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Genes candidatos e metodologias diagnósticas aplicadas ao Espectro óculo-aurículo-vertebral

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Resumo

Introdução: O espectro óculo-aurículo-vertebral (EOAV) é uma condição congênita rara e altamente heterogênea, com múltiplos achados fenotípicos. A etiologia ainda é desconhecida, entretanto, sugere-se uma possível etiologia multifatorial, com fatores genéticos e ambientais. As causas genéticas associadas ao espectro são variáveis e ainda não estão bem elucidadas. A partir do advento de novas tecnologias, uma diversidade de alterações gênicas e cromossômicas já foram reportadas em pacientes com EOAV, sendo o diagnóstico molecular um ponto-chave no acompanhamento pré e pós-natal, e no correto suporte e tratamento de portadores desse espectro.

Objetivos: Identificar genes envolvidos no desenvolvimento das características fenotípicas do EOAV e propor possíveis genes candidatos, bem como comparar a aplicabilidade de diferentes técnicas de diagnóstico citogenético molecular.

Material e Métodos: Foram realizadas duas revisões sistemáticas da literatura para responder os objetivos propostos, com busca de artigos nas bases de dados eletrônicas MEDLINE (acessada pela Pubmed) e Web of Science. Os critérios de inclusão foram específicos para cada estudo, assim como os descritores, no geral possuindo EOAV ou sinônimo como objeto principal do estudo, pacientes EOAV com resultado de teste citogenético e pacientes EOAV com alteração no cromossomo 22. A execução seguiu as diretrizes preconizadas pela Colaboração *Cochrane* e a redação seguiu as diretrizes do PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*).

Resultados: Os genes *CLTCL1*, *GSC2*, *HIRA*, *MAPK1*, *TBX1* e *YPEL1* foram encontrados como potenciais genes candidatos para o EOAV, e corroboramos com a hipótese já descrita sobre a região cromossômica 22q11 ser um importante lócus dessa condição. Array-CGH, MLPA e cariótipo de alta resolução foram as abordagens mais eficientes no diagnóstico molecular.

Conclusão: Estudos complementares referentes à interação gênica envolvida na região 22q11 são necessários para elucidar ainda mais a associação genótipo-fenótipo. Além disso, o rastreio de pacientes suspeitos de EOAV deve seguir um protocolo com metodologias adequadas, definido em conjunto com critérios clínicos, para tornar a tomada de decisões mais efetiva e proporcionar uma melhor utilização dos recursos disponíveis.

Palavras-chave: Espectro óculo-aurículo-vertebral; EOAV; síndrome de goldenhar; genes candidatos; técnicas de citogenética; revisão sistemática;

Abstract

Introduction: Oculo-auriculo-vertebral spectrum (OAVS) is a rare and highly heterogeneous congenital condition with multiple phenotypic findings. The etiology is still unknown, however, a possible multifactorial etiology with genetic and environmental factors is suggested. The genetic causes associated with the spectrum are variable and not yet well elucidated. Since the advent of new technologies, a diversity of genetic and chromosomal changes has already been reported in OAVS patients, with molecular diagnosis being a key point in prenatal and postnatal follow-up, and in the correct support and treatment of carriers of this spectrum.

Aim of study: To identify genes involved in the development of phenotypic characteristics of OAVS and propose possible candidate genes, as well as compare the applicability of different molecular cytogenetic diagnostic techniques.

Materials and methods: Two systematic literature reviews were performed in order to meet the proposed objectives, with search of articles in the electronic databases MEDLINE (accessed by Pubmed) and Web of Science. Inclusion criteria were specific for each study, as were the descriptors, generally having OAVS or synonym as the main object of the study, OAVS patients with cytogenetic test results and OAVS patients with chromosome 22 alterations. The execution followed the guidelines recommended by the Cochrane Collaboration and the writing followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Results: *CLTCL1, GSC2, HIRA, MAPK1, TBX1* and *YPEL1* genes were found to be potential candidate genes for OAVS, and we corroborate with the

hypothesis already described about the chromosomal region 22q11 being an important locus of this condition. Array-CGH, MLPA and high-resolution karyotype were the most efficient approaches in molecular diagnosis.

Conclusion: Complementary studies regarding the gene interaction involved in the 22q11 region are necessary to further elucidate the genotype-phenotype association. In addition, screening of suspected OAVS patients should follow a protocol with appropriate methodologies, defined in association with clinical criteria, to improve decision making and provide better use of available resources.

Keywords: oculo-auriculo-vertebral spectrum; OAVS; goldenhar syndrome; candidate genes; cytogenetic techniques; systematic review.

Lista de abreviaturas

Array-CGH: hibridização genômica comparativa por microarranjos

CNV: *copy number variation*

EOAV: Espectro óculo-aurículo-vertebral

FISH: hibridização in situ fluorescente

MLPA: *multiplex ligation-dependent probe amplification*

OAVS: *oculo-auriculo-vertebral spectrum*

OMS: Organização Mundial da Saúde

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SUMÁRIO

1. REFERENCIAL TEÓRICO

A Organização Mundial da Saúde (OMS) relata que doenças genéticas atingem de 3 a 10% da população[1] e estima-se que doenças raras afetam pelo menos 3,5-5,9% da população mundial[2]. O espectro óculo-aurículo-vertebral (EOAV) ou *oculo-auriculo-vertebral spectrum*, também denominado síndrome de Goldenhar ou microssomia hemifacial (OMIM 164210), é uma condição congênita rara, complexa e altamente heterogênea, com múltiplos achados fenotípicos.

A partir do primeiro caso descrito em 1845 por Carl Ferdinand Von Arlt[3], o EOAV foi denominado síndrome de Goldenhar em 1952, por Dr. Maurice Goldenhar, que identificou uma tríade comum em 3 pacientes: dermóide epibulbar, apêndices pré-auriculares e assimetria facial[4]. Achados vertebrais foram identificados como características clínicas adicionais da síndrome em 1963, por Gorlin *et al*.[3]. Ao longo do tempo, a partir de novos achados que se sobrepunham à síndrome, somado a etiologia desconhecida, passou a ser nomeada com outros termos: síndrome óculo-aurícular, síndrome óculo-vertebral, síndrome de Goldenhar-Gorlin, Displasia Óculo-aurículo-vertebral, Síndrome do primeiro e segundo arcos branquiais, Síndrome Facio-aurículovertebral e Microssomia Hemifacial[5,6,7].

Posteriormente, em 1963, após revisão de fenótipos e anormalidades congênitas entre os casos descritos, o termo espectro óculo-aurículo-vertebral passou a ser utilizado, devido à sobreposição de achados clínicos que as demais nomenclaturas possuiam[5] e a possibilidade dos indivíduos com EOAV possuírem outras alterações além das faciais.

A frequência do EOAV na população pode variar devido à heterogeneidade da condição e dificuldades de diagnóstico clínico, casos mais leves podem não ser diagnosticados e casos com malformações complexas podem receber outro diagnóstico[7]. Entretanto estima-se em 1 por 3.500-7.000 nascimentos, com leve predominância masculina[8,9,10].

O diagnóstico do EOAV é majoritariamente clínico, ou realizado através de imagens de ultrassom, se detectado no período pré-natal. Considerada a variabilidade de achados, não há um consenso universal quanto aos critérios diagnósticos mínimos. Alguns autores vêm tentando estabelecer critérios diagnósticos para o espectro, pois as manifestações clínicas são diversas. Tasse *et al*. consideram a presença de microtia ou microssomia hemifacial associada a malformações auriculares como critério mínimo[11], já Strömland *et al.* incluem pacientes com alterações clínicas em pelo menos duas de quatro áreas (oro-crânio-facial, ocular, auricular e/ou vertebral)[12]. Beleza-Meirelles *et al.* sugerem que a presença de microssomia hemifacial isolada associada a uma história familiar de EOAV também deve ser considerada como diagnóstico[13].

Assim, destaca-se a necessidade de estudos que visam auxiliar na definição do diagnóstico clínico, como a busca e associação à genes candidatos e mecanismos moleculares, que além de confirmar a suspeita clínica, podem orientar os melhores tratamentos, condutas e estabelecer o correto prognóstico para os portadores dessa condição.

1.1. Manifestações clínicas

O EOAV inclui um grupo de malformações que envolvem principalmente estruturas derivadas do primeiro e segundo arcos branquiais e primeira bolsa faríngea e fenda branquial, em particular o ouvido, a boca, a mandíbula, os olhos e a coluna cervical[14]. O quadro clínico pode incluir assimetrias e alterações faciais leves ou graves, além de anomalias esqueléticas e envolvimento de outros órgãos[15,16].

Entre as manifestações clínicas mais descritas estão incluídas alterações craniofaciais (microcefalia, assimetria craniana, deformidade craniana, macrocefalia, assimetria facial e microssomia hemifacial)[5,17,18,19]; alterações auriculares (microtia, dismorfia, baixa implantação, apêndices e fossetas pré-auriculares, atresia do canal auditivo e deficiência/perda auditiva)[5,9,20,21]; alterações oftalmológicas (fenda palpebral estreita, microftalmia, anoftalmia, dermoide epibulbar, lipodermoide, hipertelorismo, coloboma e distúrbios visuais)[5,9,17,18,19,20,22]; e alterações orais (macrostomia, fenda facial lateral, agenesia do ramo da mandíbula, micrognatia, retrognatia, fenda labial e/ou palatina)[5,9,18,19,23,24].

Adicionalmente, foram relatadas alterações esqueléticas, que são variadas, mas podem incluir escoliose, espinha bífida, hemivértebra e anomalias de costelas e vértebras[3,5,18,19,22]; malformações cardíacas (defeitos do septo ventricular e tetralogia de Fallot)[24,11,12,25,27,27,28]; e algumas alterações urogenitais (hipoplasia ou agenesia renal, ectopia renal, atresia vaginal, criptorquidia)[9,18,19,22,24]. Além disso, algumas outras anormalidades já foram identificadas como ânus imperfurado[5] e hipotireoidismo congênito[29].

Finalmente, alterações neurológicas, como atraso no desenvolvimento psicomotor, deficiência mental, autismo e dificuldade de aprendizado também já foram descritas[17,19,30].

A frequência dos sinais clínicos também é variável, entretanto, microssomia hemifacial e microtia são descritas como as características clínicas mais frequentes nos pacientes com EOAV[11,13]. Assim, a diversidade fenotípica entre os pacientes com diagnóstico clínico de EOAV é evidente, tornando clara a dificuldade para estabelecer critérios diagnósticos.

1.2. Alterações citogenéticas e moleculares

A etiologia do EOAV ainda é desconhecida. Devido a sua heterogeneidade e complexidade, sugere-se uma possível etiologia multifatorial[31], envolvendo fatores genéticos, como diferentes anomalias cromossômicas ou gênicas, e fatores ambientais, como medicação vasoativa materna, talidomida e diabetes materno[7,32].

Fatores ambientais que podem originar o EOAV estão relacionados a interrupção do desenvolvimento normal dos primeiros arcos faríngeos, pela falha na migração das células da crista neural, ou a uma lesão vascular, responsável por provocar o subdesenvolvimento de estruturas faciais[33,34].

As causas genéticas associadas ao espectro são variáveis e ainda não estão bem elucidadas. A partir do advento de novas tecnologias, como array-CGH e MLPA, diferentes alterações cromossômicas, tanto numéricas como estruturais, já foram reportadas em pacientes com suspeita clínica. As regiões cromossômicas 22q e 5p parecem ter um alto envolvimento no diagnóstico

molecular dos pacientes com esta condição, sendo a região cromossômica 22q11.1 descrita com mais frequência[35-44]. Entretanto, os resultados apresentados em diversos estudos mostram a existência de uma diversidade de achados citogenéticos em pacientes com EOAV (Tabela 1).

Tabela 1: Descrição de achados citogenéticos em pacientes com diagnóstico clínico de EOAV.

Autor	Alteração citogenética
Ala-Mello *et al.*[38]	Deleção 5p15
	Deleção 21q22.3
	Duplicação 21q22.11-q22.12
Ballesta-Martínez *et al.* [45]	Duplicação 14q23.1
Beleza-Meireles *et al.* [42]	Duplicação Xp11.21
	Duplicação 22q11.21
	Duplicação 22q13.32-33
	Deleção 14q12
	Duplicação 10p15.33
	Deleção 19q13.3
	Duplicação 22q11.1
	Duplicação 22q11.1q11.21
Bragagnolo *et al.* [70]	Duplicação 4p16.1
	Deleção 4p16.3p15.33
	Duplicação Xp22.33p22.31
	Deleção 8q13.3
	Duplicação 8q24.3
	Duplicação 10p14
	Duplicação 10p13
	Deleção 10q26.2q26.3
	Deleção 16p13.3
	Duplicação 16p13.11p12.3
	Duplicação 17q11.2
	Deleção 22q11.21
	Deleção Xp22.33

Brun *et al.*[46]	Microdeleção 15q24.1q24.2
Choong *et al.*[47]	Deleção 5p15
Colovati *et al.*[41]	Deleção 22q11.21
Derbent *et al.*[48]	Microdeleção 22q11.2
Dos Santos *et al.*[40]	Microdeleção 22q11.21
Descartes[37]	46,XX,del(5)(p15.33)
Dyggve *et al.*[49]	Deleção 5p15
Herman *et al.*[35]	46,XY,del (22)(q13.31)
Huang *et al.*[50]	Deleção 5q13.2
	Deleção 5q13.2
Josifova *et al.*[36]	46,XY,der(5)t(5;8) (p15.31;p23.1)
Kobrynski *et al.*[51]	47, XX, þ 22
Northup *et al.*[52]	46,XY,inv(14) (p11.2q22.3)
Puvabanditsin *et al.*[53]	Deleção 7q21.11
Rooryck, *et al.*[54]	47,XXX
	Isodicêntrico Y
	46,XX,t(9;18)(p23;q12)
	Deleção 12p13.33
	Duplicação 18p11.23-p11.31
	Duplicação 20p12.2
	Deleção 14q32.2
	Trissomia X
	Duplicação Yp-q11.221
	Deleção Yq11.222-q12
	Duplicação 8q11.23
	Deleção 2p11.2
	Duplicação 9q34.11
	Duplicação 4q35.1
	Duplicação 13.q13.1

	Deleção 2q11
	Duplicação 11q21
Rooryck, *et al*.[55]	46,XX, t(9;18)(p23;q12)
	Duplicação 18p11.23p11.31
Silva *et al*.[56]	47,XX,+mar mos 47,XX,+mar/46,XX 46,XX,t(6;10)(q13;q24)
Spineli-Silva *et al.*[44]	Deleção distal 22q11.2
Tasse *et al*.[11]	45,X e 47,XXX
Xu *et al*.[39]	Deleção 22q11.21–q11.23.
Zielinski *et al*.[57]	Duplicação 14q22.3

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Além dos achados cromossômicos, alguns genes potencialmente candidatos já foram propostos para o EOAV[42,54]. Lopez *et al*. identificaram o gene *MYT1*, localizado na região 22q11.2, como o primeiro gene envolvido no EOAV[58]. Entretanto, a frequência de mutações envolvendo esse gene permanece baixa[59]. Spinelli-Silva *et al*. encontraram 3 genes, localizados na região 22q11.2, associados ao desenvolvimento inadequado dos arcos faríngeos e doença cardíaca: *YPEL1*, *HIC2* e *MAPK1/ERK2*, sugerindo-os como possíveis genes candidatos[44]. Outros genes, como *BAPX1* e *TBX1* também já foram citados[11,60], mas ambos destacam a importância de mais estudos de *loci* candidatos e mecanismos moleculares associados ao desenvolvimento craniofacial. Ainda assim, até o momento nenhum gene foi confirmado como causador do fenótipo[40], mas com envolvimento nas manifestações fenotípicas.

1.3. Estratégias de investigação genética

Dada a ampla variabilidade de alterações cromossômicas e moleculares descritas em pacientes com EOAV, diferentes metodologias, como cariótipo, hibridização *in situ* fluorescente (FISH), *multiplex ligation-dependent probe amplification* (MLPA) e a hibridização genômica comparativa por microarranjos (Array-CGH) são utilizadas para realizar a investigação de alterações genéticas e auxiliar no diagnóstico clínico. A maioria dos pesquisadores opta por utilizar mais de uma metodologia para definir as alterações e corroborar com o diagnóstico.

A investigação por cariótipo com bandeamento G, técnica de citogenética clássica, é o padrão-ouro para detectar alterações cromossômicas[61]. Com capacidade de identificar rearranjos maiores que 5 Mb como inversões, inserções, translocações, deleções e duplicações, é a primeira escolha na suspeita de síndromes genéticas. Entretanto, alterações cromossômicas que afetam segmentos menores que 5 Mb, podem não ser detectadas, impossibilitando a identificação de regiões e genes envolvidos.

A técnica de FISH permite identificar rearranjos cromossômicos pequenos (200 kb-5 Mb) ou complexos, através da hibridização do DNA alvo com sondas fluorescentes[62]. O resultado pode ser obtido em até 48 horas, entretanto, sua capacidade de detecção é limitada pelo conjunto de sondas escolhidas, que são lócus específica, pode depender de cultura celular e não é capaz de detectar pequenos rearranjos em genes únicos[63,64].

A MLPA, técnica descrita por Schouten *et al*., é um ensaio multiplex que utiliza sondas específicas para determinadas sequências de DNA com o objetivo de avaliar variações no número de cópias[65]. As *copy number variations* (CNVs) são definidas como alterações genômicas envolvendo ganhos ou

perdas de um trecho de DNA, podem envolver múltiplos, um ou nenhum gene[66], e podem ser polimorfismos ou compatíveis com microdeleções ou microduplicações. Possibilita mapear várias regiões do genoma em um único ensaio, possui facilidade operacional, porém não detecta rearranjos balanceados e pequenos mosaicismos[67].

O Array-CGH permite uma análise do genoma inteiro em alta resolução. Utiliza simultaneamente o DNA de referência hibridizado com a matriz do DNA alvo, detectando até mesmo rearranjos desbalanceados e variações desconhecidas[66,69]. É uma técnica com grande aplicabilidade na triagem genômica, contudo, o custo elevado torna-a uma metodologia pouco utilizada no Brasil à nível diagnóstico.

A estratégia de investigação genética deve considerar, de forma soberana, os aspectos clínicos presentes no paciente. Um exemplo é a presença de malformações cardíacas. Alguns autores recomendam que pacientes com uma expressão fenotípica similar à do EOAV, associada a anormalidades cardíacas (especialmente defeitos conotruncais e anomalias do arco aórtico), devem ser testados para microdeleção da região 22q11 por meio de técnicas como o FISH, MLPA ou Array-CGH[25,48,70], considerando a elevada relação entre cardiopatia congênita e deleção 22q11.

O diagnóstico molecular é de grande importância para a compreensão da etiologia da condição, e pode auxiliar na definição da conduta clínica e no correto suporte e tratamento de portadores desse espectro. Além disso, também contribui com o planejamento familiar, pois através do aconselhamento genético adequado, é possível identificar os riscos relacionados à própria condição e os riscos de recorrência em outras gestações.

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3. OBJETIVOS

GERAL:

Identificar genes envolvidos no desenvolvimento das características fenotípicas do espectro óculo-aurículo-vertebral (EOAV) e propor possíveis genes candidatos.

ESPECÍFICOS:

a) Comparar a aplicabilidade de diferentes técnicas de diagnóstico citogenético molecular em pacientes com fenótipo de EOAV.
b) Identificar os achados citogenéticos já descritos e sua relação com o fenótipo dos pacientes com diagnóstico clínico de EOAV.

4. ARTIGOS CIENTÍFICOS

4.1 "*Candidate genes of oculo-auriculo-vertebral spectrum in 22q region: A systematic review"*

"Candidate genes of oculo-auriculo-vertebral spectrum in 22q region: A systematic review"

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ORIGINAL ARTICLE

Candidate genes of oculo-auriculo-vertebral spectrum in 22q region: A systematic review

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ABSTRACT

Oculo-auriculo-vertebral spectrum (hemifacial microsomia/OAVS, OMIM #164210) is a heterogenous and congenital condition caused by a morphogenesis defect of the first and second pharyngeal arches. Etiology includes unknown genetic, environmental factors and chromosomal alterations, which 22q11.2 region is the most frequently reported. Several candidate genes for OAVS have been proposed; however, none has been confirmed as causative of the phenotype. This review aims to sum up all clinical and molecular findings in 22q region of individuals diagnosed with OAVS and to investigate genes that may be involved in the development of the spectrum. A search was performed in PubMed using all entry terms to OAVS and Chromosome 22q11. After screening, 11 papers were eligible for review. Deletions and duplications in the q11.2 region were the most frequent (18/22) alterations reported and a total of 68 genes were described. Our systematic review reinforces the hypothesis that 22q11 region is a candidate locus for OAVS as well as *CLTCL1*, *GSC2*, *HIRA*, *MAPK1*, *TBX1*, and *YPEL1* as potential candidates genes for genotype–phenotype correlation. Complementary studies regarding genes interaction involved in the 22q11 region are still necessary in the search for a genotype–phenotype association, since the diagnosis of OAVS is a constant medical challenge.

KEYWORDS

22q11.2, candidate genes, hemifacial microsomia, oculo-auriculo-vertebral spectrum, systematic review

1 | INTRODUCTION

Oculo-auriculo-vertebral spectrum (OAVS) also known as Goldenhar syndrome or hemifacial microsomia (HMF/OAVS, OMIM #164210) is a congenital condition that leads to a wide variety of malformations. The cause is uncertain, but the most accepted theory is that this spectrum is caused by a morphogenesis defect of the first and second pharyngeal arches during the first 6 weeks of pregnancy (Spineli-Silva, Bispo, Gil-da-Silva-Lopes, & Vieira, 2018). This phenotype is characterized by a broad and heterogeneous spectrum of clinical features, mainly involving ears, mouth, mandible, eyes, and cervical spine (Colovati et al., 2015; Sharma & Passi, 2013). In addition, external environmental factors (vasoactive medications), maternal intrinsic factors (maternal diabetes), genetic factors (gene mutations), and chromosomal alterations may also lead to the development of this disorder (Chen, Zhao, Shen, & Dai, 2018; Hartsfield, 2007; Renkema et al., 2017).

The etiology of OAVS is still unknown, but predicted to be multifactorial, probably comprising variation in multiple genes and environmental factors. Due to the difficulty to establish an exact genetic

cause for this spectrum, the current diagnosis is based in the observation of clinical characteristics of the patient, pregnancy history, with information about twinning, placental disease, medications, drugs or retinoid acid intaken by mother, in vitro fertilization, intrauterine growth restriction, and the exclusion of differential diagnoses, which can be tested for (Chen et al., 2018; Renkema et al., 2017). Recently, descriptions of severe chromosomal rearrangements have been published in individuals with OAVS. However, its molecular basis is still unclear (Beleza-Meireles, Clayton-Smith, Saraiva, & Tassabehji, 2014). Despite the unclear etiology of OAVS, some studies have already detected loci candidates through linkage studies (Beleza-Meireles et al., 2015; Kelberman et al., 2001) and epigenetic inheritance (Fischer et al., 2006). Among chromosomal abnormalities, chromosome region 22q11.2 is the most frequently reported (Beleza-Meireles et al., 2015; Colovati et al., 2015; Derbent et al., 2003; dos Santos et al., 2014; Lafay-Cousin et al., 2009; Spineli-Silva et al., 2018; Tan et al., 2011; Torti, Braddock, Bernreuter, & Batanian, 2013; Xu, Fan, & Siu, 2008). Low copy repeats (LCRs) in 22q11.2 region have been directly implicated in its chromosomal rearrangements. Those small DNA sequences can lead to a genomic instability, mediate nonallelic homologous recombination (NAHR) or stimulate the occurrence of copy number variation that may result in the deletion or duplication of a genomic segment (Colovati et al., 2015; Stankiewicz & Lupski, 2010).

Several candidate genes for OAVS have been proposed; however, none of them has been confirmed as causative of the phenotype (dos Santos et al., 2014). This review aims to sum up all clinical and molecular findings described in 22q region of individuals diagnosed with OAVS, as well as to investigate genes that may be involved in the development of the spectrum's phenotypic characteristics.

2 | METHODS

We performed a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews. The literature search was conducted by searching PubMed, including articles published from 2000 to 2020 with the terms Goldenhar syndrome, OAVS, craniofacial microsomia, HMF, OAVS, Chromosome 22, 22q11. Two independently reviewers screened titles and abstracts. First screening should include OAVS (or any synonymous names) as the main object of the study together with a Chromosome 22 alteration. Letters that were automatically full-text read due to the lack of studies were an exception. Full-text assessment should describe the alteration found on Chromosome 22 by molecular-cytogenetic analysis and also suggest a relationship between molecular findings and OAVS. Abstracts that did not provide enough information to be included or excluded were full-text read. All duplicated articles were removed and discordant selection cases were resolved by consensus. Furthermore, we excluded papers that were not available either in English or Portuguese. After studies selection, the patients' data were extracted directly from the studies and plotted in an Excel spreadsheet. The process for screening and selecting articles for inclusion is provided in Figure 1.

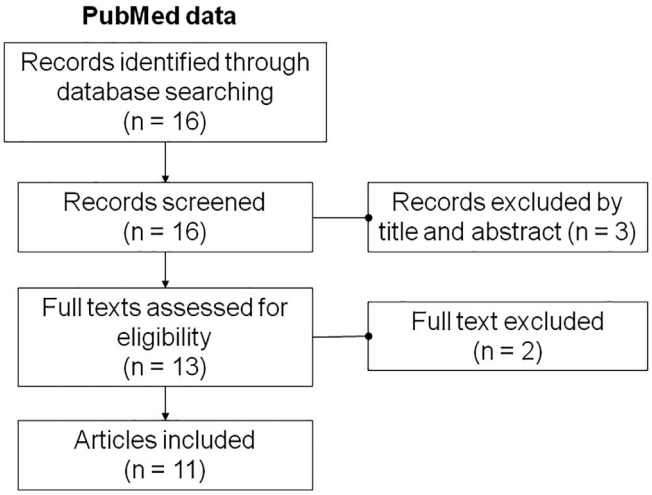

FIGURE 1 Flow diagram of article inclusion

3 | RESULTS

3.1 | Selected information

The database search resulted in 17 papers. After screening, 11 papers were eligible for extensive review. Year of publication ranged from 2003 to 2018 and the studies categories included original article (*n* = 2), case reports (*n* = 5), short report (*n* = 1), and letter (*n* = 3).

3.2 | Clinical characterization of 22 OAVS individuals

Overall, 22 individuals ranging in age from 35 days to 18 years (9 females and 13 males), presenting clinical findings compatible with OAVS and Chromosome 22 abnormalities were described. Four patients showed prenatal risk factors such as gestational diabetes, use of abortive substances and infections. Among the malformations described, ear alterations (preauricular tags, hearing loss and agenesis and atresia of external auditory canal), HMF and congenital heart disease (CHD) were the main clinical features that lead to OAVS investigation (Table 1).

3.3 | Molecular findings

Deletions and duplications in the q11.2 region were the most frequent (18/22) among all molecular alterations involving Chromosome 22 (Table 2). Then, 45 protein-coding genes, 16 pseudogenes, and 7 RNA or noncoding RNA genes were described within this region. Most frequent genes involved in the deletion were *HIC2* (OMIM *607712), *PPIL2* (OMIM *607588), *TOP3B* (OMIM *603582) (Spineli-Silva et al., 2018;Torti et al., 2013; Xu et al., 2008), *YPEL1* (OMIM *608082), *MAPK1* (OMIM *176948) (Tan et al., 2011; Torti et al., 2013; Xu et al., 2008), *UBE2L3* (OMIM *603721), *PRAME*

TABLE 1 Main clinical features of our sample

Main clinical features	Frequency (%)	Reference
HMF	72.7 (16/22)	Beleza-Meireles et al. (2015); Colovati et al. (2015); Digilio et al. (2009); dos Santos et al. (2014); Lafay-Cousin et al. (2009); Tan et al. (2011).
Microtia	36.3% (8/22)	Beleza-Meireles et al. (2015); Colovati et al. (2015); Derbent et al. (2003); Digilio et al. (2009).
Cleft lip, cleft palate, and facial cleft	27.2% (6/22)	Colovati et al. (2015); Digilio et al. (2009); Spineli-Silva et al. (2018); Torti et al. (2013); Xu et al. (2008).
Macrostomia	4.5% (1/22)	Xu et al. (2008).
Malar and mandibular hypoplasia/ asymmetria	45.4% (10/22)	Beleza-Meireles et al. (2015); Derbent et al. (2003); Digilio et al. (2009); Spineli-Silva et al. (2018); Torti et al. (2013).
Preauricular tags	54.5% (12/22)	Beleza-Meireles et al. (2015); Digilio et al. (2009); Lafay-Cousin et al. (2009); Spineli-Silva et al. (2018); Tan et al., 2011; Xu et al. (2008).
Agenesis and atresia of external auditory canal	50% (11/22)	Beleza-Meireles et al. (2015); Colovati et al. (2015); Derbent et al. (2003); Digilio et al. (2009); Torti et al. (2013); Xu et al. (2008).
Epibulbar dermoid	18.8% (4/22)	Beleza-Meireles et al. (2015); Tan et al. (2011); Lafay-Cousin et al. (2009).
Hearing loss	50% (11/22)	Beleza-Meireles et al. (2015); Colovati et al. (2015); Digilio et al. (2009); Tan et al., 2011; Torti et al. (2013).
Vertebral dysmorphia	31.8% (7/22)	Beleza-Meireles et al. (2015); Colovati et al. (2015); Derbent et al. (2003); Digilio et al. (2009); Torti et al. (2013).
Congenital heart disease	60% (13/22)	Beleza-Meireles et al. (2015); Derbent et al. (2003); Digilio et al. (2009); Spineli-Silva et al. (2018); Xu et al. (2008).
Developmental delay	22.7% (5/22)	Balcı and Engiz (2011); Beleza-Meireles et al. (2015); dos Santos et al. (2014); Lafay-Cousin et al. (2009); Spineli-Silva et al. (2018).

Abbreviation: HMF, hemifacial microsomia.

(OMIM *606021) (Torti et al., 2013; Xu et al., 2008), histone cell cycle regulator (*HIRA*; OMIM *600237) (Derbent et al., 2003), and *CLTCL1* (OMIM *601273) (Digilio et al., 2009).

Duplications in the 22q11.2 region were identified in 32% of the patients (07/22), with *GGT2* (OMIM *137181) as the most described gene (Beleza-Meireles et al., 2015). Duplication and deletion of 22q were evidenced concomitantly in an individual, although in distinct regions (q11.1 and q11.2, respectively), involving the following genes: *IL17RA* (OMIM *605461), *CECR1* (OMIM *607575), *CECR2* (OMIM *607576), *SLC25A18* (OMIM *609303), *ATP6V1E1* (OMIM *108746), *IDB*, *MICAL3* (*OMIM *608882*), *PEX26* (OMIM *608666), *TUBA8* (OMIM *605742), *USP18* (OMIM *607057), *HIC2*, *RIMBP3B* (OMIM *612700), *RIMBP3C* (OMIM *612701), *UBE2L3*, *SDF2L1* (OMIM *607551), *PPIL2*, *YPEL1*, *MAPK1*, *TOP3B*, *VPREB1* (OMIM *605141), *PRAME*, *GGTLC2* (OMIM *612339), and *RTDR1* (OMIM *605663) (Torti et al., 2013). A duplication in 22q13.3 region (*FAM19A5*, OMIM *617499) was described in a patient who also had a duplication in 22q11.2 region (*USP18*, *DGCR6*, *GGT2*) (Beleza-Meireles et al., 2015). A third finding was a derivative chromosome (47,XX + der 22t(11;22)(q23;q11)); however, involved genes were not informed (Balcı & Engiz, 2011).

Additionally, there were genes involved in both deletions and duplications in 22q11, as *RIMBP3* (OMIM *612699) (Beleza-Meireles et al., 2015), as well as its paralogous *RIMBP3B* and *RIMBP3C* (Torti et al., 2013), and *TBX1* (OMIM *602054) (Beleza-Meireles et al., 2015; dos Santos et al., 2014).

4 | DISCUSSION

OAVS is probably a group of heterogeneous disorders with the genetic etiology is still unknown.

Chromosomal alterations have been reported in several cases and regions located at 22q were the main findings described in OAVS individuals. In our review, we observed that deletions and duplications in regions 22q11.1 (Beleza-Meireles et al., 2015) and 22q11.2 (Beleza-Meireles et al., 2015; Bragagnolo et al., 2018; Colovati et al., 2015; dos Santos et al., 2014; Spineli-Silva et al., 2018; Xu et al., 2008) were the main findings in individuals with the phenotype. However, these regions comprise a lot of genes with unknown function as many pseudogenes.

The 22q11 region has eight known LCR that contain genes, pseudogenes, and other genomic sequences that are 94–99% identical both individually (within each LCR22) and between them (McDermid & Morrow, 2002). The similarity in their sequences allows the occurrence of NAHRs, a rearrangement mechanism that explains clustered breakpoints and recurrent rearrangements, such as de novo alterations (Ben-Shachar et al., 2008; Shaikh et al., 2000). Thus, the frequent description of deleterious mutations in the 22q11 region of individuals diagnosed with OAVS becomes understandable. However, the relationship between genes within this region and clinical findings remains unclear.

All cases included in this review shared some clinical features. However, phenotypic variability hinders to establish a standard

TABLE 2 Molecular findings and genes involved

Reference	Technique	Molecular findings	Genes involved
P3 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.2**	*RIMBP3*
P7 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.2**	***USP18***, ***GGT3P***, and ***DGCR6***
		Dup 22q11.2	*POM121L7*, ***GGT2***, ***BCRP2***, *KB-1592A4.15*, *KB-2A4.13*, and *FAM230B*
		Dup 22q13.3	*MIR4535* and *FAM19A5*
P10 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.2**	*AK129567*, *AK302545*, and ***GGT3P***
		Dup 22q11.2	*POM121L7*, ***GGT2***, ***BCRP2***, *KB-1592A4.15*, *KB-2A4.13*, and *FAM230B*
P11 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.2**	***USP18***, *AK129567*, *AK302545*, and ***GGT3P***
		Dup 22q11.2	*POM121L7*, ***GGT2***, ***BCRP2***, *KB-1592A4.15*, *KB-2A4.13*, and *FAM230B*
P14 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.2**	*POM121L7*, ***GGT2***, ***BCRP2***, *KB-1592A4.15*, *KB-2A4.13*, and *FAM230B*
P15 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.1**	*CCT8L2*, *FABP5P11*, ***TPTEP1***, *SLC25A15P5*, ***PARP4P3***, *ANKRD62P1-PARP4P3*, *ANKRD62P1*, ***VWFP1***, and ***XKR3***
P16 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.2**	*POM121L7*, ***GGT2***, ***BCRP2***, *KB-1592A4.15*, *KB-2A4.13*, *FAM230B*, *KB-1592A4.14*, *KB-1183D5.9*, *POM121L8P*, ***BCRP6***
		Dup 22q11.1	*CCT8L2*, *FABP5P11*, ***TPTEP1***, *SLC25A15P5*, ***PARP4P3***, *ANKRD62P1-PARP4P3*, *ANKRD62P1*, ***VWFP1***, ***XKR3***
P17 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.1**	*CCT8L2*, ***XKR3***, *FABP5P11*, ***TPTEP1***, *SLC25A15P5*, ***PARP4P3***, *ANKRD62P1-PARP4P3*, *ANKRD62P1*, ***VWFP1***
P18 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.21**	*POM121L7*, ***GGT2***, ***BCRP2***, *KB-1592A4.15*, *KB-2A4.13*, *FAM230B*, *KB-1592A4.14*, *KB-1183D5.9*, *POM121L8P*, ***BCRP6***
P51 Beleza-Meireles et al. (2015)	Array-CGH	**Dup 22q11.1**	***TBX1***+
P1 Colovati et al. (2015)	MLPA and array-CGH	**Del 22q11.2**	*ZNF74*, *KLHL22*, *MED15*, *SNAP29*, and ***LZTR1***
P7 Derbent et al. (2003)	FISH	**Del 22q11.2**	***HIRA***
P1 Digilio et al. (2009)	FISH	**Del 22q11.2**	*CLTCL1*
P2 Digilio et al. (2009)	FISH	**Del 22q11.2**	*CLTCL1*
P3 Digilio et al. (2009)	FISH	**Del 22q11.2**	*CLTCL1*
P1 dos Santos et al. (2014)	Array-CGH	**Del 22q11.2**	***GSC2***, ***TBX1***, ***SETP5***
P1 Lafay-Cousin et al. (2009)	FISH, MLPA, and array-CGH	**Del 22q11.2**	***LZTR1***, *SNRPD3*, *HIC2*, *TOP3B*, *MAPK1*, *YPEL1*, *PPIL2*, *GGT2*, *UBE2L3*, *PRAME*
P1 Spineli-Silva et al. (2018)	MLPA and array-CGH	**Del 22q11.2**	***HCI2***, ***PPIL2***, and ***TOP3B***
P2 Tan et al. (2011)	Array-CGH	**Distal Del 22q11.2**	*HIC2*, *TOP3B*, *MAPK1*, *YPEL1*, *PPIL2*, *GGT2*, *UBE2L3*, *PRAME*
P1 Torti et al. (2013)	Array-CGH	**Dup 22q11.1**	*IL17RA*, *CECR1*, *CECR2*, *SLC25A18*, *ATP6V1E1*, *BID*, *MICAL3*, *PEX26*, *TUBA8*, and ***USP18***
		Del 22q11.2	***HIC2***, *RIMBP3B*, *RIMBP3C*, ***UBE2L3***, *SDF2L1*, *MIR130B*, ***PPIL2***, ***YPEL1***, ***MAPK1***, ***TOP3B***, *VPREB1*, ***PRAME***, *GGTLC2*, and *RTDR1*
P1 Xu et al. (2008)	Array-CGH	**Del 22q11.2**	***HCI2***, *LOC220686*, ***UBE2L3***, *LOC150223*, *CCDC116 SDF2L1*, ***PPIL2***, ***YPEL1***, ***MAPK1***, *PPM1F*, ***TOP3B***, *VPREB1 LOC96610*, *SUHW2*, *SUHW1*, ***PRAME***

Note: The values marked in bold are qualitative data.
Abbreviation: FISH, fluorescence in situ hybridization.

diagnosis. Based on both this review and literature, we suggest that the minimum diagnostic criteria for OAVS in individuals with 22q11 abnormalities should include HMF and auricular alterations. Auricular alterations (preauricular tags, hearing loss, and/or agenesis/atresia of external auditory canal) were described in all individuals included in this review.

CHDs are also a frequent feature described in OAVS. In literature, the percentage of individuals with 22q11.2 deletion that have CHD is 75–80% (Azuma et al., 2015). In this review, CHD was found in 60% of the individuals diagnosed with OAVS that had a deletion and/or duplication in the 22q11 region. Therefore, the presence of any congenital heart malformation could also aid in OAVS investigation.

A total of 68 genes, pseudogenes and RNA genes were listed within all studies. A few genes were altered in individuals who share more than one malformation: HMF, auricular alterations and/or CHD. In duplicate regions, the most evidenced protein-coding genes were *GGT2* (dup22q11.2), *XKR3* (dup22q11.1), and *CCT8L2* (dup22q11.1). However, there are no descriptions in the literature about the association of these genes with HMF or OAVS phenotype.

Deleted regions (del22q11.2) in individuals diagnosed with OAVS had *MAPK1*, *YPEL1*, *HIC2*, *TOP3B*, *PRAME*, *UBE2L3*, *PPIL2*, *HIRA*, and *CLTCL1* as the most reported protein-coding genes. Tan et al. (2011) and Lafay-Cousin et al. (2009) identified two patients with deletions in the same 22q11.2 region involving LCR22-4 to LCR22-7, but they did not describe the genes involved. However, in order to have the information about which genes were comprised in the LCRs, we accessed UCSC Genome Browser assembly ID: hg38 (http://genome.ucsc.edu/). Then, we were able to list the main deleted genes common to both patients: *GGT2*, *MAPK1*, *YPEL1*, *HIC2*, *TOP3B*, *PRAME*, *UBE2L3*, and *PPIL2*.

MAPK1 and *YPEL1* play regulatory functions in cellular processes such as cell division, proliferation, differentiation, and development. Studies with animal model showed that both genes have been found to be possible candidates for the genotype–phenotype relationship with OAVS. *MAPK1* was considered to cause craniofacial and cardiac defects when inactivated at the developing neural crest, while *YPEL1* inactivation resulted in craniofacial cartilage defects and mandibular underdevelopment (Aerts et al., 2006; Newbern et al., 2008). Individuals with a 22q11.2 microdeletion of approximately 1 Mb that also presents a *MAPK1* haploinsufficiency may feature conotruncal and craniofacial anomalies due to neural crest misdevelopment. The clinical characteristics observed in this type of genetic alteration are often similar to the ones described in DiGeorge syndrome spectrum, which is often related to OAVS (Derbent et al., 2003; Digilio et al., 2009). In addition, maternal allele variants of *YPEL1*, when with incomplete penetration trait associated with genetic and/or environmental factors, could lead to OAVS phenotype. Clinical features have not been associated with neither *MAPK1* nor *YPEL1* (Zamariolli et al., 2019). Within the cases included in our review, both genes were always simultaneously deleted when cited (dos Santos et al., 2014; Spineli-Silva et al., 2018; Xu et al., 2008). However, no correlation and/or association between the two genes were found. Presence of craniofacial, auricular, and cardiac malformations in individuals with deletion in *MAPK1* and *YPEL1* may suggest an important role and strong involvement of these genes in the OAVS phenotype.

HIC2 is a transcription factor related to *HIC1* tumor suppressors, which are required for the normal cardiac development (Deltour, Pinte, Guérardel, & Leprince, 2001). The consequences of *HIC2* deletion in individuals with OAVS are still nuclear but this gene could be responsible for specific cardiac malformations when simultaneously deleted with other genes.

TOP3B has already been associated with cognitive impairment and facial dysmorphism in a patient with a minor 22q11.2 deletion (Kaufman, Genovese, & Butler, 2016). The role of *TOP3B* is often described in patients with neurological developmental delay (O'Roak et al., 2012; Stoll et al., 2013). In our review, 40% of the patients with deletion in *TOP3B* presented some developmental delay (Lafay-Cousin et al., 2009; Spineli-Silva et al., 2018). Therefore, *TOP3B* may be a possible gene candidate for OAVS phenotype, especially in cases of cognitive impairment.

PRAME, *UBE2L3*, and *PPIL2* were also found deleted in a 22q11.2 region near well-known functional genes (Lafay-Cousin et al., 2009; Tan et al., 2011; Torti et al., 2013; Xu et al., 2008). Although these genes may be acting through genetic interactions, their function in embryogenesis is still unknown. Therefore, there is no evidence that alterations in *PRAME*, *UBE2L3*, and *PPIL2* are associated with the etiology of OAVS.

Derbent et al. (2003) and Digilio et al. (2009) used TUPLE 1 and N25 probes to identify, respectively, *HIRA* and *CLTCL1* deletions through fluorescence in situ hybridization. *HIRA*, also known as *DGCR1* or *TUPLE1*, is considered the main gene for normal embryonic development and the gold standard marker for DiGeorge Syndrome diagnosis. *HIRA* probably mediates irreversible alterations in the senescent cell cellular cycle (Halford et al., 1993). In animals (mice and chickens), *HIRA* is detected at the neural crest, pharyngeal arches and heart (Gunjan, Paik, & Verreault, 2005).

CLTCL1 is a member of the family of heavy chains of clathrins and plays an essential role in the neural crest development, which is an important component for the morphogenesis of pharyngeal arches (Nahorski et al., 2015). Chromosomal alterations involving *CLTCL1* are also associated with DiGeorge syndrome, velo-cardio-facial syndrome (Long, Trofatter, Ramesh, McCormick, & Buckler, 1996), Down syndrome and cardiac malformations, mainly in typical 3 Mb deletion of LCR22A-LCR22D, a region extensively studied in DiGeorge syndrome (Hou et al., 2020).

Digilio et al. (2009) reported a patient with a 22q11.2 microdeletion in the region of DiGeorge syndrome. This deletion may lead to a phenotype similar to OAVS, which indicates a possible regulatory mechanism in the etiology of the spectrum. In addition, genes mapped in the region 22q11.2 involved in the development of neural crest cells and branchial arches would also be affected. dos Santos et al. (2014) hypothesized an altered genetic nuclear mechanism in this microdeletion carrier. Since nonoverlapping 22q11.2 deletions cause similar phenotypes, a possible regulatory mechanism acting on genes located in the 22q11.2 region and on neural crest cell development was proposed. All individuals that were tested for deletions in

CLTCL1 and *HIRA* had craniofacial malformations, auricular alterations, and CHD. Deletions of *CLTCL1* and *HIRA*, given their important roles in neurological and cardiac development, may be associated with the main clinical characteristics of individuals diagnosed with OAVS. Thus, both genes are strong candidates for genotype–phenotype association in OAVS.

TBX1 was duplicated in one individual and deleted in another (Beleza-Meireles et al., 2015; dos Santos et al., 2014). *TBX1* deficiency in mice caused distinct vascular and cardiac malformations and severe inner ear defects (Vitelli et al., 2003; Vitelli, Morishima, Taddei, Lindsay, & Baldini, 2002). The deletion found in the patient described by dos Santos et al. (2014) also involved *GSC2*. This gene is associated with velocardiofacial syndrome and is homologous to *GSC* (14q32), a gene that plays an important role in branchial arches development. A linkage study proposed that *GSC* could be a candidate gene to explain OAVS phenotype (Kelberman et al., 2001), however a deletion involving *GSC2* was described only in one patient in this review. *TBX1* and *GSC2* are considered candidate genes for the OAVS phenotype due to associations described in the literature.

Asymmetry is a clinical finding commonly described in individuals diagnosed with OAVS. Its severity is variable and affects mainly eyes, ears, and face. The etiology of this phenotype may be associated with genetic and/or environmental factors. Genetics mechanisms that may influence the asymmetric nature of OAVS comprise regulatory and nonregulatory variants as well as topologically associating domain disruption. Candidate genes suggested in this systematic review (*CLTCL1*, *GSC2*, *HIRA*, *MAPK1*, and *TBX1*) are involved in different pathways that could have an important role in the asymmetric nature of OAVS etiology (Figure 2). *YPEL1* still does not have described pathways with evident involvement in OAVS asymmetry. Although some

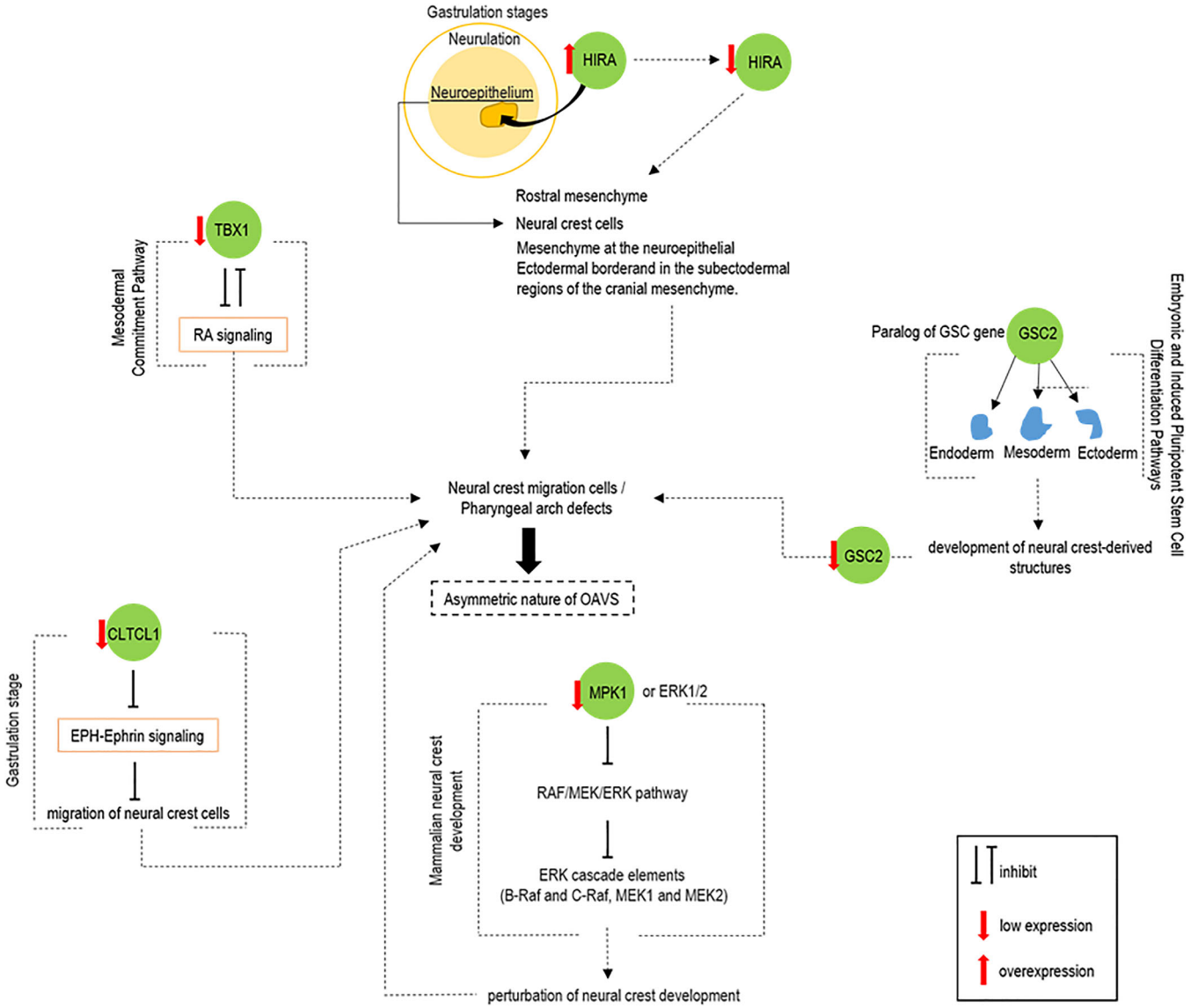

FIGURE 2 Possible pathways involved and mechanisms for oculo-auriculo-vertebral spectrum (OAVS) asymmetry [Color figure can be viewed at wileyonlinelibrary.com]

cause-consequence relationships have been hypothesized, more studies on this matter are necessary to elucidate the proper mechanism and its consequences.

Other genes as *ZNF74*, *KLHL22*, *MED15*, *SNAP29*, *LZTR1*, *RIMBP3*, *RIMBP3B*, *RIMBP3C*, *USP18*, *FAM19A5*, *IL17RA*, *CECR1*, *CECR2*, *SLC25A18*, *ATP6V1E1*, *BID*, *MICAL3*, *SDEX*, *TEXP*, *RTDR1*, *SNRPD3*, *LOC220686*, *LOC150223*, *CCDC116*, *PPM1F*, *SUHW1*, *SUHW2*, *DGCR6*, and *DGCR8* were also described in deleted/duplicated individuals diagnosed with OAVS, but in a lowest frequency. Future research is necessary to elucidate their functionality as well as their genetic interactions. Since a lot of aspects about the genetic background of OAVS is still unknown, these genes should not be ignored while performing a complete investigation of OAVS. It is possible that other genes may still be potential candidates to explain the genotype–phenotype relationship of OAVS as this study focuses only on the ones described in the studies included. For future studies, a genome-wide approach or whole genome sequencing may be important to identify other genes involved in OAVS etiology.

In conclusion, our systematic review reinforces the hypothesis that the 22q11 genomic region is a candidate locus for OAVS as well as *CLTCL1*, *GSC2*, *HIRA*, *MAPK1*, *TBX1*, and *YPEL1* as potential candidates genes for genotype–phenotype correlation. In addition, the authors suggest the possibility of investigating the 22q11 region in patients with the OAVS phenotype. Additional and complementary studies regarding genes interaction involved in the 22q11 region are still necessary in the search for a genotype–phenotype association, since the diagnosis of OAVS is a constant medical challenge.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Andressa Barreto Glaeser, Andressa Schneiders Santos, Bruna Lixinski Diniz, and Desireé Deconte made substantial contributions to the conception or design of the study; the acquisition, analysis, and interpretation of data for the study; drafting the article; and revising it critically for important intellectual content. Rafael Fabiano Machado Rosa and Paulo Ricardo Gazzola Zen revised the article critically for important intellectual content, and all authors approved the version to be published and agree 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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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4.2 *"Microarray-Based Comparative Genomic Hybridization, Multiplex Ligation-Dependent Probe Amplification, and High-Resolution Karyotype for Differential Diagnosis Oculoauriculovertebral Spectrum: A Systematic Review"*

"Microarray-Based Comparative Genomic Hybridization, Multiplex Ligation-Dependent Probe Amplification, and High-Resolution Karyotype for Differential Diagnosis Oculoauriculovertebral Spectrum: A Systematic Review"

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Microarray-Based Comparative Genomic Hybridization, Multiplex Ligation-Dependent Probe Amplification, and High-Resolution Karyotype for Differential Diagnosis Oculoauriculovertebral Spectrum: A Systematic Review

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Abstract

Oculoauriculovertebral spectrum (OAVS) is a rare class of heterogenous congenital craniofacial malformation conditions of unknown etiology. Although classic OAVS has been described as hemifacial microsomia with facial asymmetry and microtia, there is no consensus regarding clinical criteria for diagnosis or genetic cause. This systematic review aims to assess the applicability of high-resolution (HR) karyotype, fluorescence in situ hybridization, multiplex ligation-dependent probe amplification (MLPA), and microarray-based comparative genomic hybridization (array-CGH) for differential diagnosis of OAVS. A search was performed in PubMed and Web of Science using all entry terms to the following descriptors: Goldenhar's syndrome, cytogenetic analysis, hybridization in situ, fluorescent, comparative genomic hybridization, multiplex polymerase chain reaction, whole genome sequencing, and karyotype analysis methods. After screening, 25 articles met eligibility. Of the included studies, 59 individuals had a genetic alteration identified. Array-CGH, MLPA, and HR karyotype appear to be viable approaches for molecular diagnosis in OAVS. Heterogeneity is a hallmark of OAVS. Establishing an enhanced framework for diagnosis would inform clinical decision making, and better resource utilization could improve health care facility efficiency and economy.

Keywords
- Goldenhar's syndrome
- OAVS
- comparative genomic hybridization

Introduction

Oculoauriculovertebral spectrum (OAVS; OMIM 164210), including Goldenhar's syndrome and hemifacial microsomia, is a rare class of heterogenous craniofacial conditions of unknown etiology characterized by craniofacial malformations derived from the first and second branchial arches during embryonic development.[1] Numerous studies highlighted the clinical and genetic heterogeneity of OAVS,[2,3] and unsurprisingly, the birth prevalence for OAVS is inconsistent and ranges between 1:5,600 and 1:45,000.[4,5] Positive family history has been suggested to be of diagnostic value.[6] Although classic OAVS has been described as hemifacial microsomia with facial

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asymmetry and microtia, there is no consensus regarding clinical criteria for diagnosis[7] or genetic cause.[8] Several genomic and chromosomal alterations have been reported in individuals with clinical diagnosis for OAVS,[6,9–11] and there is no gold standard modality for molecular diagnosis in OAVS.

Heterogeneity is a hallmark of OAVS. Establishing an enhanced framework for diagnosis would inform clinical decision making, and better resource utilization could improve health care facility efficiency and economy. This systematic review aims to assess the applicability of high-resolution (HR) karyotype, fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), and microarray-based comparative genomic hybridization (array-CGH) for differential diagnosis of OAVS.

Methods

The question addressed by the systematic review was: for patients with clinical OAVS, is cytogenetic diagnostic testing with HR karyotype, FISH, MLPA, or array-CGH reliable to confirm diagnosis? The hypothesis was that cytogenetic testing would improve diagnosis. A systematic search of the literature was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. A search was performed in PubMed and Web of Science using all entry terms to the following descriptors: Goldenhar's syndrome, cytogenetic analysis, hybridization in situ, fluorescent, comparative genomic hybridization, multiplex polymerase chain reaction, whole genome sequencing, and karyotype analysis methods. All articles up until December 31, 2019, were selected for screening. Three reviewers independently performed article selection, and the results were stored in the authors' spreadsheet. The inclusion criteria were as follows: (1) OAVS or a synonym as the main object of the study and (2) OAVS patients with cytogenetic tests. The screening process consisted of three phases. In phase 1, articles were screened by title for inclusion of OAVS or a synonym as the main object of the study, and articles with OAVS or a synonym in the title were screened in the second phase for inclusion of cytogenetic technique in the abstract. The final phase consisted of full-text assessment for results of the cytogenetic testing and the relationship between molecular findings and OAVS. Any discrepancies were resolved by discussion until consensus was reached.

A total of 86 articles were assessed. After duplicate removal and according to the inclusion and exclusion criteria, full-text articles were assessed for eligibility, and data from 25 articles were included (►**Fig. 1**). The following variables were collected from each article: clinical findings, clinical criteria, genetic techniques performed, and molecular findings.

Results

Articles Screened and Reviewed

After screening, 25 articles published between 1988 and 2019 met inclusion criteria. Article types included: original article ($n = 11$), case reports ($n = 9$), short report ($n = 3$), and letter ($n = 2$) (►**Table 1**).

OAVS Clinical Criteria and Range of Clinical Findings

Among the screened articles, 41% did not cite a reference for OAVS clinical diagnostic criteria; 28% cite Tasse et al's criteria[7]; and 31% cite other clinical criteria. As expected, a wide range of craniofacial and other clinical features were described (►**Table 2**). Microsomia (54.5%), mandibular hypoplasia (36.3%), cleft palate (36.3%), macrostomia (27.2%), micrognathia (27.2%), retrognathia (9%), microcephaly (4.5%), and facial hypoplasia (9%) were the most frequently reported craniofacial features. Among the ocular abnormalities, epibulbar dermoid (46.1%) was most often reported, but patients with microphthalmia, anophthalmia, and ptosis ($n = 3$) were also reported. Ear alterations were described in all cases. Preauricular tags were reported in 68% of the articles and unilateral or bilateral microtia were observed in 40%. Abnormal spinal features, developmental delay, and cases of congenital heart disease were rarely reported. Family history for OAVS were described in nine studies,[1,2,6,7,9,10,12–14] and four studies included twin analysis,[1,2,15,16] two of which had no report of family history.[17,18]

Methods Applied in OAVS Investigation

Of the 25 included articles, 22 performed karyotype analysis (G-banding, $n = 14$; HR banding, $n = 6$; and R-banding, $n = 2$), 10 used FISH, 2 used MLPA, and 15 used array-CGH. Overall, of 303 individuals suspected of OAVS, only 59 individuals had associated genetic alterations (►**Table 2**). The remaining 244 individuals had no alterations or were rediagnosed based on the results with a disorder sharing clinical features with OAVS. Half of the 22 articles reporting use of karyotype analysis reported chromosomal alterations, including numerical and structural abnormalities, such as duplications, inversions, and translocations (►**Table 2**). Half of the 10 articles using FISH detected some kind of genetic alteration, including: translocations ($n = 3$), additional chromosome 22 ($n = 1$), and inversion p11.2q22.3 on chromosome 14 ($n = 1$). Both studies that performed MLPA technique found deletions in different regions 22q11.2 (►**Table 2**). Of the 15 articles reporting use of array-CGH technique to investigate OAVS, 13 identified genetic alteration, especially deletions and duplications of different chromosomal regions (►**Table 2**). Whole exome sequencing (WES) was also performed in one article but showed no significant result. All used quantitative polymerase chain reaction (qPCR) to confirm variations found by array-CGH.

Discussion

Clinical Findings

Considerable heterogeneity of clinical findings in individuals with suspected OAVS was described (►**Table 2**), including: craniofacial, limb, and organ system abnormalities. Some clinical findings were consistent in all cases, reinforcing the feasibility for a standardized clinical criteria. While many articles did not cite a diagnostic clinical criterion for OAVS, the most cited was the Tasse et al's criteria,[7] which

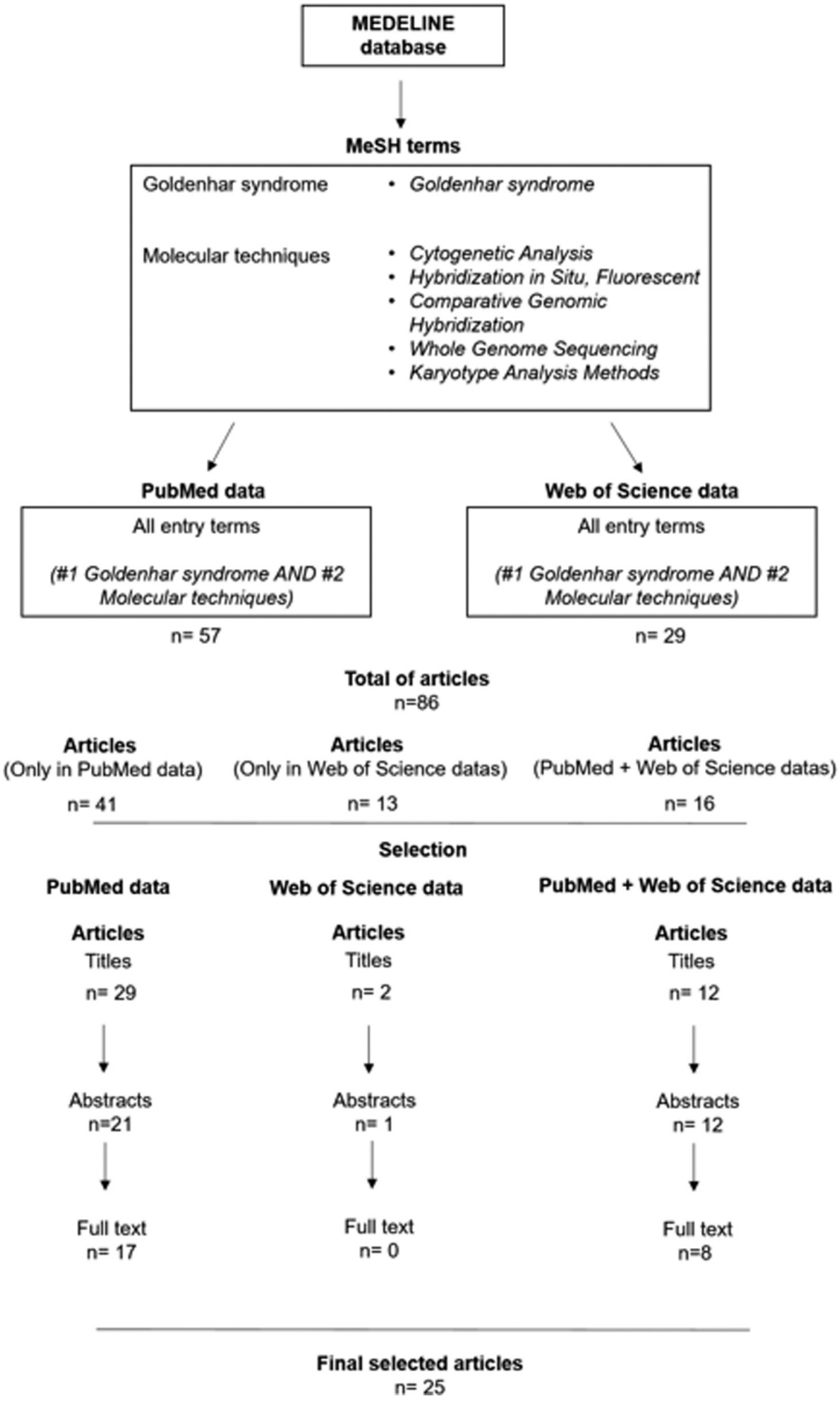

Fig. 1 Flowchart of the study selection process.

required the presence of microtia or hemifacial microsomia with or without other minor ear malformations. After evaluating 51 OAVS patients and their parents, Beleza-Meireles et al (2015) confirmed the validity of the Tasse et al's criteria and suggested adding a positive family history to the original criteria.[6] Huang et al (2010) cautioned that data suggesting an emphasis on positive family history could be skewed.[9]

Problems of Differential Diagnosis

The OAVS phenotype may overlap with other disorders that are important for differential diagnosis. These syndromes include: Treacher Collins' syndrome (OMIM 154500), auriculocondylar syndrome (OMIM 602483),[12] Townes–Brocks' syndrome (OMIM 107480),[9,19] cri du chat syndrome (OMIM 123450),[20] oculoauricular syndrome (OMIM 612109), branchio-otorenal syndrome 1 (OMIM 113650), cat eye syndrome

Table 1 Selected articles information

Author	Year	Category
Ala-Mello et al	2008	Case Report
Ballesta-Martínez et al	2013	Case Report
Beleza-Meireles et al	2015	Original Article
Bragagnolo et al	2018	Original Article
Brun et al	2012	Case Report
Colovati et al	2015	Short Report
Dos Santos et al	2014	Research Letter
Descartes	2006	Original Article
Herman et al	1988	Case Report
Huang et al	2010	Original Article
Huang et al	2010	Short Report
Josifova et al	2004	Original Article
Kobrynski et al	1993	Case Report
Northup et al	2010	Original Article
Puvabanditsin et al	2016	Case Report
Rooryck et al	2010	Original Article
Rooryck et al	2010	Short Report
Rosa et al	2010	Original Article
Silva et al	2015	Original Article
Spineli-Silva et al	2018	Case Report
Tasse et al	2005	Original Article
Verona et al	2006	Case Report
Xu et al	2008	Research Letter
Zielinski et al	2014	Original Article

(OMIM 115470), and otofaciocervical contiguous gene deletion syndrome (OMIM 166780).[1] Deletions and duplications on chromosome 22 in regions 22q11.2 causing DiGeorge's syndrome (DGS; OMIM 188400),[1,6,8,21–23] 22q11.1,[6] 22q13.32-33,[6] and 22q13.3[24] should also be considered.

Genetic Techniques

The authors in the reviewed articles used many molecular methods, and the variety of finding was great. While most molecular findings in individuals with OAVS are located within chromosome 22, it is important to highlight other alterations were also found. Molecular changes not involving chromosome 22 are of uncertain significance. Despite the high-quality information provided by array-CGH, this method does not detect balanced translocations and chromosomal inversions. Thus, it is suggested to begin the investigation through karyotyping techniques, followed by MLPA and array-CGH (►**Table 3**).

Karyotype Analysis

Karyotype was used most, with alterations found in different chromosomes and chromosome regions. The most observed alterations included chromosome 9 (inv 9),[3] t(9;18),[2,25] and chromosome 22 (del 22[23,24,26]). Chromosome banding is an essential technique used for karyotyping to identify normal and abnormal chromosomes. In an individual with suspected OAVS, Xu et al (2008)[23] found no alterations with the lower resolution GTG, but HR karyotype revealed a deletion at 22q11. GTG karyotype have a resolution of 450 to 550 bands, while HR can detect 850 band level.[27] The difference in banding resolution could explain why the GTG karyotype can miss some alterations found with HR karyotype. Therefore, for investigation of microdeletions associated with OAVS, HR and not GTG karyotyping should be used.

Table 2 Clinical and cytogenetic finding in individuals with clinical diagnosis of OAVS

Authors	Main clinical findings	Molecular techniques	Cytogenetic findings	Number of individuals
Ala-Mello et al (2008)[30]	Macrostomy Submucous cleft palate Epibulbar dermoids Preauricular tags Hemifacial microsomy Hypertelorism	G-banding karyotype FISH Array-CGH	45,XX, inv(2) (q32q37)mat, dic(5;21) (p15.3;q22.3)dn 21q and 5p were absent Deletion 5p15.33 Deletion 21q22.3 Duplication 21q22.11-q22.12	1/1 1/1 1/1
Ballesta-Martínez et al (2013)[12]	Micrognathia Macrostomia Preauricular pits and tags Auricular agenesis	High-resolution banding karyotype Array-CGH	Normal Duplication in 14q23.1	1/1 1/1
Beleza-Meireles et al (2015)[6]	Hemifacial microsomia Microtia External ear abnormalities Preauricular skin tags Cleft lip and/or cleft Palate Micrognathia Epibulbar dermoids Vertebral anomalies	Array-CGH	Duplication Xp11.21 Duplication 22q11.21 Duplication 22q13.32-33 Deletion 14q12 Duplication 10p15.33 Deletion 19q13.3 Duplication 22q11.1 Duplication 22q11.1q11.21	10/22

Table 2 (*Continued*)

Authors	Main clinical findings	Molecular techniques	Cytogenetic findings	Number of individuals
Bragagnolo et al (2018)[1]	Mandibular hypoplasia Macrostomia Micrognathia Preauricular tags and pits Epibulbar dermoid	G-banding karyotype Array-CGH	Normal Duplication 4p16.1 Deletion 4p16.3p15.33 Duplication Xp22.33p22.31 Deletion 8q13.3 Duplication 8q24.3 Duplication 10p14 Duplication 10p13. Deletion 10q26.2q26.3 Deletion 16p13.3 Duplication 16p13.11p12.3 Duplication 17q11.2 Deletion 22q11.21 Deletion Xp22.33	72/72 13/72
Brun et al (2012)[17]	Microtia Hemifacial microsomia Mandibular hypoplasia Bilateral conductive hearing loss Cleft palate	G-banding karyotype Array-CGH FISH	Normal Microdeletion in 15q24.1q24.2 Microdeletion in 15q24.1q24.2	1/1 1/1 1/1
Colovati et al (2015)[21]	Hemifacial microsomia Retrognathia Agenesis of the external auditory canal Soft cleft palate	G-banding karyotype MLPA Array-CGH	Normal Deletion in the 22q11.21 Deletion in the 22q11.21	1/1 1/1 1/1
Dos Santos et al (2014)[22]	Hemifacial microsomia Left ptosis Dysmorphic right ear Retrognathia Hearing loss	Array-CGH	Deletion in the 22q11.21	1/1
Descartes (2006)[20]	Macrostomia Midface hypoplasia Ear tags and pits Clinodactyly Frontal hemangioma	G-banding karyotype	46,XX,del(5)(p15.33)	1/1
Herman et al (1988)[24]	Hemifacial microsomia Epibulbar dermoids Macrocephaly Mandibular hypoplasia Preauricular tags Vertebral anomalies	High-resolution banding karyotype	46,XY,del (22)(q13.31)	1/1
Huang et al (2010)[9]	Preauricular tags Epibulbar dermoid Micrognathia Hypertelorism Facial dysplasia Hemifacial microsomia	G-banding karyotype Array-CGH	Normal 153 copy number variations, but without significant result	4/4 13/13
Huang et al (2010)[19]	Hemifacial microsomia Preauricular tags Atresia of the external auricular canal Vertebral anomalies	High-resolution banding karyotype Array-CGH qPCR	Normal Deletion on chromosome 5q13.2 Deletion on chromosome 5q13.2	1/1 1/1 1/1
Josifova et al (2004)[13]	Preauricular tags Right hemiparesis Developmental delay Hearing loss	G-banding karyotype FISH	Normal 46,XY,der(5)t(5;8)(p15.31;p23.1)	2/2 2/2

(*Continued*)

Table 2 (*Continued*)

Authors	Main clinical findings	Molecular techniques	Cytogenetic findings	Number of individuals
Kobrynski et al (1993)[26]	Hemifacial microsomia Epibulbar dermoid Absence of the external auditory meatus Preauricular pits Clinodactyly	G-banding karyotype FISH	47, XX, + 22 47, XX, + 22	1/1 1/1
Northup et al (2010)[14]	Ear abnormality Micrognathia Microtia Asymmetric face	G-banding karyotype FISH	46,XY,inv(14) (p11.2q22.3) 46,XY,inv(14) (p11.2q22.3)	1/1 1/1
Puvabanditsin et al (2016)[11]	Preauricular skin tags Hemifacial microsomia Microtia Absence of the auditory canal Microsomia Micrognathia	Array-CGH	Deletion in 7q21.11	1/1
Rooryck, et al (2010)[2]	Anotia or microtia Preauricular tags or pits Hearing loss Hemifacial microsomia Eye anomalies Vertebral anomalies	R-banding karyotype Array-CGH	47,XXX Isodicentric Y 46,XX,t(9;18)(p23;q12) Deletion 12p13.33 Duplication 18p11.23-p11.31 Duplication 20p12.2 Deletion 14q32.2 Trisomy X Duplication Yp-q11.221 Deletion Yq11.222-q12 Duplication 8q11.23 Deletion 2p11.2 Duplication 9q34.11 Duplication 4q35.1 Duplication 13.q13.1 Deletion 2q11 Amplification Xp22.33 Duplication 11q21	3/95 11/86
Rooryck, et al (2010)[25]	Microtia Preauricular tag Hemifacial microsomia Mandibular hypoplasia	R-banding karyotype FISH Array-CGH	46,XX, t(9;18)(p23;q12) 46,XX, t(9;18)(p23;q12) Duplication 18p11.23p11.31	1/1 1/1 1/1
Rosa et al (2010)[29]	Unilateral mandibular hypoplasia Preauricular skin tags Microtia Rib alterations Growth retardation	High-resolution banding FISH	Normal 22q11 was normal	3/3 3/3
Silva et al (2015)[3]	Orofacial clefts Micro/retrognathia Hemifacial microsomia Microtia/anotia Preauricular skin tags Auricular abnormalities Macrostomia	High-resolution banding karyotype FISH	47,XX, + mar mos47,XX, + mar/46,XX 46,XX,t(6;10)(q13;q24) 46,XX,inv(9)(p11q13) 22q11 and 5p were normal	4/23 23/23
Spineli-Silva et al (2018)[8]	Dysmorphic ears Preauricular tags Malar hypoplasia Bilateral cleft lip	G-banding karyotype MLPA Array-CGH	Normal Deletion in the 22q11.2 distal region Deletion in the 22q11.2 distal region	1/1 1/1 1/1

Table 2 (*Continued*)

Authors	Main clinical findings	Molecular techniques	Cytogenetic findings	Number of individuals
Tasse et al (2005)[7]	Preauricular pits/tags Hemifacial microsomia Microtia Orofacial clefts Anomalies of the eyes Epibulbar dermoids	G-banding karyotype FISH	Gonosomal mosaic 45,X and 47,XXX 22q11 was normal	1/49 20/20
Verona et al (2006)[18]	Facial hypoplasia Mandibular hypoplasia Micrognathia Epibulbar dermoid Preauricular tags	G-banding karyotype	Normal	1/1
Xu et al (2008)[23]	Cleft lip and palate Macrostomia Preauricular tags Microcephalic Facial dysmorphics	G-banding karyotype High-resolution banding karyotype FISH Array-CGH (BAC) Array-CGH (oligo)	Normal Deletion of 22q11.21–q11.23. 22q11 was normal Normal Deletion 22q11.21-q11.22	1/1 1/1 1/1 1/1 1/1
Zielinski et al (2014)[10]	Mandibular hypoplasia Facial asymmetry Preauricular skin tags Microtia Retrognathia	Array-CGH Exome	Duplication in 14q22.3 Normal	4/4 3/3

Abbreviations: Array-CGH, microarray-based comparative genomic hybridization; BAC, bacterial artificial chromosome; FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification; OAVS, oculoauriculovertebral spectrum; oligo, oligonucleotide; qPCR, quantitative polymerase chain reaction.

Table 3 Estimated budget of techniques and screening power

Technique	United States (US$)	Brazil (US$)	Detectable chromosomal changes					Resolution
			inv	ins	trans	del	dup	
GTG karyotype	600.00	200.00	x	x	x	x	x	~ 2 Mb
HR karyotype	800.00	240.00	x	x	x	x	x	2–5 Mb
R-banding karyotype	N/A	N/A	x	x	x	x	x	~ 2 Mb
FISH	600.00	800.00				x	x	200 kb–5 Mb
Array-CGH	600.00	1,200.00				x	x	1 kb–1 Mb
MLPA	500.00	600.00				x	x	< 5 Mb

Abbreviations: Array-CGH, microarray-based comparative genomic hybridization; del, deletion; dup, duplication; FISH, fluorescence in situ hybridization; HR, high-resolution; ins, insertion; inv, inversion; kb, kilobases; Mb, megabases; MLPA, multiplex ligation-dependent probe amplification; N/A, not available; trans, translocation.
Note: Resolution according to the International System for Human Cytogenomic Nomenclature (2016).

FISH

FISH uses fluorescent DNA probes that bind to a specific region of interest on the chromosome[28] to detect microdeletions (deletions <5 megabases), which are difficult to detect with conventional cytogenetics methods. All articles that included FISH did so simultaneously with karyotype. None of the four studies using FISH probes for 22q11.2 region found alterations,[3,7,23,29] though one study found a deletion in the 22q11.2 region through HR karyotype.[23] A possible explanation for why HR karyotype was successful when FISH had failed to show the deletion is that FISH probe used may have been for a different region. This outcome highlights the potential pitfalls of using FISH as a primary modality to investigate a poorly understood and genetically heterogenous pathology. In one instance, FISH was used to confirm a normal GTG karyotype and showed instead a translocation between telomeric regions of chromosomes 5p and 8p,[13] however. The other articles describe FISH used to confirm GTG[14,30] and R[25]-banding and array-CGH[17] findings, including: pericentric inversion 14q11-14q24[14]; a translocation between chromosomes 21q and 5p[30]; translocation (9;18)(p23;q12)[25]; and deletion in chromosome 15q.[17]

Due to the variety of chromosomal findings in patients with suspect OAVS, it may be appropriate to begin with banding techniques. Alterations identified through karyotype would facilitate the FISH probe selection to confirm the genetic diagnosis, but in individuals with a normal karyotype, MLPA and array-CGH may be better options.

MLPA

MLPA identifies abnormal copy number variations (CNVs) in different genes and is used to detect microdeletions and microduplications. Spineli-Silva et al (2018)[8] identified a deletion in 22q11.2 involving locus control regions (LCRs) D and E and genes *HIC2*, *PPIL2*, and *TOP3B*. Colovati et al (2015)[21] found a deletion in 22q11.2 involving LCRs B and D and genes *ZNF74*, *KLHL22*, *MED15*, *SNAP29*, and *LZTR1*.

Both studies that performed MLPA used the P250-B2 kit for DGS (MRC-Holland; Amsterdam, The Netherlands). This kit is an option to screen 22q11.2 locus only. While it is possible to develop a customized panel of genes that contain regions of chromosomes that have been already associated with OAVS, no specific MLPA kit for OAVS/Goldenhar's syndrome currently exists. Industry development of a standardized kit would make MLPA a promising approach for genetic screening and as a confirmatory test following another technique.

Array-CGH

Array-CGH uses simultaneously hybridized reference DNA with target DNA arrayed on a glass slide or other solid platform, allowing a HR evaluation of whole genome CNVs and identifying unbalanced chromosomal anomalies.[31] Bacterial artificial chromosome (BAC)-based and oligonucleotide (oligo)-based arrays are the two major types of array-CGH. As oligo-based arrays have better resolution and high coverage of a single chromosome, they are considered the best option for genomic screening.[32] Alterations found in the studies that have chosen the BAC-based array were heterogeneous and located in chromosomes 22[8,21,22] and 14.[10,12] Xu et al (2008)[23] used BAC-based and oligo-based arrays; the oligo-based array yielded a deletion in 21q11.21-q.11.2 region. Neither BAC-based array nor FISH identified any abnormalities,[23] again highlighting the weaknesses of those modalities in the present context. Oligo-based array showed heterogeneous alterations in 12 of 22 patients with suspected OAVS screened in one study,[6] with 10 of 12 located on chromosome 22 and showing changes predicted to be pathologic. Overall, array-CGH shows great strengths for investigating patients with suspected OAVS, with 1 of the 15 articles describing array-CGH did not observe any molecular findings.

qPCR and WES

Real-time or qPCR and WES were used adjunctively to confirm alterations found with other techniques or exclude possible variants associated with the phenotype.[9,10] The study using WES found more than 20,000 exon variants, none of which was considered to be pathologic, but a duplication in 14q22.3 was identified using array-CGH.[10] A study using qPCR confirmed a deletion in 5q13.2 by array-CGH.[19]

Conclusion

This systematic review indicates array-CGH, MLPA, and HR karyotype are reasonable approaches for differential diagnosis of OAVS and should include in the standard work-up of such patients. To reduce the diagnostic complexity caused by phenotypic and genotypic heterogeneity of OAVS, we suggest screening patients with a standardized clinical criteria for OAVS,[6,7] followed by HR karyotype to exclude structural and numerical changes, screening for 22q11 microdeletion by MLPA, and for identification or in the absence of causative molecular changes, array-CGH. Following the step-wise approach suggested by this systematic review for diagnosis can inform clinical decision making, and better resource utilization could improve health care facility efficiency and economy. Especially in developing countries, this suggested workflow conserves scarce resources while not compromising patient care in investigating a spectrum with such heterogeneous clinical and genetic findings.

Authors' Contribution

A.G., B.D., D.D., and A.S. made substantial contributions to the conception or design of the study: the acquisition, analysis, and interpretation of data for the study; drafting the article; and revising it critically for important intellectual content. R.R. and P.Z. revised the article critically for important intellectual content, and all authors (A.G., B. D., D.D., A.S., R.R., and P.Z.) approved the version to be published and agree 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.

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Conflict of Interest

None declared.

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5. CONCLUSÕES

A partir das revisões sistemáticas desenvolvidas, nossos estudos identificaram os genes *CLTCL1*, *GSC2*, *HIRA*, *MAPK1*, *TBX1* e *YPEL1* como potenciais genes candidatos para o EOAV, assim como reforçou a hipótese já descrita na literatura sobre a região genômica 22q11 ser um importante lócus para o desenvolvimento dessa condição. Este estudo foi a primeira revisão sistemática já publicada sobre o assunto.

Em relação a metodologias diagnósticas, array-CGH, MLPA e cariótipo de alta resolução (HR) foram as abordagens mais eficientes identificadas no diagnóstico diferencial. Concluímos que o rastreio de pacientes deve iniciar a partir de critérios clínicos definidos, seguido de cariótipo HR, MLPA e, se necessário ou disponível, array-CGH. Uma abordagem por etapas fornece uma melhor utilização de recursos, sem comprometer a investigação de um espectro com resultados heterogêneos.

Apesar dos importantes aspectos descritos, incluindo a identificação de genes candidatos, estudos adicionais referentes à mecanismos de ação e interação gênica são necessários para elucidar cada vez mais a associação genótipo-fenótipo do EOAV.

6. CONSIDERAÇÕES FINAIS

O Serviço de Genética Clínica da UFCSPA presta atendimentos aos pacientes atendidos pelo SUS no Hospital da Criança Santo Antônio/ISCMPA desde 1975, sendo o serviço de genética mais antigo do estado. O Serviço conta com um Laboratório de Citogenética e possui projetos de pesquisa para a investigação molecular em pacientes com suspeita clínica de doenças genéticas, intitulados “Avaliação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas”, aprovado pelo Comitê de Ética em Pesquisa do HCSA (CAAE: 74971917.2.0000.5683 – Número do Parecer: 2. 315.917), e “Investigação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas”, aprovado pelo Comitê de Ética em Pesquisa da UFCSPA (CAAE: 86036418.4.0000.5345 – Número do Parecer: 2.729.168). A partir dos projetos em andamento, o laboratório de citogenética possui um biorepositório com amostras de DNA de pacientes com diagnóstico clínico do EOAV, que consentiram em participar dos estudos.

Assim, temos como perspectiva realizar a análise citogenética molecular nos pacientes com diagnóstico clínico do EOAV, através das técnicas de MLPA e Array-CGH, com o objetivo de identificar alterações e genes envolvidos na etiologia do fenótipo, além de fornecer laudos diagnósticos a esses pacientes.

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Pseudohypoparathyroidism with Ectopic Calcification and 22q11 Deletion Syndrome: A Rare Case

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Abstract

Ectopic calcification in soft tissue is associated with several disorders including pseudohypoparathyroidism (PHP), which is characterized by resistance or nonresponse to parathyroid hormone (PTH) function. Association between PHP and 22q11DS, also known as DiGeorge syndrome, is rare, especially in children. We describe a newborn girl diagnosed with 22q11DS, presenting ectopic calcifications in soft tissue and suspicion of PHP. PTH function showed values close to the upper limit of the reference value. Radiology showed bone callus in the right wrist. PHP can be a new clinical finding associated with 22q11DS. Parathyroid function investigation in individuals with 22q11DS, presenting bone dysmorphisms and/or calcium metabolism alterations, should be considered.

Keywords
- ectopic calcification
- pseudo-hypoparathyroidism
- DiGeorge syndrome

Introduction

Ectopic calcifications in soft tissue occur sporadically or as a rare genetic condition and may be associated with fibrodysplasia ossificans progressiva (FOP; OMIM 135100), progressive osseous heteroplasia (POH; OMIM 166350), pseudohypoparathyroidism, type 1A/Albright hereditary osteodystrophy (PHP1A/AHO; OMIM 103580), hyperphosphatemic familial tumoral calcinosis (HFTC; OMIM 211900), normophosphatemic familial tumoral calcinosis (OMIM 610455), and pseudohypoparathyroidism (PHP; OMIM 203330).[1]

PHP is characterized by resistance to parathyroid hormone (PTH). Due to differences in pathogenesis and phenotype, PHP can be classified into four types: Ia, Ib, Ic, and II.[2,3] Pseudo-pseudohypoparathyroidism (pseudo-PHP) is a PHP variant characterized by the development of isolated AHO without hormonal resistance. Pseudo-PHP is caused by mutations in the GNAS gene through paternal inheritance and it is genetically related to PHP-Ia. Signs and symptoms of both conditions are similar; however, individuals with pseudo-PHP do not show resistance to PTH while individuals with PHP-Ia do.[4]

The diagnosis of PHP type Ia (PHP1a) is challenging since clinical features such as osteodystrophy, brachydactyly, round face, and symptomatic hypocalcemia, are generally developed after childhood. Although ectopic calcification may be an early sign of PHP1a, there is no well-established evidence in the beginning of its development.[2,3]

22q11 deletion syndrome (22q11DS, OMIM 611867), also known as DiGeorge syndrome, is caused by a microdeletion (1.5–3 Mb) on chromosome 22 and has an estimated prevalence of 1 per 4,500 live births.[5] Phenotypic presentation is variable and endocrinopathies are commonly observed in patients with this deletion. Generally, 22q11DS can be diagnosed when the individual presents congenital heart diseases associated with other clinical manifestations. The spectrum of clinical features that compose 22q11DS has become well characterized and some endocrine abnormalities such as growth hormone deficiency and hypothyroidism have been reported. However, the association between 22q11DS and PHP

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GATA4 Deletions Associated with Congenital Heart Diseases in South Brazil

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Abstract

The normal development of the heart comprises a highly regulated machinery of genetic events, involving transcriptional factors. Congenital heart disease (CHD), have been associated with chromosomal abnormalities and copy number variants (CNVs). Our goal was to investigate through the multiplex ligation-dependent probe amplification (MLPA) technique, the presence of CNVs in reference genes for normal cardiac development in patients with CHD. *GATA4*, *NKX2–5*, *TBX5*, *BMP4*, and *CRELD1* genes and 22q11.2 chromosome region were analyzed in 207 children with CHD admitted for the first time in a cardiac intensive care unit from a pediatric hospital. CNVs were detected in seven patients (3.4%): four had a 22q11.2 deletion (22q11DS) (1.9%), two had a *GATA4* deletion (1%) and one had a 22q11.2 duplication (0.5%). No patients with CNVs in the *NKX2–5*, *TBX5*, *BMP4*, and *CRELD1* genes were identified. *GATA4* deletions appear to be present in a significant number of CHD patients, especially those with septal defects, persistent left superior vena cava, pulmonary artery abnormalities, and extracardiac findings. *GATA4* screening seems to be more effective when directed to these CHDs. The investigation of CNVs in *GATA4* and 22q11 chromosome region in patients with CHD is important to anticipating the diagnosis, and to contributing to family planning.

Keywords
- heart defects
- congenital
- *GATA4* transcription factor
- 22q11 deletion syndrome

Introduction

Congenital heart diseases (CHDs) consists of structural changes in the heart and large vessels and are recognized as the leading cause of neonatal mortality, affecting approximately 1% of newborns.[1,2] Regarding CHDs etiology, 20% are attributed to chromosomal and/or single-gene alterations; the remaining resulting from a combination of genetic, epigenetic, and environmental factors. Chromosomal abnormalities and copy number variants (CNVs) contribute to the risk of CHD.[3]

CNVs in *GATA4*, *NKX2–5*, and *TBX5* genes have been identified among mechanisms that may explain some CHDs, since all these are transcription factors (TFs) strongly involved in cardiogenesis. The altered expression of *GATA4* (OMIM: 600576) can results in common CHDs, such as atrial septal defects (ASDs), ventricular septal defects (VSDs), and pulmonary stenosis (PS). Facial dysmorphisms and mental retardation may also be present.[4–6] It is believed that, all TFs acts together by regulating the cardiac septum formation, whereas its haploinsufficiency results in developmental heart disorders.[7] Several clinical syndromes associated with CHDs have also been related to

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Congenital Heart Defects and Dysmorphic Facial Features in Patients Suspicious of 22q11.2 Deletion Syndrome in Southern Brazil

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Abstract

22q11.2 deletion syndrome (22q11.2DS) is considered one of the most frequently observed chromosomal abnormalities in association with congenital heart disease (CHD), which can also include some combination of other features. Thus, the aim of this work was to verify the profile of dysmorphic features and heart defects found in patients referred to a reference center in Southern Brazil with clinical findings suggestive of 22q11.2DS. In the overall sample group, only patients with dysmorphic facial features (skull, eyes, ear, and nose) associated with CHD (obstructive pulmonary valve ring, truncus arteriosus, and bicuspid aortic valve associated with atrial septal defect and/or right aortic arch) had a 22q11.2 deletion. These findings proved to be reliable clinical criteria for referral to perform fluorescent *in situ* hybridization investigation for 22q11.2 deletion.

Keywords
- heart defects
- congenital
- 22q11 deletion syndrome
- facial dysmorphism

Introduction

22q11.2 deletion syndrome (22q11.2DS or DiGeorge syndrome) (OMIM 188400) is one of the most common disorders caused by a copy number variant (CNV) and nonhomologous meiotic recombination errors. 22q11.2DS is characterized by a microdeletion in region 11.2 of the long arm of chromosome 22, with approximately 90 genes being deleted.[1,2]

The prevalence and incidence in live births are still being studied due to the high phenotypic variation, which makes the immediate identification of affected individuals difficult. However, the literature reports incidences between 1 in 2,000 and 7,000 live births.[3] Currently, 22q11.2DS is considered one of the most frequently observed chromosomal abnormalities in association with congenital heart disease (CHD), only after Down syndrome.[4]

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REVIEW

WILEY

Population-based prevalence surveys during the Covid-19 pandemic: A systematic review

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Summary

Population-based prevalence surveys of Covid-19 contribute to establish the burden of infection, the role of asymptomatic and mild infections in transmission, and allow more precise decisions about reopen policies. We performed a systematic review to evaluate qualitative aspects of these studies, assessing their reliability and compiling practices that can influence the methodological quality. We searched MEDLINE, EMBASE, bioRxiv and medRxiv, and included cross-sectional studies using molecular and/or serological tests to estimate the prevalence of Covid-19 in the general population. Survey quality was assessed using the Joanna Briggs Institute Critical Appraisal Checklist for Prevalence Studies. A correspondence analysis correlated methodological parameters of each study to identify patterns related to higher, intermediate and lower risks of bias. The available data described 37 surveys from 19 countries. The majority were from Europe and America, used antibody testing, and reached highly heterogeneous sample sizes and prevalence estimates. Minority communities were disproportionately affected by Covid-19. Important risk

Abbreviations: BioS, biological sample; JBI, Joanna Briggs Institute; N/A, not applicable; NPS, nasopharyngeal swabs; MERS, Middle East respiratory syndrome; P, prevalence; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RT-PCR, reverse-transcriptase polymerase chain reaction; S, sensitivity; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.