichotomized into a- score 0 or b- scores 1, 2, and 3. The sleep duration component is classified into more than 7 hours of sleep, 6-7 hours of sleep, between 5 and 6 hours of sleep, and less than 5 hours of sleep, and the result was dichotomized into a) more than 6 hours of sleep b) less than six hours of sleep. The usual sleep efficiency component is classified as >85%, 75% to 84%, 65% to 74% and <65%; however, we dichotomized it as >75% and <74.9%. The component sleep alteration results in a score based on the weekly frequency of nocturnal awakenings and difficulty sleeping from 0

to 4. It was dichotomized into a- scores 0 to 1 and b- 2 and 3. The component related to the use of medication questions about the use of sleep medication and its frequency. We considered whether the person took it at least once a week or not. And finally, the component of daytime dysfunction evaluates the frequency of difficulty in staying awake during the day while driving, eating, or participating in a social and classical activity with a score of 0-3. We dichotomized it, however, as a-0 and b-1, 2, 3. In addition to the components, Pittsburgh allows the sum of the values attributed to the seven components to range from 0 to 21 total score of the questionnaire, indicating that the higher the number, the worse the sleep quality. A score greater than 5 indicates that the individual presents major dysfunctions in at least two components or moderate dysfunction in at least three components [18,19].

The risk of OSA was also assessed through the STOP-Bang instrument, which consists of a self-administered tool that presents subjective questions to the patient and considers gender, BMI, and neck circumference, to classify the risk of OSA as low, intermediate, and high. For anthropometric measurements, weight was collected using a portable and digital scale with G-Tech Glass 7FW (Duque de Caxias - RJ - Brazil). The height was measured through an Anthropometric measuring tape Cescorf (Porto Alegre - RS - Brazil). Body Mass Index (BMI) was calculated by the formula BMI = weight(kg)/height(m)^2. The neck circumference was obtained with a measuring tape and with the patient in a seated position. The measurement was done in the larynx region, perpendicularly to the longitudinal axis, applying minimal pressure on the skin. Body mass index classifies nutritional status as: < 18.5, underweight, 18.5–24.9, normal weight, 25.0–29.9, overweight, and $\geq$ 30.0 obesity.[20] For data analysis, the BMI result was dichotomized into lean/healthy and overweight/obesity.

The oxygen desaturation index was evaluated using the *Biologix*® device (São Paulo-SP-- Brazil) – a compact device that works as an oximeter and by actigraphy. The patient sleeps at home for one night with the equipment attached to the index finger, and an application installed on a cell phone interprets the data. The Biologix® devices were loaned to the patient and returned upon his return to UFPR. The following parameters were measured: minimum, average, and maximum SpO2; the amount of time with SpO2 levels <90% and <80%; the number of desaturations, oxygen desaturation index (ODI); heart rate (HR) minimum, average, and maximum; sound recording time and snoring time (%), and body movement. Following the following reference values: ODI <5- normal; 5 ≤ ODI <15 compatible with mild sleep apnea; 15 ≤ ODI < 30 compatible with moderate sleep apnea; ≥30 compatible with severe sleep apnea.[21]

DNA EXTRACTION, STORAGE, AND ANALYSIS

For this step, saliva samples were collected as a source of genomic DNA, following a previously published protocol.[22] During the sample collection, subjects performed a mouthwash with 5 mL of 5% saline solution for 1 minute. The mouthwash volume was packed in 15 mL centrifuge tubes (Corning Inc., Corning, NY, USA) and kept in a refrigerator.

For DNA extraction, each tube containing the saliva sample was centrifuged at 550 g for 10 minutes for *pellet* cell sedimentation. The supernatant was discarded in 2.5% sodium hypochlorite, and the *pellet* was resuspended in 1 mL extraction buffer (10 mM Tris-HCl, pH 7.8; 5 mM EDTA; 0.5% SDS). Subsequently, the sample was transferred to a 1.5 mL *Eppendorf* tube and then frozen at -20ºC until the DNA extraction.

Then, the samples were thawed and incubated with 100 ng/mL [4 µL Proteinase K (Fungal, Invitrogen Laboratories, Cat No. 25530-015) at 25 mg/mL] in a water bath at 56ºC *overnight* and then subjected to precipitation processes using 400 µL of 10M ammonium acetate solution. Next, all tubes were manually stirred for 5 minutes, centrifuged for 15 minutes (12000 rpm), and the supernatant was divided into two *Eppendorf* tubes of 700 µL each. The same volume of ice-cold isopropyl alcohol (700 µL) was added to each tube and stirred vigorously.

In each tube of the aliquots, which had been centrifuged for 20 minutes at 12000 rpm and 40ºC, a "DNA cloud" was formed. The supernatant was then carefully discarded so it would not displace the DNA *pellet,* and 1 mL of ice-cold 70% ethanol was added for further centrifugation for 15 minutes at 12000 rpm and 40ºC. Afterward, the supernatant was discarded, and the tube was opened and capsized on paper to dry for at least 30 minutes and evaporate the excess 70% ethanol solution. The DNA *pellet* was resuspended in 50 µL extraction buffer (TE) and frozen at -20ºC.

Potentially functional polymorphisms were selected, and Table 1 presents the description of the polymorphisms studied and their minimum allelic frequency. Thus, the genotyping assay of the selected polymorphisms was performed using the real-time *PCR* method through *TaqMan*® allele discrimination assays purchased from *Applied Biosystems* (Foster City, California, USA) with a solution called *TaqMan™ Genotyping Master Mix*®. Each assay includes a pair of flanking *primers* of the polymorphism region and specific probes for the identification A of alleles composed of two oligonucleotides. Each is designed for a sequence resulting from the change of base-conjugate with fluorophores that emit fluorescence at different wavelengths, called VIC™ and Fam™ using the *StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific*, Massachusetts, USA). The mentioned procedures, along with the software provided with the equipment, enabled the allelic discrimination of the samples.

STATISTICAL ANALYSIS

The results obtained were submitted to descriptive and inferential statistical analysis. Data were analyzed using the *IBM SPSS Statistics* v. 24 computer program. The polymorphisms were compared with the categorical variables using the chi-square test. A confidence interval of 95% was considered, and statistical significance was set at $p < 0.05$.

RESULTS

The sample consisted of 80 individuals, with a mean age of 31 years (±9.48), predominantly female with 52 (63.4%) women and 28 (34.1%) men, and primarily Caucasian with 65 (79.3%) whites, three blacks (3.7%) and 12 browns (14.6%). Table 1 shows descriptions of the studied polymorphisms.

TABLE 1 - DESCRIPTIONS OF THE STUDIED POLYMORPHISMS.

Gene	Official name	Chromosome position	Reference SNP (rs)	MAF *	Base Change	SNP Position
MTNR1A	Melatonin Receptor Type 1 A	4	*rs6553010*	0,36	G > A, C, T	chr4:186535189(GRCh38.p12)
			rs13140012	0,49	T > A, C, G	chr4:186544404(GRCh38.p12)
			rs6847693	0,31	T > A, C	chr4:186540490 GRCh38.p12)

Notes: *MAF, minor allele frequency. Obtained from the database: http://www.ncbi.nlm.nih.gov/snp/ SNP: single nucleotide polymorphism. Alleles: C, cytosine; G, guanine; A, adenine; T, thymine.

Table 2 shows the relationship between polymorphisms and sleep bruxism. We can verify that the dominant model of *rs6847693 was associated* with sleep bruxism *(*p = .050). It is possible to observe that 74.1% of individuals with the CC genotype presented probable sleep bruxism, while only 45.2% of the TT/CT genotype presented the condition.

TABLE 2: ASSOCIATION OF GENETIC POLYMORPHISMS *rs13140012, rs6553010, and rs6847693 OF the MTNR1A gene* WITH SLEEP BRUXISM.

Genetic polymorphisms			**Sleep bruxism (n - %)**			
Gene (polymorphism)	**Model**	**Base pairs (n)**	**Absent (11)**	**Possible (25)**	**Probable *(44)***	***p***
MTNR1A rs13140012	Additive	AA (11)	2 (18.3)	4 (36.3)	5 (45.4)	0.965
		AT (56)	7 (12.5)	17 (30.3)	32(57.2)	
		TT (13)	2 (15.4)	4 (30.8)	7 (53.8)	
	Recessive	AT-TT (69)	9 (13.0)	22 (31.8)	38 (55.0)	0.826
		AA (11)	2 (18.3)	3 (27.2)	6 (54.5)	

	Dominant	AA-AT (67)	9 (13.4)	21 (31.3)	37 (55.3)	0.983
		TT (13)	2 (15.3)	4 (30.7)	7 (53.8)	
MTNR1A rs6553010	Additive	AA (2)	0 (0.0)	0 (0.0)	2 (100)	0.504
		AG (61)	10 (16.3)	18 (29.5)	33 (54.0)	
		GG (17)	1 (5.8)	7 (41.1)	9 (52.9)	
	Recessive	AG-AA (78)	11 (14.1)	25 (32.0)	42 (53.8)	0.403
		GG (2)	0 (0.0)	0 (0.0)	2 (100)	
	Dominant	AA-AG (63)	10 (15.8)	18 (28.5)	35 (55.5)	0.385
		GG (17)	1 (5.8)	7 (41.1)	9 (52.9)	
MTNR1A rs6847693	Additive	TT (33)	5 (15.1)	11 (33.3)	17 (51.5)	0.117
		TC (20)	4 (20)	9 (45)	7 (35)	
		CC (27)	2 (7.4)	5 (18.5)	20 (74.0)	
	Recessive	TC-CC (47)	6 (12.7)	14 (29.7)	27(57.4)	0.868
		TT (33)	5 (15.1)	11 (33.3)	17 (51.5)	
	Dominant	TT-CT (53)	9 (16.9)	20 (37.7)	24 (45.2)	**0.050**
		CC (27)	2 (7.4)	5 (18.5)	20 (74.1)	

Note: Chi-square test independent samples with a significance value of 0.05. Bold values indicate statistical significance.

Regarding the *PSQI* variables, the general classification was not associated with any of the assessed genetic polymorphisms or subjective sleep quality and duration ($p > 0.05$). The other components of Pittsburgh that showed no association with the polymorphisms are shown in Table 3. It can be observed that the sleep latency component was associated with *rs6847693* in the dominant model ($p = 0.044$). The CC individuals had a higher percentage (92.59%) of high scores when compared to TT/CT individuals (73.5%). The usual sleep efficiency component showed no significant association but presented a p-borderline value in the additive model of *rs13140012* and the dominant model of *rs6553010.*

The sleep disorders component was associated with the *rs6553010* polymorphism *in the recessive model (p* = 0.39), in which 100% of GG individuals had more severe scores compared to only 30.7% of AA/AG individuals. Regarding the variable use of sleep medication, there was an association with *rs6553010* in the additive model ($p = 0.046$). None of the participants reported taking sleep medications at least once a week. However, among those with GG genotype, 50% reported using sleep medications, and 24.5% of the AG participants reported using them.

TABLE 3: ASSOCIATION OF THE GENETIC *POLYMORPHISMS rs13140012, rs6553010 and rs6847693 MTNR1A* WITH the COMPONENTS OF the PITTSBURG QUESTIONNAIRE (PSQI)

Genetic polymorphisms			**Sleep latency (n - %)**		
Gene (polymorphism)	**Model**	**Base pairs (n)**	**Score 0 (16)**	**Score 1/2/3 (64)**	***p***
MTNR1A rs13140012	Additive	AA (11)	1 (9.0)	10 (90.9)	0.153
		AT (56)	10 (17.8)	46 (82.1)	
		TT (13)	5 (38.4)	8 (61.5)	
	Recessive	AT-TT (69)	15 (21.7)	54 (78.2)	0.398
		AA (11)	1 (9.0)	10 (90.9)	
	Dominant	AA-AT (67)	11 (16.4)	56 (83.5)	0.069

		TT (13)	5 (38.4)	8 (61.5)	
MTNR1A rs6553010	Additive	AA (2)	0 (0.0)	2 (100)	0.727
		AG (61)	12 (19.6)	49 (80.3)	
		GG (17)	4 (23.5)	13 (76.4)	
	Recessive	AG-AA (78)	16 (20.5)	62 (79.4)	0.474
		GG (2)	0 (0.0)	2 (100)	
	Dominant	AA-AG (63)	12 (19.0)	51 (80.9)	0.576
		GG (17)	4 (23.5)	13 (76.4)	
MTNR1A rs6847693	Additive	TT (33)	9 (27.2)	24 (72.7)	0.130
		TC (20)	5 (25)	15 (75)	
		CC (27)	2 (7.4)	25 (92.5)	
	Recessive	TC-CC (47)	7 (14.8)	40 (85.1)	0.173
		TT (33)	9 (27.2)	24 (72.7)	
	Dominant	TT-CT (53)	14 (26.4)	39 (73.5)	**0.044**
		CC (27)	2 (7.4)	25 (92.5)	
Genetic polymorphisms			**Usual sleep efficiency (n - %)**		
Gene (polymorphism)	**Model**	**Base pairs (n)**	**>75% (42)**	**<75% (38)**	***p***
MTNR1A rs13140012	Additive	AA (11)	3 (27.2)	8 (72.7)	0.069
		AT (56)	34 (60.7)	22 (39.2)	
		TT (13)	5 (38.4)	8 (61.5)	
	Recessive	AT-TT (69)	39 (56.5)	30 (43.4)	0.128
		AA (11)	3 (27.2)	8 (72.7)	
	Dominant	AA-AT (67)	37 (55.2)	30 (44.7)	0.268
		TT (13)	5 (38.4)	8 (61.5)	
MTNR1A rs6553010	Additive	AA (2)	1 (50)	1 (50)	0.272
		AG (61)	35 (57.3)	26 (42.6)	
		GG (17)	6 (35.2)	11 (64.7)	
	Recessive	AG-AA (78)	41 (52.5)	37 (47.4)	0.943
		GG (2)	1 (50)	1 (50)	
	Dominant	AA-AG (63)	37 (58.7)	26 (41.2)	0.057
		GG (17)	5 (29.4)	12 (70.5)	
MTNR1A rs6847693	Additive	TT (33)	18 (54.5)	15 (45.4)	0.406
		TC (20)	8 (40)	12 (60)	
		CC (27)	16 (59.2)	11 (40.7)	
	Recessive	TC-CC (47)	24 (51.0)	23 (48.9)	0.759
		TT (33)	18 (54.5)	15 (45.4)	
	Dominant	TT-CT (53)	26 (49.0)	27 (50.9)	0.388
		CC (27)	16 (59.2)	11 (40.7)	
Genetic polymorphisms			**Sleep disturbance (n - %)**		
Gene (polymorphism)	**Model**	**Base pairs (n)**	**<1x (54)**	**> »1X (26)**	***p***
MTNR1A rs13140012	Additive	AA (11)	7 (63.6)	4 (36.3)	0.721
		AT (56)	37 (66.0)	19 (33.9)	
		TT (13)	10 (76.9)	3 (23.0)	
	Recessive	AT-TT (69)	48 (69.5)	21 (30.4)	0.588
		AA (11)	5 (45.4)	6 (54.5)	
	Dominant	AA-AT (67)	44 (65.6)	23 (34.3)	0.428
		TT (13)	10 (76.9)	3 (23.0)	
MTNR1A rs6553010	Additive	AA (2)	0 (0.0)	2 (100)	0.051
		AG (61)	40 (65.5)	21 (34.4)	
		GG (17)	14 (82.3)	3 (17.6)	
	Recessive	AG-AA (78)	54 (69.2)	24 (30.7)	**0.039**
		GG (2)	0 (0.0)	2 (100)	
	Dominant	AA-AG (63)	41 (65.0)	22 (34.9)	0.189
		GG (17)	13 (76.4)	4 (23.5)	
	Additive	TT (33)	23 (69.6)	10 (30.3)	0.491
		TC (20)	15 (75)	5 (25)	

MTNR1A *rs6847693*		CC (27)	16 (59.2)	11 (40.7)	
	Recessive	TC-CC (47)	31 (65.9)	16 (34.0)	0.725
		TT (33)	23 (69.6)	10 (30.3)	
	Dominant	TT-CT (53)	38 (71.6)	15 (28.3)	0.261
		CC (27)	16 (59.2)	11 (40.7)	
Genetic polymorphisms			**Sleeping medication (n - %)**		
Gene (polymorphism)	**Model**	**Base pairs (n)**	**No (64)**	**Yes (16)**	***p***
MTNR1A *rs13140012*	Additive	AA (11)	8 (72.7)	3 (27.2)	0.740
		AT (56)	46 (82.1)	10 (17.8)	
		TT (13)	10 (76.9)	3 (23.0)	
	Recessive	AT-TT (69)	57 (82.6)	12 (17.3)	0.398
		AA (11)	7 (63.6)	4 (36.3)	
	Dominant	AA-AT (67)	54 (80.5)	13 (19.4)	0.762
		TT (13)	10 (76.9)	3 (23.0)	
MTNR1A *rs6553010*	Additive	AA (2)	1 (50)	1 (50)	**0.046**
		AG (61)	46 (75.4)	15 (24.5)	
		GG (17)	17 (100%)	0 (0.0)	
	Recessive	AG-AA (78)	63 (80.7)	15 (19.2)	0.283
		GG (2)	1 (50)	1 (50)	
	Dominant	AA-AG (63)	48 (76.1)	15 (23.8)	0.025
		GG (17)	16 (94.1)	1 (5.8)	
MTNR1A *rs6847693*	Additive	TT (33)	25 (75.7)	8 (24.2)	0.426
		TC (20)	18 (90)	2 (10)	
		CC (27)	21 (77.7)	6 (22.2)	
	Recessive	TC-CC (47)	39 (82.9)	8 (17.0)	0.427
		TT (33)	25 (75.7)	8 (24.2)	
	Dominant	TT-CT (53)	43 (81.1)	10 (18.8)	0.723
		CC (27)	21 (77.7)	6 (22.2)	
Genetic polymorphisms			**Daytime dysfunction (n - %)**		
Gene (polymorphism)	**Model**	**Base pairs (n)**	**Absent (20)**	**Present (60)**	***p***
MTNR1A *rs13140012*	Additive	AA (11)	4 (36.3)	7 (63.6)	0.068
		AT (56)	10 (17.8)	46 (82.1)	
		TT (13)	6 (46.1)	7 (53.8)	
	Recessive	AT-TT (69)	16 (23.1)	53 (76.8)	0.242
		AA (11)	4 (36.3)	7 (63.6)	
	Dominant	AA-AT (67)	14 (20.8)	53 (79.1)	0.054
		TT (13)	6 (46.1)	7 (53.8)	
MTNR1A *rs6553010*	Additive	AA (2)	1 (50)	1 (50)	0.708
		AG (61)	15 (24.5)	46 (75.4)	
		GG (17)	4 (23.5)	13 (76.4)	
	Recessive	AG-AA (78)	19 (24.3)	59 (75.6)	0.408
		GG (2)	1 (50)	1 (50)	
	Dominant	AA-AG (63)	16 (25.3)	47 (74.6)	1.00
		GG (17)	4 (23.5)	13 (76.4)	
MTNR1A *rs6847693*	Additive	TT (33)	9 (27.2)	24 (72.7)	0.618
		TC (20)	6 (30)	14 (70)	
		CC (27)	5 (18.5)	22 (81.4)	
	Recessive	TC-CC (47)	11 (23.4)	36 (76.5)	0.694
		TT (33)	9 (27.2)	24 (72.7)	
	Dominant	TT-CT (53)	15 (28.3)	38 (71.6)	0.339
		CC (27)	5 (18.5)	22 (81.4)	

Note: Chi-square test independent samples with a significance value of 0.05. Bold values indicate statistical significance.

According to the reference values, an ODI of less than five is considered normal. Between five and 15 indicates mild sleep apnea, while between 15 and 30 indicates moderate sleep apnea. An ODI greater than 30 indicates severe sleep apnea. Out of the 80 individuals, 74 (92.5%) had normal ODI values. Four individuals (5%) had a mild degree, two (2.5%) had a moderate degree, and none had severe sleep apnea.

DISCUSSION

Melatonin, or 5-methoxy-N-acetyl tryptamine, is synthesized and released by the pineal gland and locally in the retina following a circadian rhythm, with low levels during the day and high levels at night. Melatonin activates two high-affinity G-protein-coupled receptors, called MT1 and MT2, to exert beneficial actions on sleep and circadian abnormalities, mood disorders, learning and memory, neuroprotection, drug abuse, and cancer. Progress in understanding the role of melatonin receptors in modulating sleep and circadian rhythms has led to the discovery of a new class of melatonin agonists for the treatment of insomnia, circadian rhythms, mood disorders, and even cancer.[23]

Variations in the *MTNR1A* gene have been linked to work shift intolerance, Alzheimer's disease, and other neurological diseases, so it is plausible that deficits in the receptor or lack of melatonin could cause sleep bruxism.[24] In this cross-sectional study, we hypothesized that genetic polymorphisms in the *MTNR1A* gene could be associated with sleep bruxism, performing the diagnosis through the DC/TMD Axis II. The OBC questionnaire was used to diagnose sleep and awake bruxism, along with a physical examination, the bruxism was diagnosed as absent possible and probable.

Our results suggest that the genetic polymorphisms *rs6553010*, *rs13140012,* and *rs6847693* of the *MTNR1A* gene may be involved in the occurrence of sleep bruxism. Subjects with the CC genotype had a higher frequency of probable sleep bruxism. Another study[25] investigated the role of these polymorphisms in sleep bruxism, however, only polysomnography (PSG) was used as a diagnostic tool and found no association between them. These different results can be explained by the fact that the PSG only considered the night of the exam for the analysis, while our diagnosis considered self-assessment, roommate feedback, and clinical signs and symptoms related with sleep bruxism. Although only three gene polymorphisms have been investigated and we have found only one association between the polymorphisms and sleep bruxism, our work suggests that melatonin may have a relationship with the condition, and this should be further investigated. There is already substantial evidence indicating a role for the MT1 receptor in modulating brain function and mood. [26] The diagnosis assessed in this study was primary sleep bruxism, considering that

sleep bruxism secondary to a respiratory factor such as obstructive sleep apnea was ruled out through type 4 polysomnography.

Other findings were related to a subjective sleep assessment. Sleep latency was associated with *rs6847693.* It is already well-established that melatonin production is regulated by the presence of light and interferes with sleep latency, which is the time it takes for a person to fall asleep. According to our findings, changes in the *MTNR1B* receptor may be associated with sleep latency. Future studies targeting melatonin receptors MT1 or MT2 may promote the development of new and more effective therapeutic agents.[23]

Still regarding sleep disorder components, the nocturnal awakenings, difficulty sleeping, and the use of sleep medication were associated with the polymorphism *rs6553010.* The complexity of the interaction between melatonin and its receptors in the CNS and their corresponding roles in sleep and circadian regulation has been studied. Further studies are needed to understand how melatonin and its agonists contribute to sleep and circadian phase changes, and how to best develop compounds that can target CNS functions specifically and effectively.[27,28,29,30] Thus, these associations found with the *rs6553010* polymorphism can help to unravel these mechanisms and new treatments.

There are some limitations to this study that need to be considered. We were unable to make a definitive diagnosis of sleep bruxism, but we used a reliable and precise method that followed international guidelines to identify probable sleep bruxism. Also, the sample size was relatively small considering the number of polymorphisms studied, which might affect the applicability of our findings. Sleep disturbances were also only subjectively assessed. In addition, it is important to remember that we evaluated only a few polymorphisms related to the *MTNR1A* gene, and that if more polymorphisms had been evaluated, other associations could have been found with our variables.

To better understand the role of the hormone melatonin with the variables analyzed, other than investigating changes in its receptors, changes in the production and secretion of melatonin should be investigated (pineal gland deficiency). Thus, further studies with melatonin quantification tests should be performed. Overall, our initial findings should be further investigated in future studies with larger samples, better diagnostic tools, and case-control studies to confirm the role of *MTNR1A* in sleep bruxism and sleep disorders.[31] We believe that knowledge of melatonin receptor structures related to these variables may lead to new treatments for sleep bruxism and sleep disorders.

CONCLUSION

The study revealed a significant between *MTNR1A* gene polymorphisms and factors like sleep bruxism, BMI, sleep latency, sleep disorders, and the usage of sleep medications. To substantiate these findings, additional research should be conducted.

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4. CONCLUSÃO

A prevalência de artralgia é maior em pacientes com DDF em tratamento ortodôntico cirúrgico quando comparado a indivíduos sem DDF. O bruxismo do sono está associado com a ocorrência de DTM articular nestes pacientes.

Em relação aos polimorfismos do gene da melatonina e a qualidade do sono, Foi encontrado associação significativa entre os polimorfismos do gene *MTNR1A* com bruxismo do sono, IMC, latência do sono, presença de distúrbios do sono e uso de medicamentos para dormir, dessa forma outros estudos devem ser realizados para confirmar os achados.

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APÊNDICE 1 – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós, Rafaela Scariot e Aline Sebastiani (professoras do Departamento de Estomatologia do curso de Odontologia da Universidade Federal do Paraná), junto com Danielle V. Bonotto e Alice V. Miotto (alunas do Programa de Pós-Graduação em Odontologia, doutorado e mestrado respectivamente), estamos convidando o(a) senhor(a) que buscou tratamento no Serviço de Odontologia da Universidade Federal do Paraná de forma voluntária para tratamento dentário ou para realização da cirurgia ortognática, a participar de um estudo intitulado "DISTÚRBIOS DO SONO E DISFUNÇÃO TEMPOROMANDIBULAR EM PACIENTES DE CIRURGIA ORTOGNÁTICA", para avaliar a qualidade do sono e a presença de dores na face ou alterações na abertura de boca. Esse estudo será importante para melhorar a compreensão e o tratamento destas alterações.

a) O objetivo dessa pesquisa é avaliar como problemas dentários e do perfil facial poderiam afetar os problemas respiratórios do sono e as dores na face e na mandíbula. E de que forma a cirurgia ortognática pode interferir nestas alterações. Verificaremos também alguns aspectos genéticos relacionados ao sono e a dor. Lembramos que é através das pesquisas clínicas que ocorrem os avanços importantes em todas as áreas, e sua participação é fundamental.

b) Caso o senhor concorde em participar da pesquisa, serão realizadas perguntas sobre a sua saúde geral (física e emocional), qualidade do seu sono, hábitos orais e sobre sintomas de dor na face. Você também será convidado a dormir com um aparelho que será conectado no dedo, e que vai demonstrar aspectos importantes do seu sono. Além disso será realizado um exame envolvendo palpação da sua face e a coleta do DNA através de um bochecho com uma solução de glicose e raspagem da bochecha com uma espátula.

c) Para tanto você deverá se dispor a participar da pesquisa durante as suas consultas nas clínicas de odontologia do campus Jardim Botânico da UFPR (Av. Prefeito Lothário Meissner, 623 - Jardim Botânico, Curitiba - PR, 80210-170). Neste estudo será utilizado um grupo de pacientes **sem deformidades dentárias e do perfil facial** e um grupo de pacientes **com deformidade dentárias e do perfil facial** que serão submetidos a cirurgia ortognática.

Participante da Pesquisa e/ou Responsável Legal:_____
Pesquisador Responsável ou quem aplicou o TCLE:_____
Orientador:_____

Comitê de ética em Pesquisa do Setor de Ciências da Saúde da FUFPR
Rua Pe. Camargo, 280 – 2º andar – Alto da Glória – Curitiba-PR –CEP:80060-240
Tel (41)3360-7259 - e-mail: cometica.saude@ufpr.br

Caso você pertença ao **grupo sem deformidades dentárias e do perfil facial** no dia do seu atendimento odontológico, você deverá responder os questionários e ser submetido ao exame clínico e a coleta do DNA. O que levará aproximadamente 20 minutos.

Caso você pertença ao grupo **com deformidades dentárias e do perfil facial** durante a consulta pré-operatória de 7 dias, e nas consultas pós-operatórias de 3 e 6 meses deverá responder os questionários e ser submetido ao exame clínico, levando em torno de 15 minutos. O DNA será coletado na consulta pré-operatória levando em torno de 5 minutos para a coleta.

Independente do grupo que pertença, você receberá emprestado o aparelho Biologix e um telefone celular com aplicativo do Biologix instalado que será utilizado em casa durante o sono (esse exame do sono será realizado em todos os participantes da pesquisa). As orientações de uso serão fornecidas pelos pesquisadores. Este aparelho deverá ser devolvido no seu próximo retorno a UFPR.

d) É possível que o (a) senhor (a) experimente algum desconforto, principalmente relacionado ao momento da realização do exame para verificar se há alguma dor ou limitação na região da face, e durante coleta da saliva e raspagem da bochecha com espátula.

e) Alguns riscos relacionados ao estudo podem ser constrangimento ao responder os questionários e durante o exame. Para evitar isso, você será avaliado individualmente em uma sala sem a presença de outros pacientes. Quanto aos riscos dos procedimentos a serem realizados, serão esclarecidos pelo cirurgião dentista responsável pelo tratamento.

g) Os benefícios esperados com essa pesquisa são compreender a associação das deformidades dentárias e do perfil facial com os problemas respiratórios do sono, função da mandíbula e as dores na face e verificar de que forma a cirurgia ortognática interfere nestas condições. E assim desenvolver novas formas de tratamento a estes indivíduos para melhorar sua qualidade de vida e a função mastigatória.

h) Os pesquisadores responsáveis por este estudo (Rafaela Scariot, Aline Sebastiani, Danielle Bonotto e Alice Miotto) poderão ser localizados no Departamento de Estomatologia no prédio de Odontologia – Campus Jardim Botânico da Universidade Federal do Paraná (Av. Prefeito Lothário Meissner, 623 - Jardim Botânico, Curitiba - PR, 80210-170) ou contactados pelo telefone (41) 3360-4020, nos dias úteis das 13:30 às 17:30 horas ou pelos e-mails rafaela_scariot@yahoo.com.br, sebastiani.aline@gmail.com, dvbonotto@gmail.com e alicevmiotto@gmail.com para esclarecer eventuais dúvidas a respeito desta pesquisa, e fornecer-lhe as informações que queira, antes, durante ou depois de encerrado o estudo. Em caso de emergência o (a) senhor (a) também pode me contatar (Danielle Bonotto), neste número (41) 99928-9982, em qualquer horário.

i) A sua participação neste estudo é voluntária e se o(a) senhor(a) não quiser mais fazer parte da pesquisa poderá desistir a qualquer momento e solicitar que lhe devolvam este Termo de Consentimento Livre e Esclarecido assinado. O seu atendimento e/ou tratamento está garantido e não será interrompido caso você desista de participar.

j) O material obtido – amostras biológicas, questionários, imagens – será utilizado unicamente para essa pesquisa e será destruído/descartado ao término do estudo, dentro de 5 anos.

k) As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas, são elas a orientadora Rafaela Scariot e responsáveis pela pesquisa Aline Sebastiani, Danielle V. Bonotto e Alice V. Miotto, sob forma codificada, para que a sua identidade seja preservada e mantida a confidencialidade.

l) O(a) senhor(a) terá a garantia de que quando os dados/resultados obtidos com este estudo forem publicados, não aparecerá seu nome.

m) As despesas necessárias para a realização da pesquisa (impressão de questionários, equipamentos utilizados) não são de sua responsabilidade e o (a) senhor(a) não receberá qualquer valor em dinheiro pela sua participação. Entretanto, caso seja necessário seu deslocamento até o local do estudo os pesquisadores asseguram o ressarcimento dos seus gastos com transporte (Item II.21, e item IV.3, sub-item g, Resol. 466/2012).

n) Quando os resultados forem publicados, não aparecerá seu nome, e sim um código.

o) Se o(a) senhor(a) tiver dúvidas sobre seus direitos como participante de pesquisa, poderá contatar também o Comitê de Ética em Pesquisa em Seres Humanos (CEP/SD) do Setor de Ciências da Saúde da Universidade Federal do Paraná, pelo e-mail cometica.saude@ufpr.br e/ou telefone 41 -3360-7259, das 08:30h às 11:00h e das 14:00h.às 16:00h.

O Comitê de Ética em Pesquisa é um órgão colegiado multi e transdisciplinar, independente, que existe nas instituições que realizam pesquisa envolvendo seres humanos no Brasil e foi criado com o objetivo de proteger os participantes de pesquisa, em sua integridade e dignidade, e assegurar que as pesquisas sejam desenvolvidas dentro de padrões éticos (Resolução nº 466/12 Conselho Nacional de Saúde).

Eu,_____ li esse Termo de Consentimento e compreendi a natureza e o objetivo do estudo do qual concordei em participar. A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper minha participação a qualquer momento sem justificar minha decisão e sem qualquer prejuízo para mim e sem que esta decisão afete meu tratamento. Eu concordo, voluntariamente, em participar deste estudo.

Curitiba, ___ de _____ de _____

_____

[Assinatura do Participante de Pesquisa ou Responsável Legal]

Eu declaro ter apresentado o estudo, explicado seus objetivos, natureza, riscos e benefícios e ter respondido da melhor forma possível às questões formuladas.

_____

[Assinatura do Pesquisador Responsável ou quem aplicou o TCLE]

Participante da Pesquisa e/ou Responsável Legal:_____
Pesquisador Responsável ou quem aplicou o TCLE:_____
Orientador:_____

ANEXO 1 - DC/TMD FORMULÁRIO DE EXAME

DC/TMD Formulário de Exame

Paciente ____________________ Examinador ____________________

Preencha a data (dd-mm-aaaa) __ __ - __ __ - __ __ __ __

1a. Local da Dor: Últimos 30 dias (Marque tudo o que se aplica)

DOR NA DIREITA: O Nenhum O Temporal O Outro M. Mast. O Estruturas Não-Mast. O Masséter O ATM

DOR NA ESQUERDA: O Nenhum O Temporal O Outro M. Mast. O Estruturas Não-Mast. O Masséter O ATM

1b. Localização da Cefaléia: Últimos 30 Dias (Marque tudo o que se aplica)

O Nenhum O Temporal O Outra — O Nenhum O Temporal O Outra

2. Relações Incisais — **Dente de Referência** O FDI #11 O FDI #21 O Outro

Trespasse Horizontal Incisal O Se negativo [ ][ ] mm — Trespasse Vertical Incisal O Se negativo [ ][ ] mm — Desvio de Linha Média: Direita O, Esquerda O, N/A O [ ][ ] mm

3. Padrão de Abertura-Fechamento (Complementar; Escolha todos que se aplicarem)

O Reto O Desvio Corrigido

Desvio não Corrigido: O Direita O Esquerda

4. Movimentos de Abertura

A. Abertura Sem Dor [ ][ ] mm

B. Abertura Máxima Não Assistida [ ][ ] mm

LADO DIREITO	Dor	Dor Familiar	Cefaleia Familiar	LADO ESQUERDO	Dor	Dor Familiar	Cefaleia Familiar
Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ	Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ
Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ		Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ	
ATM	Ⓝ Ⓢ	Ⓝ Ⓢ		ATM	Ⓝ Ⓢ	Ⓝ Ⓢ	
Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ		Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ	
Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ		Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ	

C. Abertura Máxima Assistida [ ][ ] mm

D. Interrompida? Ⓝ Ⓢ

LADO DIREITO	Dor	Dor Familiar	Cefaleia Familiar	LADO ESQUERDO	Dor	Dor Familiar	Cefaleia Familiar
Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ	Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ
Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ		Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ	
ATM	Ⓝ Ⓢ	Ⓝ Ⓢ		ATM	Ⓝ Ⓢ	Ⓝ Ⓢ	
Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ		Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ	
Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ		Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ	

5. Movimentos Laterais e Protrusivo

A. Lateralidade Direita [ ][ ] mm

LADO DIREITO	Dor	Dor Familiar	Cefaleia Familiar	LADO ESQUERDO	Dor	Dor Familiar	Cefaleia Familiar
Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ	Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ
Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ		Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ	
ATM	Ⓝ Ⓢ	Ⓝ Ⓢ		ATM	Ⓝ Ⓢ	Ⓝ Ⓢ	
Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ		Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ	
Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ		Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ	

B. Lateralidade Esquerda [ ][ ] mm

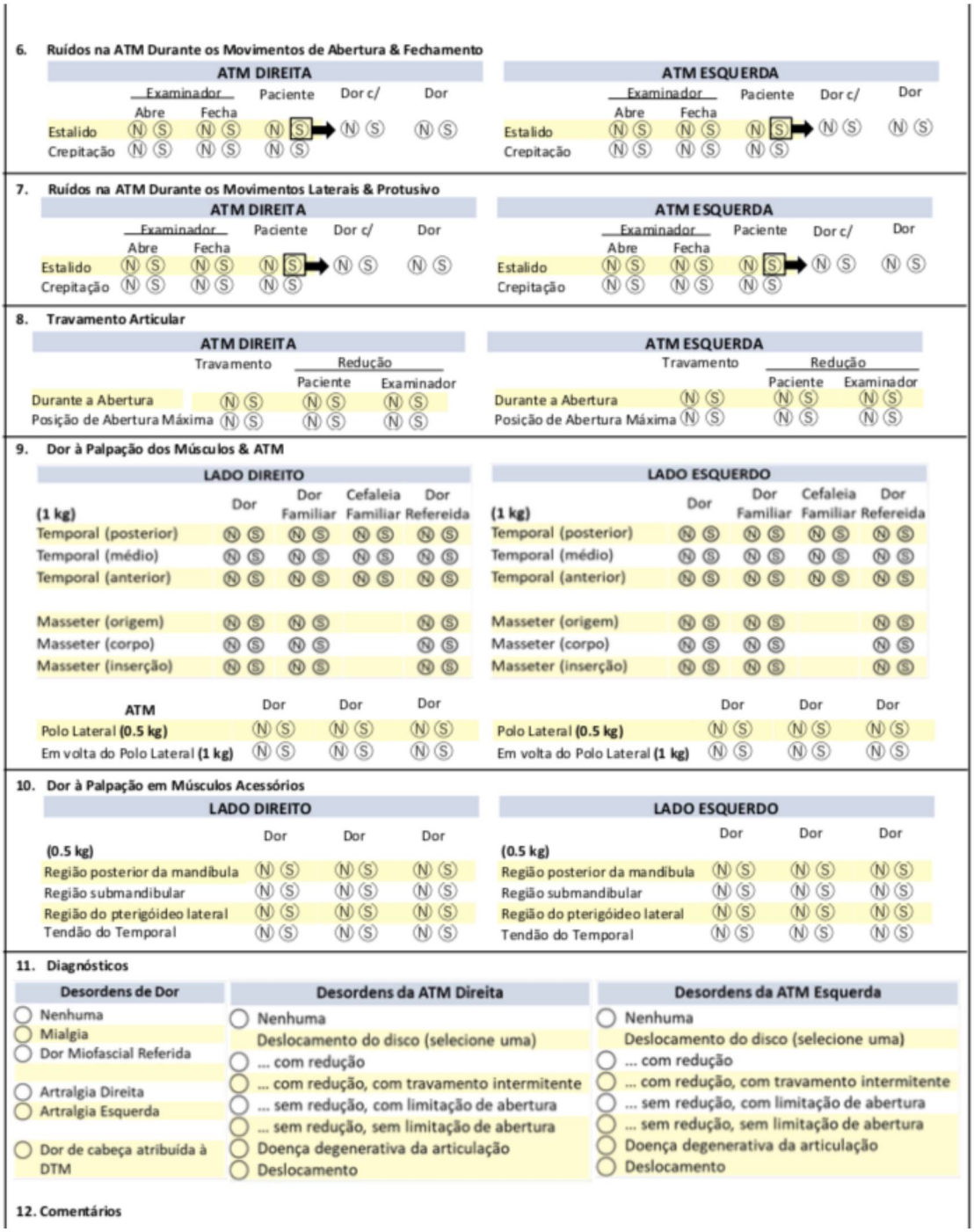
LADO DIREITO	Dor	Dor Familiar	Cefaleia Familiar	LADO ESQUERDO	Dor	Dor Familiar	Cefaleia Familiar
Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ	Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ
Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ		Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ	
ATM	Ⓝ Ⓢ	Ⓝ Ⓢ		ATM	Ⓝ Ⓢ	Ⓝ Ⓢ	
Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ		Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ	
Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ		Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ	

C. Protrusão [ ][ ] mm

O Se negativo

LADO DIREITO	Dor	Dor Familiar	Cefaleia Familiar	LADO ESQUERDO	Dor	Dor Familiar	Cefaleia Familiar
Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ	Temporal	Ⓝ Ⓢ	Ⓝ Ⓢ	Ⓝ Ⓢ
Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ		Masseter	Ⓝ Ⓢ	Ⓝ Ⓢ	
ATM	Ⓝ Ⓢ	Ⓝ Ⓢ		ATM	Ⓝ Ⓢ	Ⓝ Ⓢ	
Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ		Outros Músc M	Ⓝ Ⓢ	Ⓝ Ⓢ	
Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ		Não-mast.	Ⓝ Ⓢ	Ⓝ Ⓢ	

ANEXO 1 - DC/TMD Formulário de Exame - Continuação



6. Ruídos na ATM Durante os Movimentos de Abertura & Fechamento

ATM DIREITA	Examinador: Abre	Examinador: Fecha	Paciente	Dor c/	Dor
Estalido	N S	N S	N S	N S	N S
Crepitação	N S	N S	N S		

ATM ESQUERDA	Examinador: Abre	Examinador: Fecha	Paciente	Dor c/	Dor
Estalido	N S	N S	N S	N S	N S
Crepitação	N S	N S	N S		

7. Ruídos na ATM Durante os Movimentos Laterais & Protusivo

ATM DIREITA	Examinador: Abre	Examinador: Fecha	Paciente	Dor c/	Dor
Estalido	N S	N S	N S	N S	N S
Crepitação	N S	N S	N S		

ATM ESQUERDA	Examinador: Abre	Examinador: Fecha	Paciente	Dor c/	Dor
Estalido	N S	N S	N S	N S	N S
Crepitação	N S	N S	N S		

8. Travamento Articular

ATM DIREITA	Travamento	Redução: Paciente	Redução: Examinador
Durante a Abertura	N S	N S	N S
Posição de Abertura Máxima	N S	N S	N S

ATM ESQUERDA	Travamento	Redução: Paciente	Redução: Examinador
Durante a Abertura	N S	N S	N S
Posição de Abertura Máxima	N S	N S	N S

9. Dor à Palpação dos Músculos & ATM

LADO DIREITO (1 kg)	Dor	Dor Familiar	Cefaleia Familiar	Dor Referida
Temporal (posterior)	N S	N S	N S	N S
Temporal (médio)	N S	N S	N S	N S
Temporal (anterior)	N S	N S	N S	N S
Masseter (origem)	N S	N S		N S
Masseter (corpo)	N S	N S		N S
Masseter (inserção)	N S	N S		N S

ATM	Dor	Dor	Dor
Polo Lateral (0.5 kg)	N S	N S	N S
Em volta do Polo Lateral (1 kg)	N S	N S	N S

LADO ESQUERDO (1 kg)	Dor	Dor Familiar	Cefaleia Familiar	Dor Referida
Temporal (posterior)	N S	N S	N S	N S
Temporal (médio)	N S	N S	N S	N S
Temporal (anterior)	N S	N S	N S	N S
Masseter (origem)	N S	N S		N S
Masseter (corpo)	N S	N S		N S
Masseter (inserção)	N S	N S		N S

	Dor	Dor	Dor
Polo Lateral (0.5 kg)	N S	N S	N S
Em volta do Polo Lateral (1 kg)	N S	N S	N S

10. Dor à Palpação em Músculos Acessórios

LADO DIREITO (0.5 kg)	Dor	Dor	Dor
Região posterior da mandíbula	N S	N S	N S
Região submandibular	N S	N S	N S
Região do pterigóideo lateral	N S	N S	N S
Tendão do Temporal	N S	N S	N S

LADO ESQUERDO (0.5 kg)	Dor	Dor	Dor
Região posterior da mandíbula	N S	N S	N S
Região submandibular	N S	N S	N S
Região do pterigóideo lateral	N S	N S	N S
Tendão do Temporal	N S	N S	N S

11. Diagnósticos

Desordens de Dor	Desordens da ATM Direita	Desordens da ATM Esquerda
○ Nenhuma	○ Nenhuma	○ Nenhuma
○ Mialgia	Deslocamento do disco (selecione uma)	Deslocamento do disco (selecione uma)
○ Dor Miofascial Referida	○ ... com redução	○ ... com redução
○ Artralgia Direita	○ ... com redução, com travamento intermitente	○ ... com redução, com travamento intermitente
○ Artralgia Esquerda	○ ... sem redução, com limitação de abertura	○ ... sem redução, com limitação de abertura
○ Dor de cabeça atribuída à DTM	○ ... sem redução, sem limitação de abertura	○ ... sem redução, sem limitação de abertura
	○ Doença degenerativa da articulação	○ Doença degenerativa da articulação
	○ Deslocamento	○ Deslocamento

12. Comentários

ANEXO 2 – QUESTIONÁRIO DE SAÚDE DO PACIENTE - SÍNTOMAS FÍSICOS (PHQ-15)

Questionário de Saúde do Paciente – 15: Sintomas Físicos

Durante as últimas 4 semanas, o quanto você tem se incomodado com os problemas abaixo? Por favor, marque no quadrado para indicar a sua resposta.

		Não incomodou nada	Incomodou um pouco	Incomodou muito
		0	1	2
1.	Dor de estômago	☐	☐	☐
2.	Dor nas costas	☐	☐	☐
3.	Dor nos braços, pernas, ou articulações (joelhos, quadris, etc)	☐	☐	☐
4.	Cólicas menstruais ou outros problemas relacionados à sua menstruação [apenas para mulheres]	☐	☐	☐
5.	Dores de cabeça	☐	☐	☐
6.	Dor no peito	☐	☐	☐
7.	Tontura	☐	☐	☐
8.	Períodos de desmaios	☐	☐	☐
9.	Sentir o seu coração bater forte ou acelerar	☐	☐	☐
10.	Falta de ar	☐	☐	☐
11.	Dor ou problemas durante a relação sexual	☐	☐	☐
12.	Prisão de ventre, intestino solto ou diarréia	☐	☐	☐
13.	Náuseas, gases ou indigestão	☐	☐	☐
14.	Sentir-se cansado(a) ou com pouca energia	☐	☐	☐
15.	Dificuldade de dormir	☐	☐	☐
SOMA TOTAL =				

ANEXO 3 – DESORDEM DE ANSIEDADE GENERALIZADA (GAD - 7)

Desordem de Ansiedade Generalizada – 7 (GAD 7)

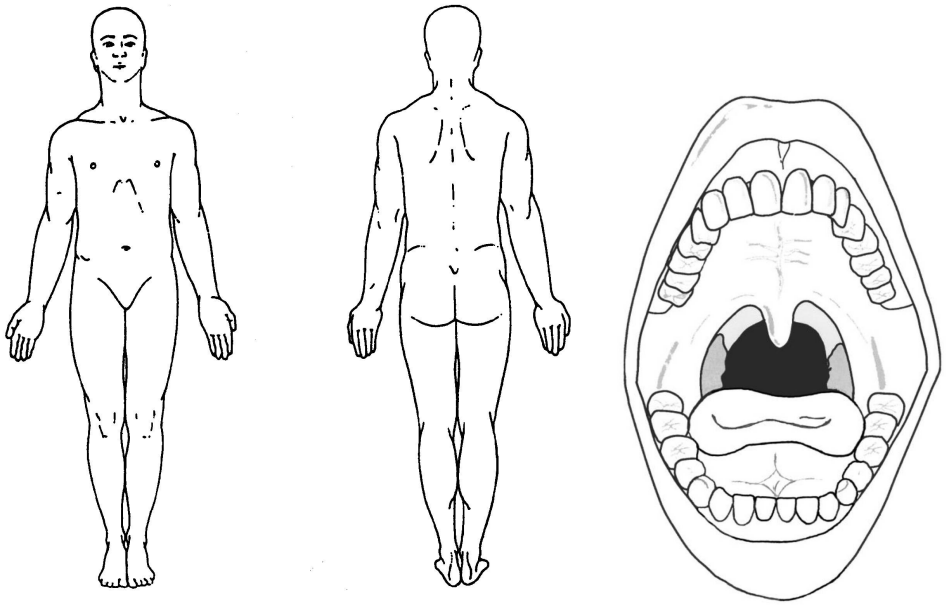
Durante as últimas 2 semanas, com que frequência você tem se incomodado com os problemas abaixo? Por favor, marque no quadrado para indicar a sua resposta.

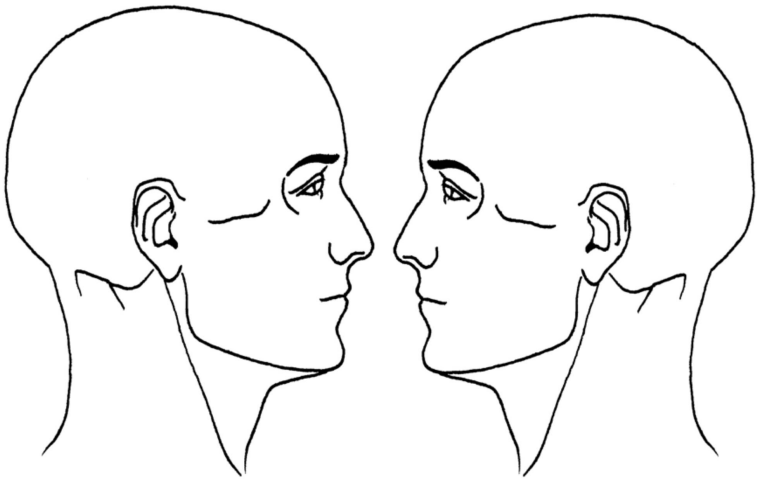
	Nenhuma vez	Vários dias	Mais da metade dos dias	Quase todos os dias
	0	1	2	3
1. Sentir-se nervoso(a), ansioso(a) ou irritado(a)	☐	☐	☐	☐
2. Não ser capaz de parar ou controlar suas preocupações	☐	☐	☐	☐
3. Preocupar-se sem necessidade com diversas coisas	☐	☐	☐	☐
4. Dificuldade para relaxar	☐	☐	☐	☐
5. Estar tão agitado(a) que é difícil ficar sentado(a) sem se mexer	☐	☐	☐	☐
6. Se tornar facilmente aborrecido(a) ou irritável	☐	☐	☐	☐
7. Sentir medo como se algo terrível fosse acontecer	☐	☐	☐	☐
SOMA TOTAL =				

Se você marcou algum dos problemas, o quanto esses problemas têm dificultado você para trabalhar, cuidar das coisas de casa, ou se relacionar com outras pessoas?			
Nada difícil	Um pouco difícil	Muito difícil	Extremamente difícil
☐	☐	☐	☐

ANEXO 4 – DESENHO DA DOR – *PEN DRAWING*

Indique a localização de TODAS as suas diferentes dores sombreando a área, usando os diagramas que são mais relevantes. Se existir um ponto exato onde a dor estiver localizada, indique com um ponto sólido (□). Se sua dor se move de um ponto para outro, use setas para mostrar o caminho.





ANEXO 5 – ORAL BEHAVIORS CHECKLIST (OBC) – LISTA DE COMPORTAMENTOS ORAIS

Lista de Verificação dos Comportamentos Orais (OBC)

Com qual frequência você fez cada uma das seguintes atividades, baseado no último mês? Se a frequência das atividades variar, escolha a opção mais frequente. Marque (√) uma resposta para cada item e não pule nenhum item. Se você mudar de ideia, preencha a marcação incorreta completamente e, em seguida, marque (√) na nova resposta.

	Atividades durante o sono	Nenhuma vez	<1 noite/ mês	1-3 noites/ mês	1-3 noites/ semana	4-7 noites/ semana
1	Aperta ou range os dentes **quando está dormindo**, baseado em qualquer informação que você possa ter.	☐	☐	☐	☐	☐
2	Dorme numa posição que coloque pressão sobre a mandíbula (por exemplo, de barriga para baixo, de lado).	☐	☐	☐	☐	☐
	Atividades durante a vigília (acordado)	**Nunca**	**Uma pequena parte do tempo**	**Alguma parte do tempo**	**A maior parte do tempo**	**O tempo todo**
3	Range os dentes **quando está acordado**	☐	☐	☐	☐	☐
4	Aperta os dentes **quando está acordado**	☐	☐	☐	☐	☐
5	Pressiona, toca ou mantém os dentes em contato além de quando está comendo (ou seja, faz contato entre dentes superiores e inferiores).	☐	☐	☐	☐	☐
6	Segura, enrijece ou tensiona os músculos, sem apertar ou encostar os dentes.	☐	☐	☐	☐	☐
7	Mantém ou projeta a mandíbula para frente ou para o lado	☐	☐	☐	☐	☐
8	Pressiona a língua com força contra os dentes	☐	☐	☐	☐	☐
9	Coloca a língua entre os dentes	☐	☐	☐	☐	☐
10	Morde, mastiga, ou brinca com a língua, bochechas ou lábios	☐	☐	☐	☐	☐
11	Mantém a mandíbula em posição rígida ou tensa, tal como para segurar ou proteger a mandíbula	☐	☐	☐	☐	☐
12	Segura entre os dentes ou morde objetos, como cabelo, cachimbo, lápis, canetas, dedos, unhas, etc	☐	☐	☐	☐	☐
13	Faz uso de goma de mascar (chiclete)	☐	☐	☐	☐	☐
14	Toca instrumento musical que envolve o uso da boca ou mandíbula (por exemplo, instrumentos de sopro, metal ou corda)	☐	☐	☐	☐	☐
15	Inclina com a mão na mandíbula, tal como se fosse colocar ou descansar o queixo na mão	☐	☐	☐	☐	☐
16	Mastiga os alimentos apenas de um lado	☐	☐	☐	☐	☐
17	Come entre as refeições (ou seja, alimento que requer mastigação)	☐	☐	☐	☐	☐
18	Fala prolongadamente (por exemplo, ensinando, vendas, atendimento ao cliente)	☐	☐	☐	☐	☐
19	Canta	☐	☐	☐	☐	☐
20	Boceja	☐	☐	☐	☐	☐
21	Segura o telefone entre a cabeça e os ombros	☐	☐	☐	☐	☐

ANEXO 6 – *STOP-BANG* – QUESTIONÁRIO DE RISCO PARA APNEIA OBSTRUTIVA DO SONO

Questionário STOP BANG

1- **S** (Ronco): Você ronca alto (mais alto do que falar ou alto o suficiente para o ronco ser ouvido mesmo de portas fechadas)?
Sim ( ) Não( )

2- **T** (Cansaço) Você frequentemente sente-se cansado, com fadiga ou sonolência durante o dia?
Sim ( ) Não ( )

3- **O** (Observação de apneia): Alguém observou se você teve parada respiratória durante o sono?
Sim ( ) Não ( )

4- **P** (Pressão): Você é hipertenso ou está fazendo tratamento para hipertensão?
Sim ( ) Não ( )

5- **B** (Índice de massa corpórea - IMC): IMC maior que 35 kg/m^2?
Sim ( ) Não ( )

6- **A** (Idade): Idade maior que 50 anos?
Sim ( ) Não ( )

7- **N** (Circunferência do pescoço): circunferência do pescoço maior que 40 cm?
Sim ( ) Não ( )

8- **G** (gênero): gênero masculino?
Sim ( ) Não ( )

Três ou mais respostas positivas: **Alto risco** para apneia obstrutiva do sono
Menos que três respostas positivas: **Baixo risco** para apneia obstrutiva do sono.

ANEXO 7 – *PITTSBURGH SLEEP QUALITY INDEX - PSQI*

ÍNDICE DE QUALIDADE DE SONO DE PITTSBURG
<INSTRUÇÕES PARA PONTUAÇÃO>

Componente 1: Qualidade subjetiva do sono: examine a questão 6 e atribua a pontuação da seguinte maneira:

Resposta	Escore
Muito boa	0
Boa	1
Ruim	2
Muito ruim	3

Pontuação do componente 1 .....

Componente 2: Latência do sono:

1. Examine a questão 2 e atribua a pontuação de a seguinte maneira:

Resposta	Escore
< ou = 15 minutos	0
16 a 30 minutos	1
31 a 60 minutos	2
> 60 minutos	3

2. Examine a questão 5a e atribua a pontuação da seguinte maneira:

Resposta	Escore
Nenhuma vez	0
Menos de 1 vez/semana	1
1a 2 vezes/semana	2
2 a 3 vezes/semana	3

3. Some a pontuação da questão 2 e 5a

ANEXO 7 – *Pittsburgh Sleep Quality Index - PSQI* - continuação

4. Atribua a pontuação do componente 2 da seguinte maneira:

Soma	Escore
0	0
1 a 2	1
3 a 4	2
5 a 6	3

Pontuação do componente 2 .....

Componente 3: Duração do sono:
1. Examine questão 4 e atribua a pontuação da seguinte maneira:

Resposta	Escore
> 7 horas	0
6 a 7 horas	1
5 a 6 horas	2
< 5 horas	3

Pontuação do componente 3 .....

Componente 4: Eficiência habitual do sono:
1. Examine a questão 2 e atribua a pontuação da seguinte maneira:
- ✓ Escreva o número de horas dormidas (questão 4)
- ✓ Calcule o número de horas no leito:

{horário de levantar (questão 3) – horário de deitar (questão 1)}
- ✓ Calcule a eficiência do sono:

{no de horas dormidas/no de horas no leito} x 100 = eficiência do sono(%)
- ✓ Atribua a pontuação do componente 4 da seguinte maneira:

Eficiência do sono (%)	Escore
> 85%	0
75 a 84%	1
65 a 74%	2
<65%	3

ANEXO 7 – *Pittsburgh Sleep Quality Index - PSQI* - continuação

Pontuação do componente 4 .....

Componente 5: Distúrbios do sono:

1. Examine as questões de 5b a 5j e atribua a pontuação:

Resposta	Escore
Nenhuma vez	0
Menos de 1 vez/sem	1
1 a 2 vezes/semana	2
3 vezes/sem ou mais	3

2. Some a pontuação de 5b a 5j:

3. Atribua a pontuação do componente 5 da seguinte forma:

Soma de 5b a 5j	Escore
0	0
1 a 9	1
10 a 18	2
19 a 27	3

Pontuação do componente 5 .....

Componente 6: Uso de medicação para dormir:

1.Examine a questão 7 e atribua a pontuação da seguinte maneira:

Resposta	Escore
Nenhuma vez	0
Menos de 1 vez/sem	1
1 a 2 vezes/semana	2
3 vezes/sem ou mais	3

Pontuação do componente 6 .....

Componente 7: Disfunção durante o dia:

1. Examine a questão 8 e atribua a pontuação da seguinte maneira:

ANEXO 7 – *Pittsburgh Sleep Quality Index - PSQI* - continuação

Resposta	Escore
Nenhuma vez	0
Menos de 1 vez/sem	1
1 a 2 vezes/semana	2
3 vezes/sem ou mais	3

2. Examine a questão 9 e atribua a pontuação da seguinte maneira:

Resposta	Escore
Nenhuma	0
Pequena	1
Moderada	2
Muita	3

3. Some a pontuação das questões 8 e 9

4. Atribua a pontuação do componente 7 da seguinte maneira:

Soma	Escore
0	0
1 a 2	1
3 a 4	2
5 a 7	3

Pontuação do componente 7 .....

Os escores dos sete componentes são somados para conferir uma pontuação global do PSQI, a qual varia de 0 a 21.

Pontuação	Qualidade do sono
0 a 4	boa
5 a 10	ruim
> 10	presença de distúrbio do sono

NORMAS DA REVISTA

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GUIDE FOR AUTHORS

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4 Instructions for Authors

Shortcuts

- Manuscript Submission Overview
- Manuscript Preparation
- Preparing Figures, Schemes and Tables
- Supplementary Materials, Data Deposit and Software Source Code
- Research and Publication Ethics
- Reviewer Suggestions
- English Corrections
- Preprints and Conference Papers
- Authorship
- Editorial Independence
- Conflicts of Interest
- Editorial Procedures and Peer-Review
- Promoting Equity, Diversity and Inclusiveness within MDPI Journals
- Resource Identification Initiative

5 Submission Checklist

Please:

1. Read the **Aims & Scope** to gain an overview and assess if your manuscript is suitable for this journal;
2. Use the **Microsoft Word template** or **LaTeX template** to prepare your manuscript;

3. Make sure that issues about **publication ethics**, **research ethics**, **copyright**, **authorship**, **figure formats**, **data** and **references format** have been appropriately considered;
4. Ensure that all authors have approved the content of the submitted manuscript.
5. Authors are encouraged to add a **biography** (optional) to the submission and post it to **SciProfiles**.

6 Manuscript Submission Overview

7 Types of Publications

Full experimental details must be provided so that the results can be reproduced. *Diagnostics* requires that authors publish all experimental controls and make full datasets available where possible (see the guidelines on **Supplementary Materials** and references to unpublished data).

Manuscripts submitted to *Diagnostics* should neither be published previously nor be under consideration for publication in another journal. The main article types are listed below and a comprehensive list of article types can be found **here.**

- *Article:* These are original research manuscripts. The work should report scientifically sound experiments and provide a substantial amount of new information. The article should include the most recent and relevant references in the field. The structure should include an Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, and Conclusions (optional) sections, with a suggested minimum word count of 4000 words.
- *Guidelines:* These papers cover step-by-step systematical procedural instructions to clinicians for the care of patients with specific conditions. They can be consensus-based or clinical practice guidelines.
- *Protocol:* Protocols provide a detailed step-by-step description of a method. They should be proven to be robust and reproducible and should accompany a previously published article that uses this method. Any materials and equipment used should be explicitly listed. Conditions, quantities, concentrations, etc., should be given. Critical timepoints and steps, as well as warnings, should be emphasized in the text. The structure should include an Abstract, Keywords, Introduction, Experimental Design, Materials and Equipment, Detailed Procedure, and Expected Results, with a suggested minimum word count of 4000 words.

- *Review:* Reviews offer a comprehensive analysis of the existing literature within a field of study, identifying current gaps or problems. They should be critical and constructive and provide recommendations for future research. No new, unpublished data should be presented. The structure can include an Abstract, Keywords, Introduction, Relevant Sections, Discussion, Conclusions, and Future Directions, with a suggested minimum word count of 4000 words.
- **Interesting Images**: *Diagnostics* encourages the submission of **Interesting Images**.

8 Submission Process

Manuscripts for *Diagnostics* should be submitted online at **susy.mdpi.com**. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the **criteria to qualify for authorship**) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the **submission website**. Once you have registered, **click here to go to the submission form for *Diagnostics***. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

9 Accepted File Formats

Authors are encouraged to use the **Microsoft Word template** or **LaTeX template** to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the Editorial Office **diagnostics@mdpi.com**. Accepted file formats are:

- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, we encourage you to use the ***Diagnostics* Microsoft Word template file**. Please insert your graphics (schemes, figures, *etc.*) in the main text after the paragraph of its first citation.
- *LaTeX:* Manuscripts prepared in LaTeX must be collated into one ZIP folder (including all source files and images, so that the Editorial Office can recompile the submitted PDF). When preparing manuscripts in LaTeX, we encourage you to use the ***Diagnostics* LaTeX template files**. You can now also use the online

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- We do not have strict formatting requirements, but all manuscripts must contain the required sections: Author Information, Abstract, Keywords, Introduction, Materials & Methods, Results, Conclusions, Figures and Tables with Captions, Funding Information, Author Contributions, Conflict of Interest and other Ethics Statements. Check the Journal ***Instructions for Authors*** for more details.
- Your references may be in any style, provided that you use the consistent formatting throughout. It is essential to include author(s) name(s), journal or book title, article or chapter title (where required), year of publication, volume and issue (where appropriate) and pagination. DOI numbers (Digital Object Identifier) are not mandatory but highly encouraged. The bibliography software package *EndNote*, ***Zotero***, *Mendeley*, *Reference Manager* are recommended.
- When your manuscript reaches the revision stage, you will be requested to format the manuscript according to the journal guidelines.

11 Cover Letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work. It should explain why the manuscript fits the scope of the journal.

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All cover letters are required to include the statements:

- We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.
- All authors have approved the manuscript and agree with its submission to (journal name).

12 Author Biography

Authors are encouraged to add a biography (maximum 150 words) to the submission and post it to **SciProfiles**. This should be a single paragraph and should contain the following points:

1. Authors' full names followed by current positions;
2. Education background including institution information and year of graduation (type and level of degree received);
3. Work experience;
4. Current and previous research interests;
5. Memberships of professional societies and awards received.

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13 Manuscript Preparation

14 General Considerations

- **Research manuscripts** should comprise:
    - **Front matter**: Title, Author list, Affiliations, Abstract, Keywords.
    - **Research manuscript sections**: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).
    - **Back matter**: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, **References**.
- **Review manuscripts** should comprise the **front matter**, literature review sections and the **back matter**. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the **PRISMA** guidelines.
- **Case reports** should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case

presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.

- **Interesting Images**: *Diagnostics* encourages the submission of **Interesting Images**. The number of images are at the discretion of the author. No regular manuscript text (introduction/methods/results/discussion) should be included. Instead, images should be accompanied by detailed legends with no restriction in length. Reference citations should appear in the legends. Also, an unstructured abstract of no more than 200 words should be included as well as list of 3 to 10 keywords. Image files can be included either in the template or uploaded separately in high resolution. There are no restrictions on use of color or image size, however features should be sharp and not blurred. For readability, we recommend that any text in figures is at least 12 pt in size. Submitted images will be peer-reviewed under the same process as a regular research article.
- **Graphical Abstract:**

  A graphical abstract (GA) is an image that appears alongside the text abstract in the Table of Contents. In addition to summarizing the content, it should represent the topic of the article in an attention-grabbing way. Moreover, it should not be exactly the same as the Figure in the paper or just a simple superposition of several subfigures. Note that the GA must be original and unpublished artwork. Any postage stamps, currency from any country, or trademarked items should not be included in it.

  The GA should be a high-quality illustration or diagram in any of the following formats: PNG, JPEG, TIFF, or SVG. Written text in a GA should be clear and easy to read, using one of the following fonts: Times, Arial, Courier, Helvetica, Ubuntu or Calibri.

  The minimum required size for the GA is 560 × 1100 pixels (height × width). The size should be of high quality in order to reproduce well.

- **Acronyms/Abbreviations/Initialisms** should be defined the first time they appear in each of three sections: the abstract; the main text; the first figure or table. When defined for the first time, the acronym/abbreviation/initialism should be added in parentheses after the written-out form.
- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.

- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on **Deposition of Sequences and Expression Data**.
- **Equations:** If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.
- **Research Data and supplementary materials:** Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Disclose at the submission stage any restrictions on the availability of materials or information. Read the information about **Supplementary Materials** and Data Deposit for additional guidelines.
- **Preregistration:** Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.
- **Guidelines and standards:** MDPI follows standards and guidelines for certain types of research. See **https://www.mdpi.com/editorial_process** for further information.

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These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used. Please do not include abbreviated or short forms of the title, such as a running title or head. These will be removed by our Editorial Office.
- **Author List and Affiliations:** Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, and country. At least one author should be designated as the corresponding author. The email addresses of all authors will be displayed on published papers, and hidden by Captcha on the website as standard. It is the responsibility of the corresponding author to ensure that consent for the display of email addresses is obtained from all authors. If an author (other than the corresponding author) does not wish to have their email addresses displayed in this way, the

corresponding author must indicate as such during proofreading. After acceptance, updates to author names or affiliations may not be permitted. Equal Contributions: authors who have contributed equally should be marked with a superscript symbol (†). The symbol must be included below the affiliations, and the following statement added: "These authors contributed equally to this work". The equal roles of authors should also be adequately disclosed in the author contributions statement. Please read the criteria to qualify for authorship.

- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.
- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

16 Research Manuscript Sections

- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.
- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.

- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.
- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.
- **Conclusions:** This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.
- **Patents:** This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

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- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.
- **Funding:** All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.

  Please add: "This research received no external funding" or "This research was funded by [name of funder] grant number [xxx]" and "The APC was funded by [XXX]" in this section. Check carefully that the details given are accurate and use the standard spelling of funding agency names at https://search.crossref.org/funding, any errors may affect your future funding.
- **Acknowledgments:** In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

- **Author Contributions:** Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
  For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.", please turn to the **CRediT taxonomy** for the term explanation. For more background on CRediT, see **here**. "**Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully**".

- **Institutional Review Board Statement:** In this section, please add the Institutional Review Board Statement and approval number for studies involving humans or animals. Please note that the Editorial Office might ask you for further information. Please add "The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." OR "Ethical review and approval were waived for this study, due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans or animals. You might also choose to exclude this statement if the study did not involve humans or animals.

- **Informed Consent Statement:** Any research article describing a study involving humans should contain this statement. Please add "Informed consent was obtained from all subjects involved in the study." OR "Patient consent was waived due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans. You might also choose to exclude this statement if the study did not involve humans.
  Written informed consent for publication must be obtained from participating patients

who can be identified (including by the patients themselves). Please state “Written informed consent has been obtained from the patient(s) to publish this paper” if applicable.

- **Data Availability Statement:** In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section “**MDPI Research Data Policies**”. You might choose to exclude this statement if the study did not report any data.
- **Conflicts of Interest:** Authors must identify and declare any personal circumstances or interest that may be perceived as influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. *Diagnostics* does not publish studies funded partially or fully by the tobacco industry. Any projects funded by industry must pay special attention to the full declaration of funder involvement. If there is no role, please state “The sponsors had no role in the design, execution, interpretation, or writing of the study”. For more details please see **Conflict of Interest**.
- **References:** References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as **EndNote**, **ReferenceManager** or **Zotero** to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. If available online, you may use reference style 9. below.
- Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for **Endnote** and **Zotero** are available.

References should be described as follows, depending on the type of work:

❑ Journal Articles:

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.

❑ Books and Book Chapters:

2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196.

3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196.

❑ Unpublished materials intended for publication:

4. Author 1, A.B.; Author 2, C. Title of Unpublished Work (optional). Correspondence Affiliation, City, State, Country. year, *status* (*manuscript in preparation*; *to be submitted*).

5. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* year, *phrase indicating stage of publication* (*submitted*; *accepted*; *in press*).

❑ Unpublished materials not intended for publication:

6. Author 1, A.B. (Affiliation, City, State, Country); Author 2, C. (Affiliation, City, State, Country). Phase describing the material, year. (phase: Personal communication; Private communication; Unpublished work; etc.)

❑ Conference Proceedings:

7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

❑ Thesis:

8. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.

❑ Websites:

9. Title of Site. Available online: URL (accessed on Day Month Year). Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as **WebCite**. Archived websites should be cited using the link provided as follows:

10. Title of Site. URL (archived on Day Month Year).

See the **Reference List and Citations Guide** for more detailed information.

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18 Preparing Figures, Schemes and Tables

- File for Figures and Schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.
- *Diagnostics* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.
- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).
- All Figures, Schemes and Tables should have a short explanatory title and caption.
- All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.
- Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

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19 Supplementary Materials, Data Deposit and Software Source Code

MDPI Research Data Policies

MDPI is committed to supporting open scientific exchange and enabling our authors to achieve best practices in sharing and archiving research data. We encourage all authors of articles published in MDPI journals to share their research data. Individual journal guidelines can be found at the journal 'Instructions for Authors' page. Data sharing policies concern the minimal dataset that supports the central findings of a published study. Generated data should be publicly available and cited in accordance with journal guidelines.

MDPI data policies are informed by **TOP Guidelines** and **FAIR Principles**.

Where ethical, legal or privacy issues are present, data should not be shared. The authors should make any limitations clear in the Data Availability Statement upon submission. Authors should ensure that data shared are in accordance with consent provided by participants on the use of confidential data.

Data Availability Statements provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

Below are suggested Data Availability Statements:

- Data available in a publicly accessible repository
  The data presented in this study are openly available in [repository name e.g., FigShare] at [**doi**], reference number [reference number].
- Data available in a publicly accessible repository that does not issue DOIs
  Publicly available datasets were analyzed in this study. This data can be found here: [link/accession number]
- Data available on request due to restrictions eg privacy or ethical
  The data presented in this study are available on request from the corresponding author. The data are not publicly available due to [insert reason here]
- 3rd Party Data
  Restrictions apply to the availability of these data. Data was obtained from [third party] and are available [from the authors / at URL] with the permission of [third party].
- Data sharing not applicable
  No new data were created or analyzed in this study. Data sharing is not applicable to this article.
- Data is contained within the article or supplementary material
  The data presented in this study are available in [insert article or supplementary material here]

Data citation:

- [dataset] Authors. Year. Dataset title; Data repository or archive; Version (if any); Persistent identifier (e.g., DOI).

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository or uploading as supplementary information to the publication. The name and version of all software used should be clearly indicated.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of

the peer-review process. Any file format is acceptable, however we recommend that common, non-proprietary formats are used where possible. For more information on supplementary materials, please refer to **https://www.mdpi.com/authors/layout#_bookmark83**.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal **Data** also accepts submissions of data set papers.

Deposition of Sequences and Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited into an acceptable repository such as **GenBank**, **EMBL**, or **DDBJ**. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the GEO database or in the NCBI's Sequence Read Archive.
- *New microarray data* must be deposited either in the GEO or the ArrayExpress databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.

- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool SPIN).

All sequence names and the accession numbers provided by the databases should be provided in the Materials and Methods section of the article.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

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20 Research and Publication Ethics

21 Research Ethics

22 Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (**https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/**), revised in 2013. According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study

has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

A written informed consent for publication must be obtained from participating patients. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent for publication from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions. A **template permission form** is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission. Editors reserve the right to reject any submission that does not meet these requirements.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

23 Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (**arriveguidelines.org/**) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at **https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20**

Questionnaire.pdf. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

1. NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: **https://www.animalethics.org.au/three-rs**
2. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: **https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf**
3. American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: **https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use**
4. European Animal Research Association. EU regulations on animal research. Available online: **https://www.eara.eu/animal-research-law**

24 Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

25 Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the **Convention on Biological Diversity** and the **Convention on the Trade in Endangered Species of Wild Fauna and Flora**.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

26 Clinical Trials Registration

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) **guidelines** which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include **clinicaltrials.gov**, **the EU Clinical Trials Register** and those listed by the World Health Organisation **International Clinical Trials Registry Platform**.

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper

without trial registration for further peer-review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 **checklist** and **flow diagram** as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (**http://www.consort-statement.org**) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

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27 Sex and Gender in Research

We encourage our authors to follow the **'Sex and Gender Equity in Research – SAGER – guidelines'** and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full **guidelines** before submission.

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28 Borders and Territories

Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

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29 Publication Ethics Statement

Diagnostics is a member of the Committee on Publication Ethics (**COPE**). We fully adhere to its **Code of Conduct** and to its **Best Practice Guidelines**.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Diagnostics* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Diagnostics* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- The journal accepts exact translations of previously published work. All submissions of translations must conform with our **policies on translations**.
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our **policy regarding Updating Published Papers**.
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the **Rights and Permissions** page.
- Plagiarism, data fabrication and image manipulation are not tolerated.

- **Plagiarism is not acceptable** in *Diagnostics* submissions.

  Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

  Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

  All MDPI submissions are checked for plagiarism using the industry standard software iThenticate. If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, an investigation will take place and action taken in accordance with our policies.

- **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

  Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

  If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

30 Citation Policy

Authors should ensure that where material is taken from other sources (including their own published writing) the source is clearly cited and that where appropriate permission is obtained.

Authors should not engage in excessive self-citation of their own work.

Authors should not copy references from other publications if they have not read the cited work.

Authors should not preferentially cite their own or their friends', peers', or institution's publications.

Authors should not cite advertisements or advertorial material.

In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on **citation manipulation** with recommendations for best practice.

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31 Reviewer Suggestions

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last three years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

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32 English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found **here**.

MDPI provides minor English editing by native English speakers for all accepted papers, included in the APC. The APC does not cover extensive English editing. Your paper could be returned to you at the English editing stage of the publication process if extensive editing is required. You may choose to use a paid language-editing service, such as MDPI's **Author Services**, before submitting your paper for publication. If you use an alternative service that provides a confirmation certificate, please send a copy to the Editorial Office. Authors from economically developing countries or nations should consider registration with **AuthorAid**, a

global research community that provides networking, mentoring, resources and training for researchers.

33 Preprints and Conference Papers

Diagnostics accepts submissions that have previously been made available as preprints provided that they have not undergone peer review. A preprint is a draft version of a paper made available online before submission to a journal.

MDPI operates ***Preprints***, a preprint server to which submitted papers can be uploaded directly after completing journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. Check the *Preprints* **instructions for authors** for further information.

Expanded and high-quality conference papers can be considered as articles if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper. *Diagnostics* does not publish pilot studies or studies with inadequate statistical power.

Unpublished conference papers that do not meet the above conditions are recommended to be submitted to the **Proceedings Series journals**.

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34 Authorship

MDPI follows the International Committee of Medical Journal Editors (**ICMJE**) guidelines which state that, in order to qualify for authorship of a manuscript, the following criteria should be observed:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND

- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments. More detailed guidance on authorship is given by the **International Council of Medical Journal Editors (ICMJE)**.

Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication. We reserve the right to request confirmation that all authors meet the authorship conditions.

For more details about authorship please check **MDPI ethics website**.

35 Reviewers Recommendation

Authors can recommend potential reviewers. Journal editors will check to make sure there are no conflicts of interest before contacting those reviewers, and will not consider those with competing interests. Reviewers are asked to declare any conflicts of interest. Authors can also enter the names of potential peer reviewers they wish to exclude from consideration in the peer review of their manuscript, during the initial submission progress. The editorial team will respect these requests so long as this does not interfere with the objective and thorough assessment of the submission.

36 Editorial Independence

37 Lack of Interference with Editorial Decisions

Editorial independence is of utmost importance and MDPI does not interfere with editorial decisions. All articles published by MDPI are peer reviewed and assessed by our independent editorial boards, and MDPI staff are not involved in decisions to accept manuscripts. When making an editorial decision, we expect the academic editor to make their decision based only upon:

- The suitability of selected reviewers;
- Adequacy of reviewer comments and author response;
- Overall scientific quality of the paper.

In all of our journals, in every aspect of operation, MDPI policies are informed by the mission to make science and research findings open and accessible as widely and rapidly as possible.

38 Editors and Editorial Staff as Authors

Editorial staff or editors shall not be involved in processing their own academic work. Submissions authored by editorial staff/editors will be assigned to at least two independent outside reviewers. Decisions will be made by other Editorial Board Members who do not have a conflict of interest with the author. Journal staff are not involved in the processing of their own work submitted to any MDPI journals.

39 Conflicts of Interest

According to The International Committee of Medical Journal Editors, "Authors should avoid entering into agreements with study sponsors, both for-profit and non-profit, that interfere with authors' access to all of the study's data or that interfere with their ability to analyze and interpret the data and to prepare and publish manuscripts independently when and where they choose."

All authors must disclose all relationships or interests that could inappropriately influence or bias their work. Examples of potential conflicts of interest include but are not limited to financial interests (such as membership, employment, consultancies, stocks/shares ownership, honoraria, grants or other funding, paid expert testimonies and patent-licensing arrangements) and non-financial interests (such as personal or professional relationships, affiliations, personal beliefs).

Authors can disclose potential conflicts of interest via the online submission system during the submission process. Declarations regarding conflicts of interest can also be collected via the **MDPI disclosure form**. The corresponding author must include a summary statement in the manuscript in a separate section "Conflicts of Interest" placed just before the reference list. The statement should reflect all the collected potential conflicts of interest disclosures in the form.

See below for examples of disclosures:

Conflicts of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stocks in Company Y. Author C has been involved as a consultant and expert witness in Company Z. Author D is the inventor of patent X.

If no conflicts exist, the authors should state:

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

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40 Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether they are properly prepared and whether they follow the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals' *Editor-in-Chief* or *Associate Editors* to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board Members* and Guest Editors of the journal. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past three years and should not currently work or collaborate with any of the institutions of the co-authors of the submitted manuscript.

Optional Open Peer-Review

The journal operates optional open peer-review: *Authors are given the option for all review reports and editorial decisions to be published alongside their manuscript. In addition, reviewers can sign their review, i.e., identify themselves in the published review reports.* Authors can alter their choice for open review at any time before publication, but once the paper has been published changes will only be made at the discretion of the *Publisher* and *Editor-in-Chief*. We encourage authors to take advantage of this opportunity

as proof of the rigorous process employed in publishing their research. To guarantee impartial refereeing, the names of referees will be revealed only if the referees agree to do so, and after a paper has been accepted for publication.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.
- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. A maximum of two rounds of major revision per manuscript is normally provided. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments. If the required revision time is estimated to be longer than 2 months, we will recommend that authors withdraw their manuscript before resubmitting so as to avoid unnecessary time pressure and to ensure that all manuscripts are sufficiently revised.
- *Reject and Encourage Resubmission*:
  If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.
- *Reject*:
  The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments using an **appeal form**. Appeals can only be submitted

following a “reject and decline resubmission” decision and should be submitted within three months from the decision date. Failure to meet these criteria will result in the appeal not being considered further. The *Managing Editor* will forward the manuscript and related information (including the identities of the referees) to a designated *Editorial Board Member*. The Academic Editor being consulted will be asked to provide an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. This decision will then be validated by the *Editor-in-Chief*. A reject decision at this stage is final and cannot be reversed.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the **www.mdpi.com** website.

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41 Promoting Equity, Diversity and Inclusiveness within MDPI Journals

Our Managing Editors encourage the Editors-in-Chief and Associate Editors to appoint diverse expert Editorial Boards. This is also reflective in our multi-national and inclusive workplace. We are proud to create equal opportunities without regard to gender, ethnicity, sexual orientation, age, religion, or socio-economic status. There is no place for discrimination in our workplace and editors of MDPI journals are to uphold these principles in high regard.

42 Resource Identification Initiative

To improve the reproducibility of scientific research, the **Resource Identification Initiative** aims to provide unique persistent identifiers for key biological resources, including antibodies, cell lines, model organisms and tools.

We encourage authors to include unique identifiers - RRIDs- provided by the **Resource Identification Portal** in the dedicated section of the manuscript.

To help authors quickly find the correct identifiers for their materials, there is a single **website** where all resource types can be found and a ‘cite this’ button next to each resource, that contains a proper citation text that should be included in the methods section of the manuscript.