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Bruna Lixinski Diniz

Análise das metodologias moleculares aplicadas ao diagnóstico da síndrome de deleção 22q11 e atualização de resultados entre indivíduos suspeitos e diagnosticados: enfoque cardíaco.

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Orientador: Dr. Paulo Ricardo Gazzola Zen
Coorientador: Dr. Rafael Fabiano Machado Rosa

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Resumo da Tese

Introdução: A síndrome de DiGeorge ou deleção 22q11.2 (22q11.2DS; OMIM #188400) é a síndrome de microdeleção cromossômica mais frequente, originada a partir de eventos de recombinação meiótica não homóloga que ocorrem em aproximadamente 1 em cada 1.000 – 7.000 recém-nascidos. Os defeitos cardíacos congênitos (CHDs) são uma das características clínicas mais prevalentes descritas em indivíduos diagnosticados com a 22q11.2DS, sendo o principal achado que motiva o encaminhamento para a investigação da síndrome, especialmente em indivíduos com poucas manifestações fenotípicas. Sabe-se que 4 LCRs, do inglês, *low copy repeats*, (A, B, C e D) definem essa região e dependendo do tamanho da deleção e LCRs envolvidas, o fenótipo pode ser sucinto ou até mais abrangente. Atualmente, há diferentes metodologias citogenéticas que são usadas rotineiramente em laboratórios clínicos e de pesquisa. Portanto, a escolha de uma tecnologia eficiente e a interpretação precisa dos achados clínicos são cruciais para o diagnóstico dos pacientes com 22q11.2DS.

Objetivos: Analisar as metodologias moleculares utilizadas para o diagnóstico da 22q11.2DS em associação com a cardiopatia congênita e determinar o tamanho de deleção e genes envolvidos através da técnica de MLPA em pacientes com diagnóstico molecular para 22q11.2DS após triagem pela FISH.

Material e Métodos: Foi realizada uma revisão sistemática dos últimos 20 anos de pesquisa sobre pacientes com 22q11.2DS em associação com CHD e o processo de investigação por trás de cada diagnóstico. Posteriormente, uma reavaliação dos casos identificados pelo Serviço de Genética Clínica foi

realizada para determinar o tamanho real da deleção e possíveis outras causas moleculares de pacientes previamente triados pela metodologia de FISH. **Resultados:** Este trabalho originou dois artigos, uma revisão sistemática e um artigo original. Na revisão sistemática, 60 artigos foram elegíveis para análise. Apresentamos uma nova visão do defeito do septo ventricular como um possível achado cardíaco fundamental em indivíduos com 22q11.2DS. Além disso, descrevemos as tecnologias moleculares e a avaliação cardíaca como ferramentas valiosas para orientar os pesquisadores em investigações futuras. Já no artigo original, todos os pacientes que haviam sido diagnosticados anteriormente por FISH (n=10) apresentaram exatamente o mesmo tamanho de deleção, LCRs e genes envolvidos quando avaliados por MLPA. Em pacientes com FISH-, o *GATA4* deletado ou duplicado em diferentes exons (1 e 6), apresentou fenótipos distintos de defeitos cardíacos congênitos. Os pacientes que não tiveram seu diagnóstico definido por FISH apresentaram resultados moleculares diferentes, variando de achados normais a alterações nos genes *GATA4* e *NXK2-5*.

Conclusão: A diversidade molecular nas malformações cardíacas é uma realidade e um grande desafio, uma vez que a correlação genótipo-fenótipo é limitada. Portanto, novas percepções sobre esse assunto devem ser consideradas: a síndrome de deleção 22q11.2 deve estar ligada apenas à região do cromossomo 22 ou há uma variabilidade fenotípica a ser analisada que envolve um ambiente genômico mais amplo?

Palavras-chave: síndrome de deleção 22q11.2; cardiopatia congênita; FISH; MLPA.

Abstract

Introduction: DiGeorge syndrome or 22q11.2 deletion (22q11.2DS; OMIM #188400) is the most frequent chromosomal microdeletion syndrome, originating from non-homologous meiotic recombination events that occur in approximately 1 in every 1,000 - 7,000 newborns. Congenital heart defects (CHDs) are one of the most prevalent clinical features described in individuals diagnosed with 22q11.2DS and are the main finding to be referred for investigation of the syndrome, especially in individuals with a mild phenotype. It is known that 4 LCRs (A, B, C and D) define this region and depending on the deletion size and LCRs involved, phenotype can be brief or even more comprehensive. Currently, there are different cytogenetic methodologies that are routinely used in clinical and research laboratories. Therefore, the choice of an efficient technology and the accurate interpretation of clinical findings are crucial for the diagnosis of patients with 22q11.2DS.

Aim of study: Analyzing the molecular methodologies used for 22q11.2DS diagnosis in association with congenital heart disease and determining deletion size and genes involved through the MLPA technique in patients with molecular diagnosis for 22q11.2DS after being screened by FISH.

Materials and methods: We carried out a systematic review of the last 20 years of research on patients with 22q11.2DS in association with CHD and the investigation process behind each diagnosis. Subsequently, a re-evaluation of the cases identified by the Clinical Genetics Service was carried out to determine the actual size of the deletion and possible other molecular causes in patients previously screened by FISH methodology.

Results: This work resulted in two articles, a systematic review and an original article. In the systematic review, 60 articles were eligible for analysis. We present a new view of ventricular septal defect as a possible key cardiac finding in individuals with 22q11.2DS. In addition, we describe molecular technologies and cardiac assessment as valuable tools to guide researchers in future investigations. In the original article, all the patients who had previously been diagnosed by FISH (n=10) had the same size of deletion, LCRs and genes involved. FISH- patients, *GATA4* deleted or duplicated in different exons (1 and 6), showed distinct phenotypes of congenital heart defects. Patients whose diagnosis was not defined by FISH had different molecular results, ranging from normal findings to alterations in the *GATA4* and *NXK2-5* genes.

Conclusion: Molecular diversity in cardiac malformations is a reality and a great challenge since genotype-phenotype correlation is hindered. Therefore, new insights on that matter should be considered: 22q11.2 deletion syndrome should only be linked to the chromosome 22 region, or is there a phenotype variability to be looked at that involves a broader genomic environment?

Keywords: 22q11 deletion syndrome; congenital heart defects; FISH; MLPA.

Lista de abreviaturas

CGH do inglês, *Comparative Genomic Hybridization*

CNV do inglês, *copy number variation*

DNA do inglês, *deoxyribonucleic acid*

EDTA do inglês, *ethylenediaminetetraacetic acid*

FISH do inglês, *Fluorescence in situ Hybridization*

LCR do inglês, *low copy repeat*

MLPA do inglês, *Multiplex Ligation-dependent Probes Amplification*

PCR do inglês, *Polymerase Chain Reaction*

SGC do inglês, *contiguous gene syndromes*

SMM do inglês, *microdeletion or microduplication syndromes*

SNP do inglês, *single nucleotide polymorphism*

SV do inglês, *structural variation*

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1. REFERENCIAL TEÓRICO

1.1 Malformações congênitas

As malformações congênitas são anomalias estruturais ou funcionais originadas por eventos fisiológicos ou não que precedem o nascimento.[1] As manifestações clínicas são diversificadas, podendo variar desde dismorfias menores até alterações complexas e raras.[2] A etiologia das malformações pode estar relacionada a alterações mendelianas, cromossômicas, ambientais, entre outros, porém muitas vezes não é possível determinar a sua causa. Sugere-se que 6% das malformações congênitas estejam relacionadas a anomalias cromossômicas, podendo esta proporção aumentar quando são associados os desequilíbrios genômicos abaixo da resolução (<5Mb), ou seja, não diagnosticáveis pela análise cromossômica padrão, o cariótipo.[3]

As microdeleções e microduplicações (MMs) podem estar presentes no período pré-natal, as quais apenas são identificadas por métodos mais sofisticados de diagnóstico citogenético, como por exemplo, a hibridização *in situ* fluorescente (FISH), *Multiplex Ligation-dependent Probe Amplification* (MLPA) ou microarray cromossômico (CMA).[4] As microduplicações e microdeleções cromossômicas estão envolvidas em diversas malformações congênitas, sendo a mais comum e uma das principais causas de morbidade infantil, a cardiopatia congênita (*congenital heart disease*: CHD).[5] As anomalias congênitas podem contribuir para a incapacidade motora de longo prazo, o que pode resultar em impactos significativos nos indivíduos acometidos, além dos familiares, sistemas de

saúde e da sociedade como um todo. Com isso, é importante que se faça um diagnóstico completo de cada síndrome, obtendo um maior número de informações possíveis a fim de proporcionar melhor acompanhamento e tratamento dos pacientes. Assim, a partir de uma atenta avaliação clínica se pode traçar uma estratégia de investigação visando a otimização do uso dos diferentes testes genéticos.

1.1.1 Cardiopatia congênita

A CHD é um conjunto de anormalidades estruturais e funcionais do coração e grandes vasos, que podem surgir durante a embriogênese cardíaca.[5] Possui incidência de 19-75 casos a cada mil nascidos vivos. A etiologia é complexa e multifatorial, com cerca de 80% das CHDs surgindo através da combinação de fatores genéticos e ambientais.[6] A malformação cardíaca oriunda de anomalias genéticas ou cromossômicas, como a síndrome de Down, está presente em 1 a cada 100 crianças.[7]

A base genética, epigenética e ambiental precisa da CHD ainda não está totalmente compreendida. A maioria das CHDs ocorrem como malformações de forma isolada, enquanto 25-30% estão associadas a alterações extracardíacas. Alguns defeitos específicos são frequentemente encontrados em associação com síndromes genéticas conhecidas.[8] Cerca de 20% dos casos podem ser atribuídos a anomalias cromossômicas, síndromes mendelianas, desordens genéticas não sindrômicas ou teratógenos.[9]

Aneuploidias e microdeleções de regiões cromossômicas estão associadas ao desenvolvimento da CHD (Tabela 1), porém os mecanismos

pelos quais esse desequilíbrio cromossômico altera a cardiogênese são pouco conhecidos e provavelmente muito mais complexos do que um efeito de dosagem gênica. Já se sabe que mutações em genes associados as CHDs humanas afetam um conjunto heterogêneo de moléculas que coordenam o desenvolvimento cardíaco, além de frequentemente alterarema dosagem de proteínas.[5,10]

Tabela 1. Cardiopatias congênitas – condições mais comuns.

Mecanismo genético	*Cromossomo/Região*	*Síndrome genética*	*Características dos defeitos cardíacos*
Tetrassomia	22pter-q11	Olho do Gato	Retorno total/parcial venoso pulmonar anormal; Veia cava superior esquerda persistente
Trissomia	13	Patau	Defeito do septo ventricular; *Ductus arteriosus* patente; Defeito do septo atrial; Dextroposição.
	18	Edwards	Defeito do septo ventricular; Defeito do septo atrial; *Ductus arteriosus* patente.
	21	Down	Defeito do septo ventricular; Defeito do septo atrioventricular; Defeito do septo atrial.
Monossomia	X	Turner	Via de saída do ventrículo esquerdo; Malformação da aorta.
Deleção	3p	-3p	Defeito do septo atrioventricular
	4p	Wolf-Hirschhorn	Defeito do septo atrial
	5p	Cri-du-chat	Variável (em 30%)
	8p	-8p	Defeito do septo atrioventricular
	9p	-9p	Defeito do septo ventricular; *Ductus arteriosus* patente; Estenose da valva pulmonar.
Microdeleção	7q11	Willams	Estenose supravalvar aórtica; Estenose periférica da artéria pulmonar; Estenose da valva pulmonar.

17p11.2	Smith-Magenis	Estenose da valva pulmonar; Defeito do septo atrial; Defeito do septo ventricular; Malformação da valva atrioventricular.
17p13.3	Miller-Dieker	Tetralogia de Fallot; Defeito do septo ventricular; Estenose da valva pulmonar.
22q11.2	DiGeorge	Anomalias da via de saída e do arco aórtico.

A biologia molecular e as novas técnicas disponíveis têm possibilitado a descoberta de genes que podem interagir entre si ou com fatores externos gerando uma pré-disposição ao desenvolvimento da doença.[09,11,12] Mudanças no número de cópia de segmentos específicos do DNA são frequentemente associados à causa ou pré-disposição para doenças. Tais alterações podem incluir desde a presença de uma cópia extra de um cromossomo inteiro, a deleções e duplicações de inúmeros pares de bases ou pequenos fragmentos cromossômicos envolvendo apenas um único éxon. Esta perda ou ganho de material genético desencadeia um efeito direto sobre a dosagem gênica, aumentando ou diminuindo o padrão de expressão dos genes afetados. [9]

Os genes responsáveis pelo desenvolvimento cardíaco são os mais afetados por estas mutações, atualmente, mais de 50 genes são potenciais candidatos a serem estudados.[13,14] As mutações podem ser herdadas ou ocorrer de forma esporádica ou de novo.[14] A CHD ocorre associada a uma síndrome ou de forma isolada. Dentre as principais causas da CHD, a síndrome de deleção 22q11.2 (22q11.2DS) é a segunda mais comum, ficando atrás da síndrome de Down. A CHD é a manifestação mais crítica e principal fator de morbimortalidade na 22q11.2DS afetando entre 74% e

80% dos pacientes. Dentre diversas CHDs relatadas, os defeitos conotruncais e/ou do arco aórtico são os mais prevalentes. [15,16]

1.2 Síndrome de deleção 22q11.2

A síndrome de deleção 22q11.2 (22q11.2DS ou DiGeorge) (OMIM #188400) é uma das mais comuns desordens relacionadas a variantes do número de cópias (CNVs) e erros de recombinação meiótica não-homóloga. A 22q11.2DS é caracterizada por uma microdeleção no braço longo (q), região 11.2 do cromossomo 22, com perda de, aproximadamente, 90 genes.[17,18] A prevalência e incidência em nascidos vivos ainda é objeto de estudo devido à grande variação no fenótipo que dificulta a imediata identificação de indivíduos acometidos. Entretanto, na literatura são relatadas incidências nos nascimentos entre 1 em 2000 – 7000 nascidos vivos.[19]

A maioria (90-95%) dos indivíduos recém identificados com 22q11.2DS possui deleções de novo, ou seja, nenhum dos pais tem a deleção 22q11.2. No entanto, devido à melhora da sobrevida e, portanto, maior aptidão reprodutiva dos indivíduos com 22q11.2DS, a prevalência, especialmente dos tipos hereditários, deve aumentar.[20,21] A 22q11.2DS é conhecida pela heterogeneidade de achados clínicos e a dificuldade de se estabelecer uma análise fidedigna baseado no fenótipo. A primeira caracterização da 22q11.2DS veio por meio da descrição de achados clínicos em crianças que apresentavam a tríade clínica de imunodeficiência, hipoparatireoidismo e CHD, pelo Dr. Angelo DiGeorge, em 1965.[22] Após o surgimento das técnicas citogenéticas convencionais e

moleculares, foi possível a identificação da deleção da região q11.2 do cromossomo 22 que estava presente frequentemente nesses indivíduos diagnosticados clinicamente como síndrome de DiGeorge.[23,24]

A 22q11.2DS é agora conhecida por ter uma apresentação heterogênea que inclui múltiplas anomalias congênitas e condições identificadas no decorrer da investigação clínica, tais como anomalias palatais, gastrointestinais e renais, doença autoimune, atrasos cognitivos variáveis, fenótipos comportamentais e doenças psiquiátricas.[25] A partir da identificação da CHD nesses indivíduos, criou-se hipóteses de que um mecanismo que leva a alteração no desenvolvimento embrionário poderia estar envolvido.[26] Dentre as CHDs, as mais comumente observadas são: arco aórtico tipo B interrompido (52%), truncus arteriosus (34%), Tetralogia de Fallot (16%) e defeitos do septo ventricular (5-10%).[27]

A relação entre os genes presentes na região 22q11.2 deletados em heterozigose e fenótipos ainda é pouco compreendida. Sabe-se que 4 LCRs, do inglês, *low copy repeats*, (A, B, C e D) definem essa região. Estas LCRs, devido à semelhança substancial de sequência, facilitam a recombinação meiótica homóloga não-alélica, resultando em translocação, deleção ou duplicação desequilibrada. Dependendo do tamanho da deleção e LCRs envolvidas, o fenótipo pode ser sucinto ou até mais abrangente. Mais de 85% da recombinação ocorre entre LCR A e LCR D, resultando em uma deleção "típica" de 3 Mb (Figura 1a). A deleção de 1,5 Mb entre LCR A e LCR B pode ser identificada em 10% dos indivíduos afetados.[28] Indivíduos com deleção LCR22A – LCR22B possuem o espectro completo de fenótipos que também é encontrado em indivíduos

com a deleção típica LCR22A – LCR22D, sugerindo que os fenótipos chave da 22q11.2DS são, na sua maior parte, devidos à diminuição da dosagem dos genes localizados na região das LCR22A – LCR22B. Defeitos cardiovasculares também parecem ser 3 vezes mais frequentes em deleções de LCR22A – LCR22B ou LCR22A – LCR22D.62 Já as deleções em regiões distais, LCR22B – LCR22D ou LCR22C – LCR22D, são associadas a alterações no desenvolvimento cardíaco semelhantes àquelas associadas a deleção típica, embora apresentadas em menor frequência (Figura 1b). Existem ~90 genes conhecidos presentes no locus típico de 3-Mb da região 22q11.2, incluindo 46 genes codificadores de proteínas, sete microRNAs (miRNAs), dez RNAs não codificantes e 27 pseudogenes.[29]

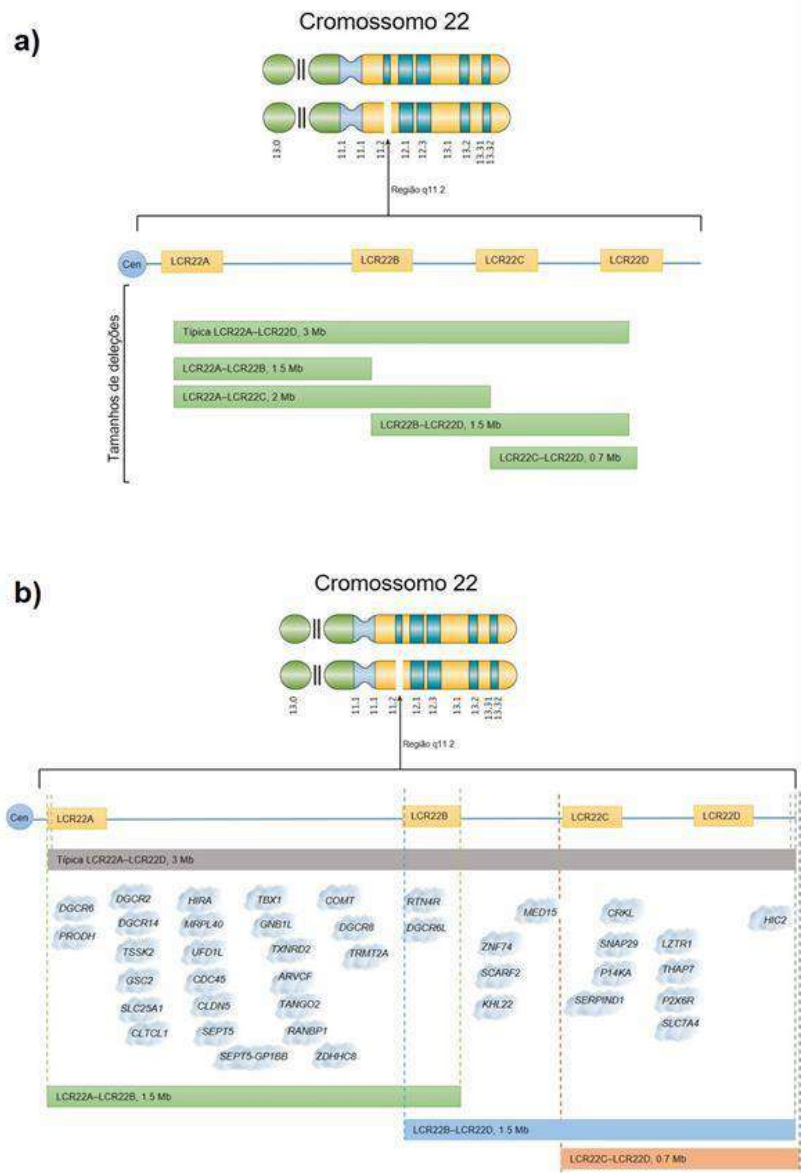


Figura 1. Deleção 22q11.2. a) Tamanho de deleções entre LCRs na região 22q11.2. b) Genes envolvidos nas deleções entre LCRs na região 22q11. Adaptado de McDonald-McGinn et al., 2015, Motahari et al., 2019.[25,29]

1.3 Metodologias utilizadas para a investigação da 22q11.2DS

O início da citogenética clássica deu-se no final do século 19, através da realização de analises microscópicas de cromossomos em células de carcinoma e sarcoma.[30] A partir disso, com o desenvolvimento de técnicas mais aprimoradas com o intuito de aumentar a resolução dos cromossomos, a citogenética humana tornou-se não só uma ciência

valiosa, como uma estratégia de diagnóstico tradicional para detecção de anormalidades cromossômicas pré-natais, pós-natais e adquiridas.[31] A primeira visualização de padrão de bandas cromossômicas foi através do método de bandamento Q e logo após, desenvolveu-se a técnica mais usada em tempos atuais, o bandamento G.[32]

A cariotipagem com bandamento G é considerada padrão-ouro para analises de doenças cromossômicas, como aneuploidias, trissomias ou monossomias, além de grandes rearranjos estruturais (>5-10Mb). Esta metodologia baseia-se em cultura de células que varia de 3 a 5 dias, associado com a confecção de lâminas, seguida da coloração de Giemsa, com uso de uma enzima proteolítica (tripsina), e análise microscópica que possibilita a visualização habitual de um padrão de 450 a 550 bandas nos cromossomos. No entanto, a técnica não possibilita a visualização de alterações pequenas, a nível submicroscópico, como no caso de microdeleções cromossômicas.[33]

A introdução e aplicação de abordagens moleculares aprimoraram a resolução da análise de cromossomos, resultando na obtenção rápida de resultados e detecção mais precisa de anormalidades cromossômicas. A FISH foi a primeira metodologia molecular aplicada na pesquisa citogenética,[34] além de ser atualmente uma das principais técnicas para diagnósticos clínicos e análises de microdeleções e microduplicações.[35] Através da hibridização do DNA alvo com sondas fluorescentes, esta técnica permite identificar cromossomos envolvidos em aneuploidias e rearranjos cromossômicos, podendo ser obtido o resultado em 48 horas. Por mais que esta abordagem seja de nível mais específica, a capacidade

de detecção é considerada limitada, pois dependendo dos conjuntos de sondas escolhidas, as alterações podem não ser possíveis de análise.[36]

A FISH é a técnica mais utilizada para o diagnóstico devido à sua alta sensibilidade e baixo custo relativo. As sondas comerciais para 22q11.2DS têm como alvo o segmento de DNA entre as LCR22A - LCR22B da região 22q11.2, mas não podem precisar o tamanho da deleção. A sonda LSI TUPLE1-HIRA Spectrum Orange/LSI ARSA Spectrum Green possui duas sequências de DNA marcadas com duas cores (vermelho e verde), na qual uma hibridiza na banda alvo 22q11.2 (LSI TUPLE Spectrum Orange) e a outra na banda controle 22q13 (ARSA Spectrum Green).[35] A TUPLE1-HIRA é específica para a banda 22q11.2, mais precisamente no gene *HIRA* que está posicionado entre a LCR22A-LCR22B. A sonda apresenta microssatélites de DNA (D22S553, WI-326, D22S942) localizados em diferentes posições no gene *HIRA* (Figura 2). A ausência do sinal vermelho no cromossomo 22 indica deleção do locus HIRA em 22q11.2 (Figura 3a). A ARSA é específica para a banda 22q13 que hibridiza na extremidade telomérica do cromossomo 22, diretamente marcada com verde e é usada como um controle interno, além de verificar a região 22q13. As sondas hibridizadas fluorescem com luminosidade moderada a intensa tanto em núcleos de interfase como em metáfases cromossômicas. Em núcleos de interfase e metáfases de células normais, a sonda geralmente aparece como 2 sinais vermelhos e dois sinais verdes distintos (Figura 3b).

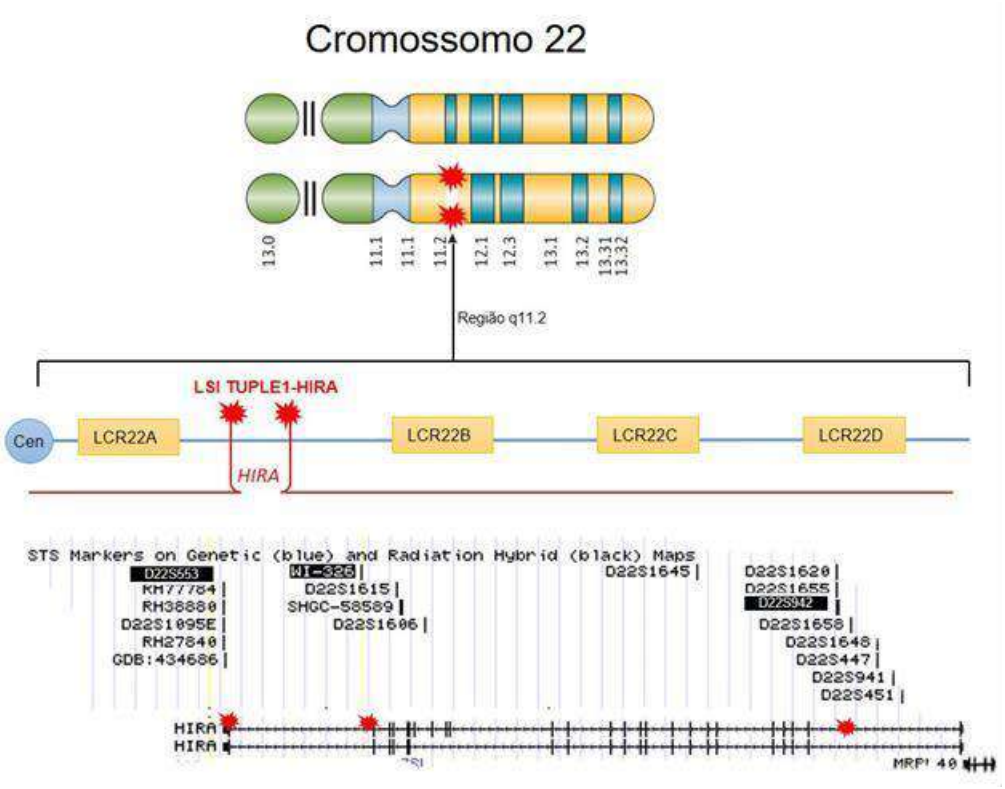
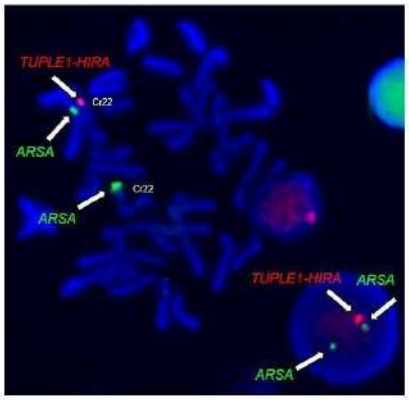


Figure 2. Localização dos microssatélites de DNA presentes na sonda TUPLE1-HIRA. Adaptado de McDonald-McGinn et al., 2015; USCS genome browser (http://genome.uscs.edu).[25]

a)



b)

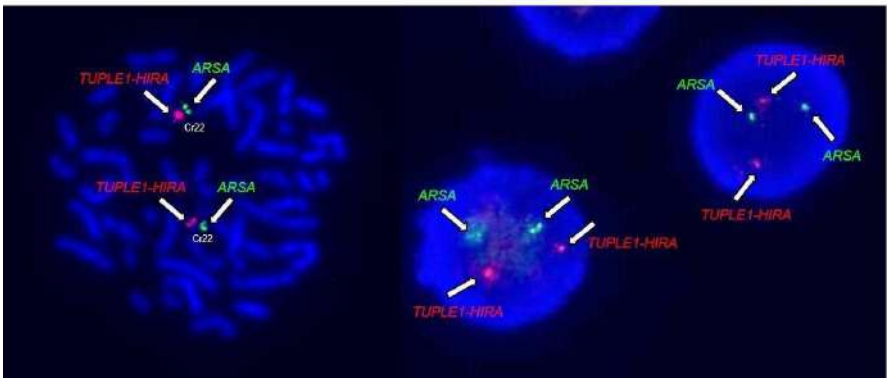


Figure 3. Imagens de análise da FISH do Laboratório de Citogenética da UFCSPA. a) Células alteradas: ausência do sinal vermelho (TUPLE1-HIRA) em metáfase e em intérfase, indicando deleção do locus HIRA em 22q11.2. b) Células normais: núcleos de intérfase e metáfase de células normais apresentando 2 sinais vermelhos (TUPLE1-HIRA) e dois sinais verde (ARSA) distintos.

Outras técnicas, como a amplificação de sonda multiplex dependente de ligação (MLPA) e o array-CGH são úteis para definir o tamanho da deleção. O array-CHG permite detectar rearranjos cromossômicos muito pequenos e inicialmente esta abordagem era feita para identificar desequilíbrios cromossômicos através da detecção de

CNVs em tumores, no intuito de distinguir genes que poderiam estar envolvidos na patogênese do câncer.[37,38] Pouco depois, começou então a ser utilizada como uma poderosa tecnologia genômica, auxiliando o diagnóstico clínico de pacientes que apresentavam atraso mental idiopático, deficiência intelectual, anormalidades congênitas, esquizofrenia e outros transtornos neuropsiquiátricos. Através desta técnica foi possível analisar o genoma inteiro em alta resolução, detectando variações até então desconhecidas.[39]

Em relação à detecção de genes com deleções/duplicações, podemos destacar a técnica de MLPA. Por ser um método sensível e rápido, simples e econômico, é indicado para caracterizar desordens congênitas e hereditárias, analisando quantitativamente mais de 40 dosagens diferentes de sequências em uma única reação de PCR.[40] Além disso, o MLPA pode analisar até 96 amostras simultaneamente, com resultados em até 24h, seguindo o protocolo descrito em basicamente quatro passos (desnaturação, hibridização do DNA, reação de ligação e amplificação por PCR). Já para interpretação dos resultados, é necessário realizar etapas de separação dos produtos por eletroforese capilar e a análise dos dados por programas específicos. Mais de 300 sondas (probes) estão sendo comercializadas para a investigação de doenças genéticas, porém a técnica é incapaz de detectar rearranjos balanceados e mosaicismos de baixo grau, além de ser sensível a qualidade da amostra de DNA testada.[41,42,43]

No Brasil, embora o Sistema Único de Saúde (SUS) realize alguns testes genéticos dependendo da condição apresentada, a falta de

disponibilização destes exames diagnósticos em grande escala, torna-se quase impossível garantir um bom aconselhamento genético para a população. Assim, a possibilidade de realização de exames como FISH, MLPA, aCGH/SNP array, sequenciamento de genes ou do exoma é muito dependente de projetos de pesquisa. A realização de testes genéticos específicos possibilita a obtenção de diagnósticos mais precisos, além de se entender melhor a gênese do fenótipo cardíaco, levando a um planejamento terapêutico mais otimizado.

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3. OBJETIVOS

GERAL:

Ampliar o a análise molecular de pacientes cardiopatas e dismórficos com suspeita ou diagnosticados previamente com síndrome de DiGeorge.

ESPECÍFICOS:

a) Analisar as metodologias moleculares utilizadas para o diagnóstico da síndrome de DiGeorge em associação com a cardiopatia congênita através de uma revisão sistemática.
b) Determinar o tamanho de deleção e genes envolvidos através da técnica de MLPA em pacientes com diagnóstico molecular para síndrome de DiGeorge após triagem pela FISH.
c) Associar genes alterados identificados através da técnica de MLPA com o fenótipo cardíaco de pacientes com síndrome de DiGeorge.

4. ARTIGO CIENTÍFICO REDIGIDO EM INGLÊS

4.1 Artigo 1 - publicado

"Congenital Heart Defects and 22q11.2 Deletion Syndrome: A 20-Year Update and New Insights to Aid Clinical Diagnosis"

Bruna Lixinski Diniz, Desirée Deconte, Kerolainy Alves Gadelha, Andressa Barreto Glaeser, Bruna Baierle Guaraná, Andreza Ávila de Moura, Rafael Fabiano Machado Rosa, Paulo Ricardo Gazzola Zen.

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Congenital Heart Defects and 22q11.2 Deletion Syndrome: A 20-Year Update and New Insights to Aid Clinical Diagnosis

Bruna Lixinski Diniz[1] Desirée Deconte[1] Kerolainy Alves Gadelha[2] Andressa Barreto Glaeser[1] Bruna Baierle Guaraná[1,3] Andreza Ávila de Moura[2] Rafael Fabiano Machado Rosa[1,3] Paulo Ricardo Gazzola Zen[1,3]

[1] Graduate Program in Pathology, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil
[2] Underdegree Program in Biomedicine, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil
[3] Department of Internal Medicine, Clinical Genetics Service, Federal University of Health Sciences of Porto Alegre (UFCSPA) and Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Porto Alegre, RS, Brazil

Address for correspondence Paulo Ricardo Gazzola Zen, PhD, Clinical Genetics Service/UFCSPA, Rua Sarmento Leite, 245/404–Bairro Centro, Porto Alegre, RS – Brasil CEP, 90050-170, Brasil (e-mail: paulozen@ufcspa.edu.br).

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Abstract

Congenital heart defects (CHDs) are one of the most prevalent clinical features described in individuals diagnosed with 22q11.2 deletion syndrome (22q11.2DS). Therefore, cardiac malformations may be the main finding to refer for syndrome investigation, especially in individuals with a mild phenotype. Nowadays, different cytogenetic methodologies have emerged and are used routinely in research laboratories. Hence, choosing an efficient technology and providing an accurate interpretation of clinical findings is crucial for 22q11.2DS patient's diagnosis.

This systematic review provides an update of the last 20 years of research on 22q11.2DS patients with CHD and the investigation process behind each diagnosis. A search was performed in PubMed, Embase, and LILACS using all entry terms to DiGeorge syndrome, CHDs, and cytogenetic analysis. After screening, 60 papers were eligible for review. We present a new insight of ventricular septal defect as a possible pivotal cardiac finding in individuals with 22q11.2DS. Also, we describe molecular technologies and cardiac evaluation as valuable tools in order to guide researchers in future investigations.

Keywords
- 22q11.2 deletion syndrome
- congenital heart defects
- DiGeorge syndrome
- ventricular heart septal defects

Introduction

22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge syndrome (OMIM #188400), is characterized by a microdeletion in the long arm of chromosome 22. The estimated incidence of this disorder ranges from 1 per 2,000 to 1 per 7,000 births.[1] 22q11.2SD is known for its clinical and molecular heterogeneity. Around 90 genes have been described in 22q11.2DS and more than 180 clinical features are reported. However, several aspects of this syndrome are still unclear and in need of further analysis to elucidate both molecular and clinical characterization.[2]

Congenital heart defects (CHDs) are the most prevalent characteristics among individuals diagnosed with 22q11.2DS and may be the main finding to refer for syndrome investigation.[3] CHDs, also known as a congenital heart

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Georg Thieme Verlag KG,
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70469 Stuttgart, Germany

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anomaly and congenital heart disease, are a group of structural anomalies of the heart and great vessels that rise during cardiac development.[4,5] About 20% of patients with CHD have a genetic or a chromosomal abnormality: Down syndrome is the most common condition associated with CHD, followed by 22q11.2DS.[6] Overall, 60 to 80% of the individuals diagnosed with 22q11.2DS have some cardiac malformation that can vary in severity, which includes many different aortic arch and/or cardiac outflow tract anomalies.[3-5]

Different cytogenetic methodologies have emerged and are in routine use in research laboratories. Fluorescence in situ hybridization (FISH) is the gold standard method for 22q11.2DS diagnosis. However, in the last two decades, multiplex ligation-dependent probe amplification (MLPA) and microarray analysis (array-comparative genomic hybridization [CGH] and single-nucleotide polymorphism [SNP] array) became effective tools alongside FISH.[7-11] The increase in the use of molecular technologies allowed individuals to be diagnosed more frequently, providing new insights into the molecular mechanism behind 22q11.2DS. Different methodologies are able to detect genome variants in individuals with phenotypes that vary from minimal clinical presentations to complex and severe physical manifestations.[2,6,10] However, the molecular and clinical heterogeneity observed within individuals with 22q11.2DS made the genotype-phenotype correlation challenging. Hence, understanding which technology will provide a better and efficient deoxyribonucleic acid screening as well as how to interpret clinical findings is essential to provide an assertive and accurate diagnosis for 22q11.2DS patients.[1,5] This systematic review provides an update of the last 20 years of research on 22q11.2DS patients with CHD and the investigation process behind each diagnosis.

Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines. We systematically searched PubMed (Medline), EMBASE, and BVS (Lilacs/Medline) for articles published between January 1, 2000 and January 1, 2020. DeCS, MeSH, and Emtree descriptors were used to index articles with the following terms: 22q11 deletion syndrome (22q11.2DS), CHDs, and cytogenetics methodologies. The exact search terms employed are provided in online ►Supplementary Methods S1. All results of the systematic selection process are illustrated in a PRISMA flowchart (►Fig. 1).

Articles were selected in a two-steps analysis: title and abstract screening followed by a full-text read. Authors were divided in three pairs for independent screening and further discussion of potential disagreements. If the disagreement remained, a "senior reviewer" decided if the study would be included or excluded. In order to reduce a potential bias, all three pairs were rearranged between the first and second step. Inclusion criteria for the first step were as follows: have a case or cases of 22q11.2DS with any indication of CHD involvement. In the second step the inclusion criteria encompassed 22q11.2DS cases that were diagnosed clinically and molecularly with a well-delineated CHD. In studies with multiple patients, only individuals with both 22q11.2DS and CHD were included. For all cytogenetics techniques performed, only studies that had complete information about molecular analysis were included. The studies should provide probes, kits, and/or the genomic address of the deletion in order to be eligible. Only articles written in English were included. Additional inclusion and exclusion criteria are described in ►Fig. 1.

Data Extraction

Published metadata were obtained using a data extraction template that was created and modified according to all the studies reviewed. The publication details were captured and summarized in a tabular format developed by the authors of this review. The data extracted from all articles were as follows: number of patients, age, type(s) of CHD(s), molecular technique(s) performed, genetic alterations found in each patient, and probe(s) or kits used.

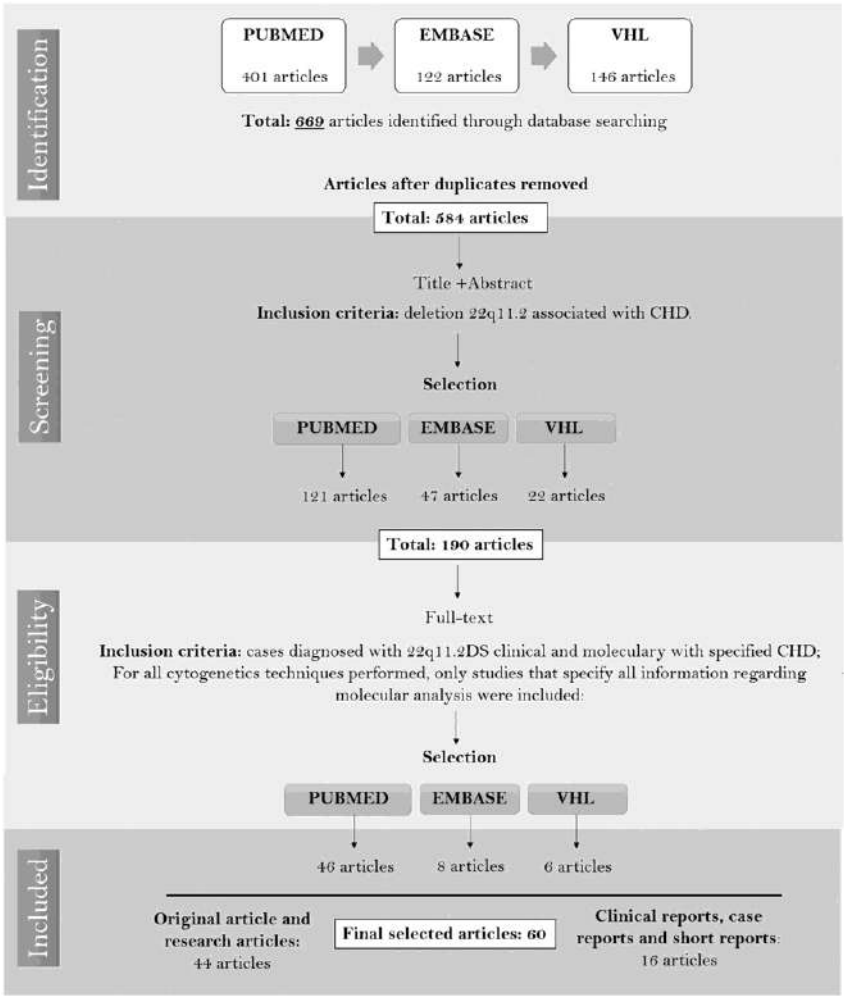
Result

Sixty articles (►Supplementary Material S2) were considered eligible for analysis. The included studies varied between original article, research articles, clinical reports, case reports, and short reports. Data from 657 cases diagnosed (clinically and molecularly) with 22q11.2DS and CHD were obtained.

Congenital Heart Defects and 22q11.2DS

Individuals with 22q11.2DS presented different cardiac malformations. Cases with single and multiple CHDs were described. For further analysis, we classified all included patients into five groups: group 1 (G1): individuals with 1 CHD; group 2 (G2): individuals with two CHDs; group 3 (G3): individuals with three CHDs; group 4 (G4): individuals with four CHDs; and group 5 (G5): individuals with five CHDs.

G1 comprised the majority of 22q11.2DS individuals (60.7%). Tetralogy of Fallot (TOF) and ventricular septal defect (VSD) were the most prevalent heart defects described in all groups (►Fig. 2). TOF was observed in 50.5% of the individuals diagnosed with 22q11.2DS, while 25.9% had VSD. However, VSD is one of the four cardiac malformations that comprises TOF.[12,13] Therefore, VSD when isolated, with other CHD or as a part of TOF was the main cardiac defect found in 22q11.2DS cases (76.4%, $n = 502$). Double-chambered right ventricle, double aortic arch, cervical aortic arch, mitral valve dysplasia, and hypoplastic right heart syndrome were described only as an isolated heart defect. On the other hand, major aortopulmonary collateral arteries, aberrant left subclavian artery, patent foramen ovale, aortic-pulmonary collateral arteries, persistent left superior vena cava, aberrant right subclavian artery, tricuspid atresia, transposition of the great arteries, overriding aorta, and aortic valve were observed only in association with other CHDs. The remaining CHDs were described in either ways (isolated or in association with other CHDs) (►Table 1).



Studies should inform the following informations in order to be eligible:

:

CHD → describe which type of CHD(s) each individual diagnosed with 22q11DS have.

Karyotype → inform the karyotype result (normal or abnormal); when abnormal, the study should describe the alteration found.

FISH → describe the probe used (comercial probes) or the designed region (in house probes) as well as FISH results (normal or abnormal). When more than one probe was used, the study should describe which probe detected the deletion.

MLPA → brief which kit was used as well as MLPA results (normal or abnormal); when abnormal the results should specify which genes were deleted.

CMA (array-CGH or SNP-array) → show the results whether normal or abnormal; when abnormal the study should inform the start-stop region of the deletion (genomic address and deletion size).

STR analysis → inform which STRP markers were used and the results found; when altered, the study should provide the information regarding which markers were deleted.

Exclusion criteria (First (title + abstract) and second (full-text) selection steps):

1. DiGeorge type 2 syndrome;
2. 22q11 distal deletion syndrome;
3. Translocations/duplication/microduplication/triplication or any other alteration associated with 22q11.2DS;
4. Overlapping syndromes or any other molecular alteration that is not associated with 22q11.2DS;
5. Articles focus on molecular techniques review;
6. No abstract available;
7. No access to full-text article;

Fig. 1 Flowchart of the study selection process for eligible articles.

Molecular technologies used for 22q11.2DS analysis included karyotype, FISH, MLPA, array-CGH, and SNP array. Polymerase chain reaction (PCR) and gene sequencing were performed only for validation of results.[14,15] Short tandem repeat (STR) analysis was carried out alongside FISH in three studies.[16-18] Karyotype did not show any deletion. Therefore, it was considered as null when performed with other technologies.

The use of a single molecular technique was enough to diagnose the 22q11.2DS in 86.7% (n ¼ 570) of the individuals. Note that 12.8% were diagnosed by two methodologies while 0.5% used three different technologies to confirm the deletion. Array-CGH, SNP array, and STR analysis were able to detect a deletion in all 22q11.2DS individuals whenever they were performed (►Table 2).

FISH diagnosed 613 individuals with 22q11.2DS by commercial probes (DiGeorge/VCFS TUPLE1 probe, DiGeorgeTBX1 probe, and/or DiGeorge Region N25 probes), bacterial artificial chromosome (BAC) clones, and/or cosmids. Probes were used individually or combined and all commercial probes had a high detection rate (►Table 3). MLPA, array-CGH, and SNP array were used alongside FISH to either confirm the diagnosis or enhance the results by searching for other deleted genes and break-points.[19-21] When FISH failed to diagnose, these three methodologies were applied to follow-up the investigation.[15,21-23]

MLPA diagnosed 98.6% of the 22q11.2DS individuals. SALSA MLPA Probemix P250 DiGeorge and SALSA MLPA Probemix P311 Congenital Heart Disease were the two kits used for investigation. *CDC45*, *GP1BB*, and *DGCR8* are

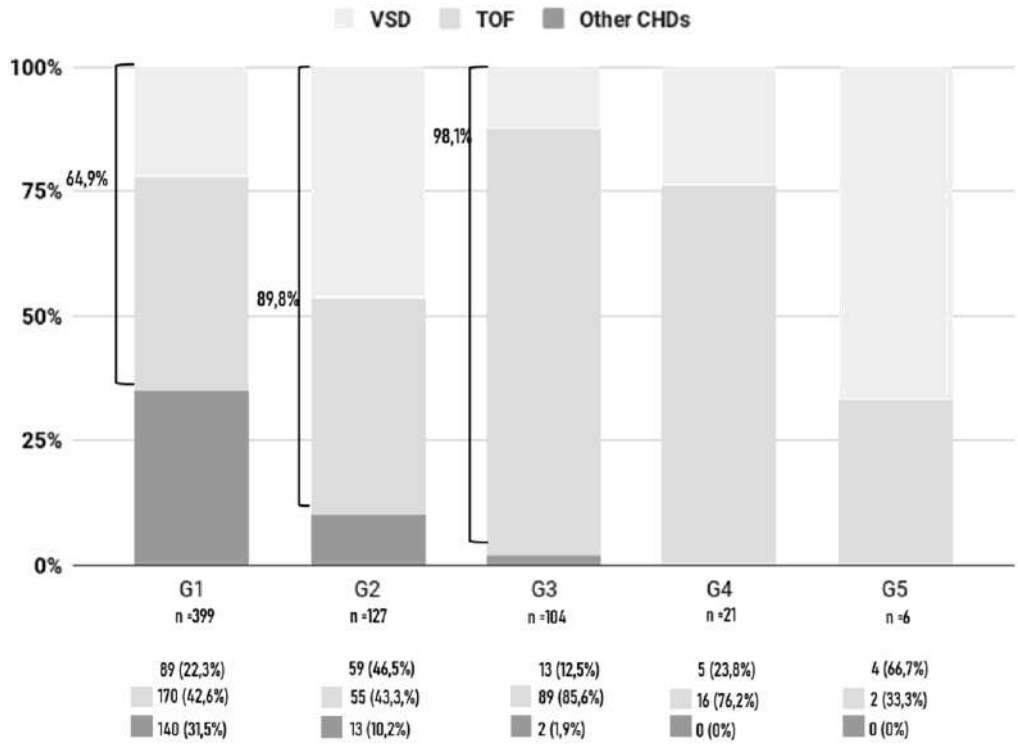


Fig. 2 Ventricular septal defect (VSD) and tetralogy of Fallot (TOF) prevalence among all five congenital heart defects (CHDs) groups. Square brackets point out VSD plus TOF percentage in all deleted individuals of each group.

common genes between both kits. SALSA MLPA Probemix P311 comprises genes that are associated with CHDs, but not necessarily located in the 22q11 region. However, our results showed that only the genes located within the 22q region were found deleted (*CDC45*, *GP1BB*, and *DGCR8*). SALSA MLPA Probemix P250 detected all the deletions. Low copy repeat (LCR)-A to LCR-B covered the location of most deletions found in patients with 22q11.2DS. Deleted genes are described in ►Fig. 3.

Array-CGH and SNP array were performed in 36 individuals and detected 100% of the deletions. Start-stop sequence wide-ranged from chr22:17.2 to chr22:22.1. STR analysis was always used with FISH as a confirmatory approach. D22S1638, D22S941, D22S1623, D22S311, D22S1709, D22S1648, D22S1144, D22S264, D22S306, D22S944, D22S303, D22S301, D22S308, D22S156, D22S257, D22S425, D22S427, and TOP1P2 were all markers used to investigate 22q11.2 deletion syndrome. D22S1144, D22S420, and TOP1P2 were the only three markers that did not detect deletions in any patient. Markers' specific locations within the 22q11 region were not provided by the authors. Due to regular updates in Genome Browser we could not infer the location as well.

VSD x Molecular Methodologies

VSD (isolated, with other CHD or as a part of TOF) was the main CHD described in individuals diagnosed by FISH (76% [*n* ¼ 466]) with 91.8% of the deletions detected by N25 probe (►Table 3). In individuals diagnosed with MLPA, 78.3% (*n* ¼ 54) had VSD. LCR-A to LCR-B covered the location of most deletions while genes located between LCR-D and LCR-E (*HIC2*, *PPIL2*, and *TOP3B*) were rarely deleted (►Fig. 3). Among the individuals diagnosed by array-CGH and SNP array, 88.8% (*n* ¼ 32) had VSD. In these patients 19 different deletions were found. Although deletion breakpoints were heterogeneous, a common deleted location between chr:22:18.9 and chr22:21,4 (LCR A to LCR D) was observed in the majority of the 22q11.2DS individuals (►Fig. 4).

Discussion

22q11.2 deletion syndrome is the second most common cause of CHD, developmental delays, and syndromic palatine anomalies in children.[4] Literature shows that TOF and pulmonary atresia with VSD are regular findings in patients with 22q11.2DS. However, their prevalence is yet to be established.[24] Due to 22q11.2DS heterogeneous phenotype, individuals with that disorder are usually hard to diagnose. Moreover, the genotype spectrum in 22q11.2DS is diverse. In order to elucidate the clinical and molecular correlation of this syndrome, we analyzed the last 20 years' of research on 22q11.2DS patients with CHD and the investigation process behind each diagnosis. We reviewed all molecular technology used in the 22q11.2DS investigation in order to provide an improved and accurate diagnosis—which makes our findings relevant.

VSD and TOF

TOF is the most prevalent cyanotic congenital heart disease. However, the exact cause of this cardiac malformation remains unknown. TOF is a combination of four related heart defects: OA, right ventricular outflow tract (RVOT)

Table 1 CHDs described in all 22q11 deletion syndrome patients included in the study

CHD	*n*	%
TOF	332	50.5
VSD	170	25.9
PA	153	23.4
MAPCA	76	11.5
RAA	63	9.6
TA	49	7.4
ASD	48	7.3
PDA	44	6.7
IAA	42	6.3
DORV	18	2.7
APV	9	1.3
PS	8	1.2
ALSA	7	1.0

Abbreviations: ALSA, aberrant left subclavian artery; APCA, aortic-pulmonary collateral arteries; APV, absent pulmonary valve; ARSA, aberrant right subclavian artery; ASD, atrial septal defect; AVI, aortic valve insufficiency; BAV, bicuspid aortic valve; CAA, cervical aortic arch; CHD, congenital heart defect; CoA, coarctation of aorta; DAA, double aortic arch; DCRV, double chambered right ventricle; DORV, double-outlet right ventricle; HRHS, hypoplastic right heart syndrome; IAA, interrupted aortic arch; MAPCA, major aortopulmonary collateral arteries; MVD, mitral valve dysplasia; MVP, mitral valve prolapse; OA, overriding aorta; PA, pulmonary atresia; PDA, patent ductus arteriosus; PFO, patent foramen ovale; PLSVC, persistent left superior vena cava; PS, pulmonary stenosis; RAA, right aortic arch; TA, truncus arteriosus; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; TRC.A, tricuspid atresia; VSD, ventricular septal defect.
Note: CHDs < 1%: CoA; PFO; APCA; PLSVC; BAV; ARSA; TRC.A; DCRV; TGA; DAA; MVP; OA; CAA; AVI; MVD; HRHS.

obstruction, right ventricular hypertrophy, and VSD.[12,13] VSD is a defect in the septum between the right and left ventricle and the extent of the opening may vary from a pin size to a complete absence of the ventricular septum, creating one common ventricle.[25]

We identified that TOF (50.5%) and VSD (25.9%) were the two most described cardiac malformations in individuals diagnosed with 22q11.2DS (►Table 1). Ryan et al[26] and Botto et al[27] estimated that VSD when isolated from other CHDs has a prevalence of 14 and 20%, respectively. In our study, isolated VSD was found in 13.5% (*n* ¼ 89) of the cases, a similar percentage to the abovementioned studies. However, VSD is found not only isolated but with other CHDs as well. Therefore, it is important to analyze the frequency of this cardiac finding as both isolated and in association with other CHDs to provide a reliable analysis of 22q11.2DS etiology.

VSD may play an important role in the development of other cardiac malformations that are related to TOF. The interventricular septum constitutes a major part of the heart and contributes to both left and right ventricular physiological function. Congenital anomalies of the interventricular septum may vary from the absence of the septum to a small and hemodynamically insignificant VSDs.[28,29] During cardiac development, TOF occurs when the conal or infundibular portion of the ventricular septum is displaced anteriorly into the RVOT. This displaced septum projects into the pulmonary outflow tract, often resulting in obstruction and hypoplasia of downstream structures, including the pulmonary valve, main pulmonary artery, and pulmonary artery branches.[30,31] Since VSD is an error in the interventricular communication (interventricular septum is one of the first to close in the heart development),[28] it could be responsible for the progression of other malformations within TOF. This highlights the importance of the VSD in 22q11.2DS cases, whether as isolated, with other CHD, or within TOF.

If we consider that VSD is a part of TOF, VSD becomes the most observed CHD in 22q11.2DS individuals (76.9%). Ryan et al[26] and Botto et al[27] found that 41 and 66% of the patients had VSD (isolated and in association with other CHDs), respectively. Both studies used FISH or microsatellite markers for diagnosis (the majority of patients were investigated for a single probe), but no conclusions could be drawn between the size of the deletion and phenotype. Since a variety of molecular-genetic approaches were implemented in laboratory routine in early 2000s,[32,33] some cases of Ryan

Table 2 Individual's molecular investigation stratified by methodologies

		Individuals tested	FISH þ	MLPA þ	Array þ	STRþ
Single methodology	FISH	542	542			
	MLPA	12		12		
	Array (CGH/SNP)	16			16	
Two methodologies	FISH þ MLPA	47	44	47		
	FISH þ Array (CGH/SNP)	8	5		8	
	FISH þ STR	20	20			20
	MLPA þ Array (CGH/SNP)	9		9	9	
Three methodologies	FISH þ MLPA þ Array (CGH/SNP)	3	2	2	3	
	Total	657	613	70	36	20

Abbreviations: CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification; SNP, single-nucleotide polymorphism; STR, short tandem repeat.
Note: STR was performed as a confirmatory tool for FISH results.

Table 3 Summary of all FISH probes and MLPA kits used to diagnose 22q11.2 individuals

FISH probes[a]	22q11.2DS cases investigated	22q11 region of interest	Deleted individuals	Deleted individuals with VSD (isolated or with other CHD or as a part of TOF)
Commercial FISH probes				
DiGeorge/VCFS TUPLE1 probe	547	HIRA gene, D22S55, D22S609, and D22S942 markers	539 (98.5%)	402 (74.5%)
DiGeorgeTBX1 probe	5	TBX1	4 (80%)	2 (50%)
DiGeorge Region N25 probe	129	D22S75 marker and the centromeric end of the CLTCL1 gene	123 (95.3%)	113 (91.8%)
BAC clones	29	Genes: HIRA; PI4KA; SNAP29, CRKL; AIFM; UFD1L; COMT; HCF2. Markers: D22S1649; D22S1694; D22S264; D22S935; D22S111; D22S553; D22S941; D22S163.	29 (100%)	22 (75.8%)
Cosmids				
Sc11.1	4	DGCR6	4 (100%)	4 (100%)
MLPA kits[a]				
	22q11.2DS cases investigated	22q11 region of interest		Individuals
SALSA P311	6	BID; CDC45, CLDN5		3 (50%)
SALSA P250	67	CDC45, CLDN5, CLTCL1, DGCR8, GNAZ, GP1BB, HIC2, HIRA, IL17RA, KLHL22, LZTR1, MED15, MICAL3, PPIL2, RAB36, RSPH14, SLC25A18, SMARCB1, SAP29, SNRPD3, TBX1, TOP3B, TXNRD2, USP18, ZNF74		67 (100%)

Abbreviations: CHD, congenital heart defect; FISH, fluorescence in situ hybridization; MLPA, multiplex ligation-dependent probe amplification; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
Note: MLPA bolded genes: deleted. For more details refer to Fig. 3.
[a]Patients in some studies were tested for more than one probe and/or kits.

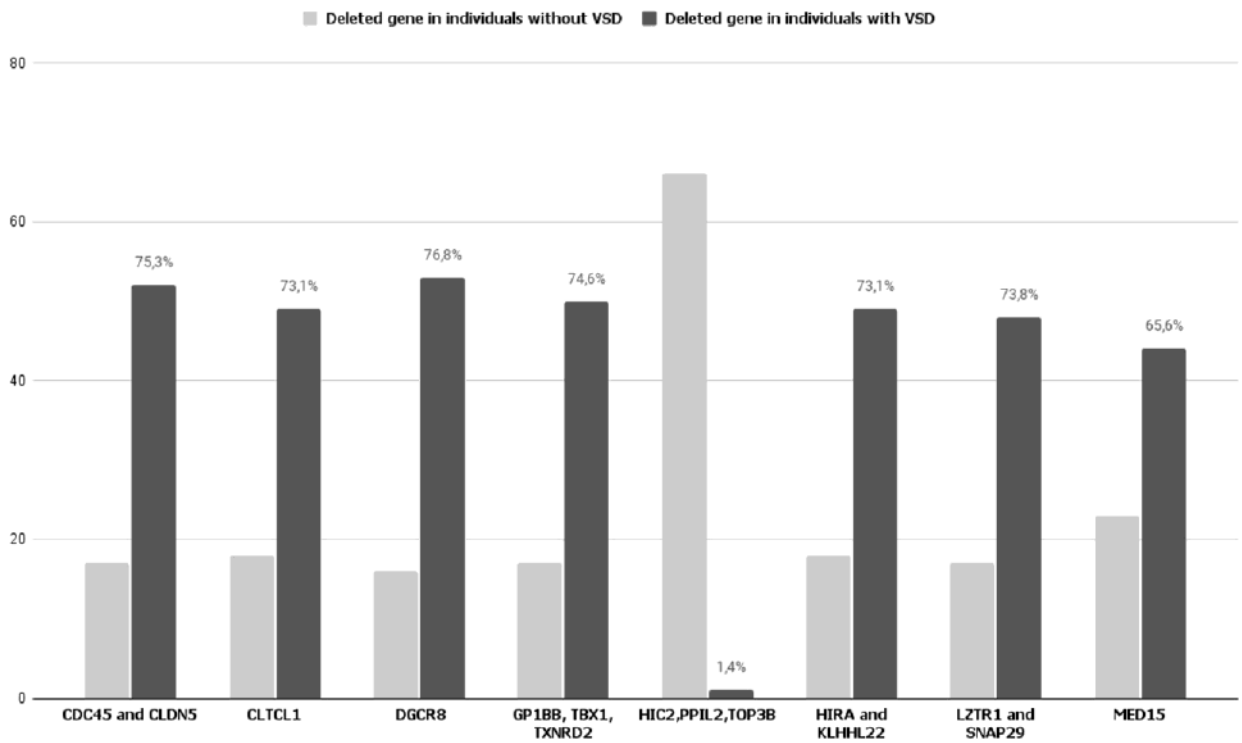


Fig. 3 Multiplex ligation-dependent probe amplification (MLPA) deleted genes in individuals with and without ventricular septal defect (VSD) (n ¼ 71).

et al[26] and Botto et al[27] may have been underdiagnosed. The evolution of echocardiography and the technology update resulted in a better interpretation of cardiopathies based on the highest definition of both anatomy and cardiac function.[34] We combined the results obtained from a single molecular technique with the combined use of three complementary technologies, which allowed a larger number of VSD cases in comparison with previous studies.

Overview of Molecular Technologies

FISH is currently the golden standard cytogenetic method for 22q11.2DS diagnosis due to its high sensitivity and relatively low cost. The usual 22q commercial probes target the segment between LCR22A and LCR22B, but cannot precise the size of the deletion. On the other hand, other techniques such as MLPA, array-CGH, and analysis of copy number variation (CNV) are useful to define the size of the deletion.[35] In this review, karyotype, FISH, MLPA, array-CGH, SNP array, and STR analysis were identified as molecular tools for 22q11.2DS investigation.

Only three studies applied STR analysis, but all used FISH to confirm the results.[16–18] STR markers are used to define the size and the parental origin of the deletion in order to identify possible 22q11.2DS familial cases. However, the identification and the extensive characterization of 22q11.2 deletions is possible only if the STRs analysis is extended to the parents.[36] Microsatellites precise linkage location were hard to determine due to the lack of primary information to interpret the results. Markers are constantly updated through genome databases as well, varying the genomic location and hindering the methodology's replicability. 22q11.2DS has a heterogeneous molecular diagnosis, therefore, in order to replicate the methodology applied in previous successful studies it is essential to perform a proper investigation and aid the molecular elucidation of this syndrome.

FISH was the most performed technique used to investigate 22q11.2DS. However, array-CGH and SNP array—also known as chromosomal microarray analysis (CMA)—were the two exclusive methodologies that achieved a 100% detection rate. CMA can detect submicroscopic CNVs, which enables whole-genome screening for chromosomal imbalances at a higher resolution than is possible with conventional karyotyping. Also, CMA is gradually replacing traditional karyotype analysis in the prenatal setting.[11] CNVs can be also detected by chromosomal analysis and FISH. Although large CNVs have been described through chromosomal analysis and FISH, microarray technologies were responsible for a great number of smaller CNVs addition in CNVs databases.[37] Previous clinical studies have indicated a large number of CNVs diagnostically associated with CHDs and have recommended that CMA should be used as the first-line genetic diagnostic test for CHD patients.[38,39] However, there is limited knowledge about the involvement of CNVs in isolated or nonisolated VSDs 22q11.2 deletion.[3,11]

In this study, we observed that molecular alterations found by CMAs in individuals with 22q11.2DS and CHD ranged from region 17.2 to region 22.1 of chromosome 22q11. All VSD cases were spotted throughout this region as well. However, a prevailing region (chr:22:19,0 to chr:22:22,1) was observed in these individuals, which may indicate a possible hotspot location for VSD molecular causes (►Fig. 4). This finding supports the hypothesis that VSD is the main cardiac malformation described in 22q11.2DS and may be the most relevant clinical finding to initiate 22q11.2DS molecular investigation. Therefore, further studies on VSD and 22q11.2DS should be done in order to deepen our understanding of this correlation.[40]

MLPA is a multiplex PCR method, detecting small scale CNVs, partial deletions/duplications of specific genes. It is widely used for the validation of array CGH results and screening CNVs in known genes.[41,42] Compared to microarrays, MLPA has a low cost and is easier to use.[43] The studies included in this review used two different MLPA kits: P311 and P250. Probemix P311 kit is exclusive for reference genes associated with cardiac malformations. However, only *CDC45*, *GP1BB*, and *DGCR* are located within the 22q11 region. Therefore, the use of P311 is more indicated when no alterations in chromosome 22q11 were found. On the other hand, Probemix P250 is specific for DiGeorge syndrome investigation, including reference genes and LCRs for both type 1 and 2. Compared to P311, P250 diagnosed more individuals with both 22q11.2DS and CHD (►Table 3).

The following genes analyzed by P250 MLPA kit were not found deleted in any of the cases investigated: *BID*, *GNAZ*, *IL17RA*, *MICAL3*, *RAB36*, *RSPH14*, *SLC25A18*, *SMARCB1*, *SNRPD3*, and *USP18*. This result may indicate that these genes might not be involved in the molecular etiology of both 22q11.2DS and CHD. *DGCR8* was the most deleted gene in individuals with VSD (►Fig. 3). The inclusion of MLPA in clinical settings as a complementary or screening method can significantly increase the detection rate of CNVs.[43,44] Molecular alterations found either by CMAs or MLPA reinforces that genes located between the distal region of LCR22A and the distal region of LCR22D may be important genetic keys for cardiac development during embryogenesis, primarily in individuals with 22q11.2DS and VSD.

FISH tests use commercial probes such as TUPLE1, TBX1, and N25 for chromosomal investigation (►Table 3). In individuals with VSD, probe N25 had the highest detection percentage (91.8%). Botto et al[27] used probe N25 to detect the deletion in all individuals with 22q11.2DS. However, Fernández et al[45] also used this probe and failed to detect deletions in the included patients. In these cases, array-CGH was performed as a complementary tool to screen possible molecular alterations and was able to find a deletion in a more distal region of the chromosome. The study[45] revealed a deleted region (chr22:19.0 ~ 20.8) in a patient with 22q11.2DS and VSD similar to those found in this review. The author also pointed out *SNAP29*, *LZTR1*, and *HIC2* (genes outside the probe N25 range detection) as possible candidate genes that could be involved in 22q11.2DS phenotype. Therefore, we reinforce the importance of complementary molecular methodologies in the investigative process of heterogeneous syndromes in order to provide a more assertive diagnosis. FISH, for example, is unable to screen a wide

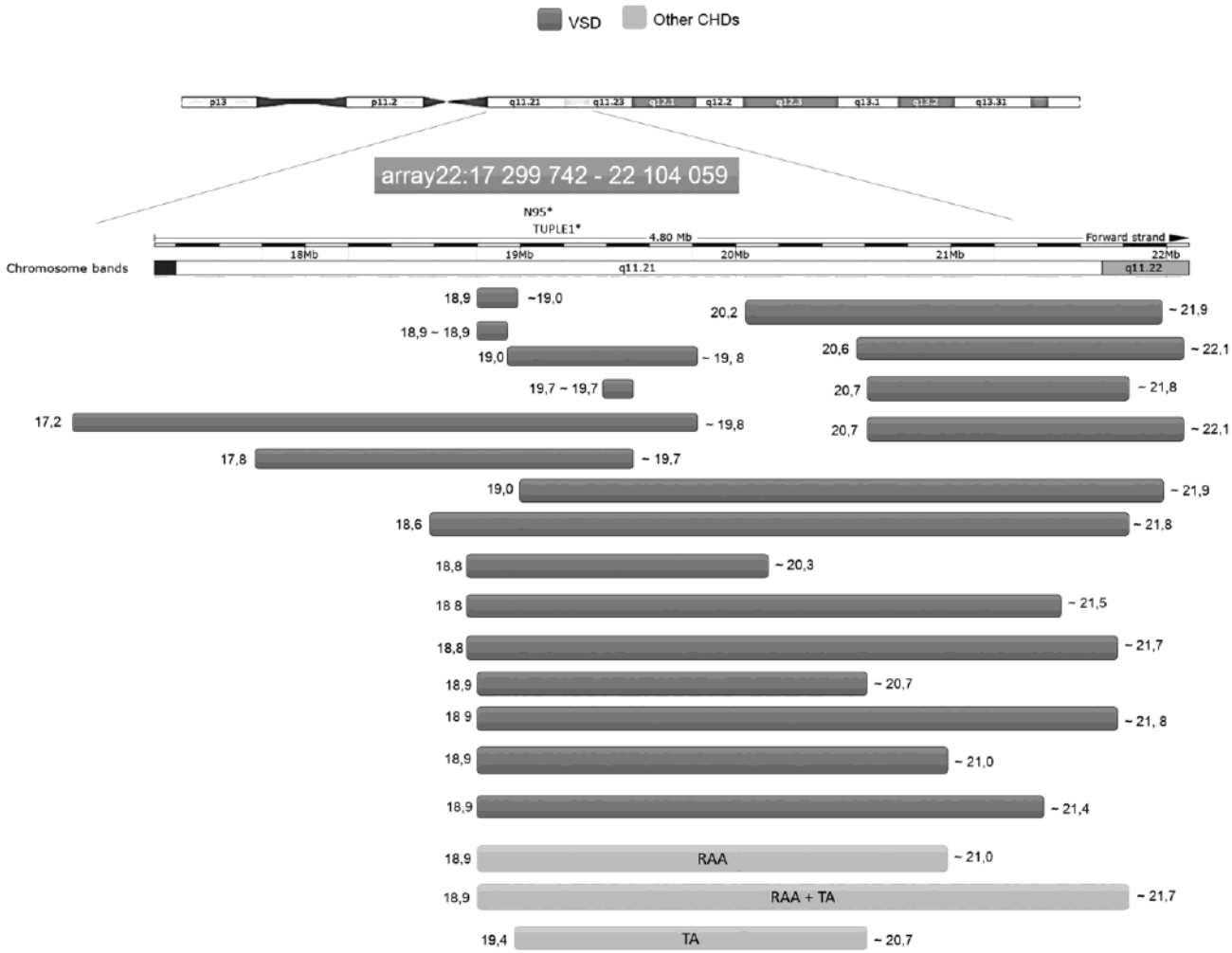


Fig. 4 Genomic addresses (CRCh37/hg19) of all cases (n ¼ 36) diagnosed with 22q11.2DS by array-comparative genomic hybridization (CGH) and/or single-nucleotide polymorphism (SNP) array. LCR A: chr22:18,640,00 - 18,910,000; LCR B: chr22:20,250,000 - 20,680,000; LCR C: chr22:21,020,000 - 21,090,000; LCR D: chr22: 21,470,000 - 21,920,000; LCR E: 22,960,000 - 23,050,000; LCR F: 23,650,000 - 23,820,000. ωN25 (chr22:19,166,986 - 19,279,239); TUPLE1 (chr22:19,318,221 - 19,435,224).

portion of the 22q11 region and, sometimes, can underdiagnose some patients.

TUPLE1 is the gold standard probe used in FISH for 22q11.2DS diagnosis. However, our review showed that the N25 probe seems to be more assertive when investigating individuals with CHDs, especially VSD. *HIRA*, also known as *DGCR1* or *TUPLE1*, is considered a candidate gene in some haploinsufficiency syndromes such as 22q11.2DS, and its insufficient activity may interrupt normal embryonic development. Research to elucidate *HIRA* mechanisms and role in human cells were performed, but its activity is still not clear.[46] Therefore, CHD may not be the best clinical finding to insight TUPLE1 probe investigation. Individuals suspicious of 22q11.2DS that have other malformation such as facial dysmorphism and hypocalcemia may have a more successful diagnosis with TUPLE1 probe. BACs clones, an in-house alternative for FISH probes, were also used in some studies. However, this type of methodology needs to go under validation processes as well as to be standardized according to the AGT Cytogenetics Laboratory Manual.[47] Hereby, the use of BACs clones is a labor-intensive technique that demands a large amount of time and cannot be applied for a large group analysis.

Our review suggests that VSD may be the main CHD found in individuals with 22q11.2DS. Moreover, the chromosome location between chr:22:19.0 and chr:22:22.1 may be a hotspot region for genes associated with cardiac malformation. CMA seems to be a more sensitive technique to molecularly diagnose 22q11.2DS individuals with VSD and mild phenotype.[8] Moreover, microarrays can be used when karyotype and FISH results are normal and with no abnormalities.[48] Whole-exome sequencing should be also considered when array analysis results are negative.[40]

MLPA is a cost-effective method and can also detect smaller deletions missed by FISH, especially when classic clinical findings are absent in the individual phenotype.[7] Therefore, when choosing MLPA as a diagnostic tool, P250 should be the primer choice for 22q11.2DS associated with CHD investigations. Moreover, P311 should be used as an alternative kit when no molecular alterations were found in

the 22q11 region. In order to aid researchers in future investigations regarding 22q11.2DS associated with CHDs we propose a small—and hopefully helpful—guideline.

Guideline Highlights

Light or mild phenotype þ *CHD*. Methodologies: CMA and MLPA.

CMA seems to be a more sensitive technique to molecularly diagnose 22q11.2DS individuals with VSD and light/mild phenotype. If the technology is not available, we suggest the MLPA SALSA P250 kit to proceed with the investigation. When P250 fails to detect molecular alteration associated with 22q11.2DS, SALSA P311 kit can be used to investigate the CHD etiology.

Severe phenotype þ *CHD*, Methodology: FISH

In individuals with severe phenotype and VSD the FISH N25 probe seems to be a great sensitive approach to diagnose 22q11.2DS. When other CHDs are involved in the phenotypic spectrum of the patient, TUPLE1 may be more assertive in the diagnosis.

Further studies are needed to provide a more accurate prevalence of VSD in individuals with 22q11.2DS and to elucidate possible causative genes of 22q11.2DS phenotype. We emphasize that studies with clear and replicable methodologies are extremely important to ensure a proper investigation and further diagnosis of 22q11.2DS individuals. The results found in this study may have analytical biases due to a single molecular methodology investigation in the majority of the individuals included. Since the 22q11.2DS critical region has 45 known protein coding genes, 7 micro-ribonucleic acid, and 10 non-coding genes that map to the 3 Mb[2] the use of more methodologies in 22q11.2DS molecular investigation could provide a more accurate diagnosis.

Another possible bias of our review is that we only included studies that have a proper replicable methodology described. In order to be considered replicable, the study should provide the reader basic information regarding each methodology performed as well as detailed results of each individual analyzed. If the study used more than one molecular approach, a result for each technique should be informed. All information is essential in order to interpret and analyze the real applicability as well as the significance of each molecular technique in the investigation of 22q11.2DS individuals.

Ethical Approval

This study did not require ethical approval.

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Conflict of Interest

None declared.

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4.2 Artigo 2 - submetido

"*HIRA*, *NKX2-5* and *GATA4* alterations versus cardiac malformations related to 22q11.2 deletion syndrome."

Bruna Lixinski Diniz, Desirée Deconte, Ana Kalise Böttcher, Rafaela Mergener, Bruna Baierle Guaraná, Natasha Malgarezi de Moraes, Rafael Fabiano Machado Rosa, Paulo Ricardo Gazzola Zen.

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Bruna Lixinski Diniz[1], Desirée Deconte[1], Ana Kalise Böttcher[2], Rafaela Mergener[1], Bruna Baierle Guaraná[1], Natasha Malgarezi de Moraes[1], Rafael Fabiano Machado Rosa[3], Paulo Ricardo Gazzola Zen[3].

[1]Graduate Program in Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Brazil

[2]Underdegraduate Program in Biomedicine, UFCSPA, Porto Alegre, Brazil

[3]Department of Internal Medicine, Clinical Genetics, UFCSPA, Porto Alegre, Brazil

These authors contributed equally to this work.

Corresponding author: Paulo Ricardo Gazzola Zen
Clinical Genetics Service - UFCSPA
Rua Sarmento Leite, 245/403 – Bairro Centro
Porto Alegre, RS, Brazil.
CEP: 90050-170,
E-mail address: paulozen@ufcspa.edu.br

Abstract:

Congenital heart disease (CHD) comprises a wide spectrum of structural defects. However, the etiology of a large proportion of CHDs remains undefined. Among the genetic causes, 22q11.2 deletion syndrome is the condition which most stands out. This association is related to many cardiac embryonic development genes being in the chromosome 22 region, as well as being a region with a high probability of errors in gene recombination, influencing normal levels of gene expression and affecting a gene's copy number. Our research carried out screening with ten patients previously diagnosed with 22q11.2DS by FISH and randomized another ten patients who did not have a previous molecular diagnosis by the same methodology. All patients had congenital heart disease and facial dysmorphia. All patients who had been previously diagnosed by FISH were found to have the exact same deletion size, LCRs and genes involved. *GATA4* when deleted or duplicated in different exons (1 and 6) showed distinct congenital heart defect phenotypes. Patients who did not have their diagnosis defined by FISH showed different molecular results, ranging from normal findings to alterations in the *GATA* and *NXK2* genes. Molecular diversity in cardiac malformations is a reality and a great challenge since genotype-phenotype correlation is hindered. Therefore, new insights on that matter should be considered: 22q11.2 deletion syndrome should only be linked to the chromosome 22 region, or is there a phenotype variability to be looked at that involves a broader genomic environment?

Keyword: *HIRA* gene, *NKX2-5* gene, *GATA4* gene, 22q11.2 deletion syndrome

Introduction

22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge syndrome (OMIM #188400), is characterized by a microdeletion in the long arm of chromosome 22 and is widely known for its clinical and molecular heterogeneity.[1] Among the more than 180 clinical characteristics already described, congenital heart disease (CHD) stands out as one of the indicative pieces of evidence for beginning investigations into the syndrome.[2]

CHDs constitute a developmental malformation of the heart, aorta, or other large blood vessels that make up the most common forms of major birth defects,[3] affecting 1.3– 1.7% of newborns per year in Brazil (Brazil Ministry of Health, 2021, http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinasc/cnv/nvuf.def.). CHD has its genetic basis determined by the association of single or multiple genes, chromosomal changes, and multifactorial causes. Studies have demonstrated a high frequency of copy number variation (CNV) in the human genome, which are likely to contribute to genetic heterogeneity and phenotypic variability.[4,5] Other genes outside the 22q region can also influence cardiac development. CNVs in *GATA4* and *NKX2–5* genes have been identified among mechanisms that may explain some CHDs, since all these are transcription factors (TFs) strongly involved in cardiogenesis.[6]

Molecular biology and the arrival of several new technologies have made it possible to discover genes that can interact with each other or with external factors, generating a predisposition profile to the development of the disease.[7,8] Overall, 60 to 80% of the individuals diagnosed with 22q11.2DS have cardiac malformation that can vary in severity.[1,2] Tetralogy of Fallot and pulmonary atresia with ventricular septal defect are regular findings in patients with 22q11.2DS, however, their prevalence is yet to be established.[9] Through the identification of CHD in these individuals, hypotheses were created that a mechanism that leads to an alteration in embryonic development could be involved.[10]

Different cytogenetic methodologies have emerged and have been improving genetics research in the last decade. Fluorescence in situ hybridization (FISH) is the gold standard method for 22q11.2DS diagnosis. However, in the last two decades, multiplex ligation-dependent probe amplification (MLPA) and microarray analysis became effective tools alongside FISH.[11,12] Multiplex ligation-dependent probe amplification (MLPA) is a variation of the multiplex polymerase chain reaction method. It is used to identify CNVs, including deletions and duplications in CHDs predisposition genes.[13]

The variety of molecular technologies allowed individuals to be diagnosed more frequently, providing new insights into the molecular mechanism behind 22q11.2DS. Furthermore, since the 22q11.2DS critical region has a complex molecular structure, combining more methodologies in 22q11.2DS molecular investigation may be a crucial ally in order to provide a more accurate diagnosis for patients as well as deepen our understanding of this syndrome's challenging and intricate genetic roots.[14]

Knowledge of normal cardiac development and the mechanisms of CHDs are essential to daily practice, as much for the daily evaluation of heart diseases as for genetic counseling.[15] This study assessed two groups of individuals presenting CHDs, suspected, or diagnosed with 22q11.2DS, who have previously used only one molecular approach. Our intent was to determine the deletion size of subjects previously diagnosed by FISH and investigate the genetic profile of individuals that were yet to be molecularly diagnosed.

Methodology

Here we made a molecular evaluation update and provided a result description of patients previously described by DINIZ, B et al 2020.[16] Participants were recruited from cardiac intensive care units (ICU) of three health institutions in Southern Brazil.

Our research carried out screening with ten patients previously diagnosed with 22q11.2DS by FISH and randomized another ten patients who did not have a previous molecular diagnosis by the same methodology.

All patients had congenital heart disease and facial dysmorphia. CHDs were described based on echocardiography, cardiac catheterization, and surgical description following the classification suggested by BOTTO et al. 2003.[17]

Molecular cytogenetic testing by FISH was previously performed using the commercial Vysis LSI DiGeorge/*TUPLE1* (Abbott Molecular) region dual-color probe, which identifies deletions of band 22q11.2. Blood samples in EDTA were taken for subsequent DNA extraction according to the PUREGENE protocol (*https://web.emmes.com/study/hbb/public/EN-Gentra-PuregeneHandbook-2011.pdf*).

For subjects with positive result for 22q11.2DS by FISH, MLPA assay was performed using the SALSA MLPA P250-B2 DiGeorge to determine deletion size throughout LCRs involved in the deletion. This kit detects not only deletions or duplications in the human 22q11.2 region, but also contains probes that can assess the copy number status at 4q, 8p, 9q, 10p, 17p and 22q13 chromosome regions. All alterations detected are causative of DiGeorge syndrome (DGS), DGS type II or disorders with phenotypic characteristics of DGS. Probe sequences are shown in **Table 1.**

Table 1. P250 probes arranged according to chromosomal location

Gene	Partial sequence (24 nt adjacent to ligation site)
Cat Eye Syndrome (CES) region:	
IL17RA	**GCAGAGTTATCT-GTCCTGCAGCTG**
SLC25A18	**GCAGTGAGAAGA-GTCGAGTGAAGC**
BID	**CTACTGGTGTTT-GGCTTCCTCCAA**
MICAL3	**GAACTACCGCCT-GTCCCTGAGGCA**
USP18	**CTCAGTCCCGAC-GTGGAACTCAGC**
End of CES region; Start DiGeorge (DGS) region; probes in region LCR22A – LCR22B:	
CLTCL1	**TGTTGCCTTGGT-GACCGAGACCGC**
HIRA	**GGAGCTGCTGAA-GGAGCTGCTACC**
CDC45	**ATGTTCGTGTCC-GATTTCCGCAAA**

CLDN5	**TTCGCCAACATT-GTCGTCCGCGAG**
GP1BB	**CACAACCGAGCT-GGTGCTGACCGG**
TBX1	**CCGGGTGAAGCT-TCGCTGGCTGCC**
TBX1	**TCCCTTCGCGAA-AGGCTTCCGGGA**
TXNRD2	**GGAGGGTCAGGA-GAGGAGCTGCAG**
DGCR8	**GGTAATGGACGT-TGGCTCTGGTGG**
Probes in region LCR22B – LCR22C:	
ZNF74	**CAGGCAGATTAT-TCCTCGATGCTG**
KLHL22	**TCTTCGATGTTG-TGCTGGTGGTGG**
MED15 (PCQAP)	**TGGCATTTGGAT-GAAGACACAGGT**
Probes in region LCR22C – LCR22D:	
SNAP29	**AGGAGCAAGATG-ACATTCTTGACC**
LZTR1	**ATGATGAAGGAG-TTCGAGCGCCTC**
End of the commonly-deleted DiGeorge (DGS) region; probes in region LCR22D – LCR22E:	
HIC2	**GTTCCAGCAGAT-CTTGGACTTCAT**
PPIL2	**GAAGAGCCCTCA-ACCAGTGCCACT**
TOP3B	**GAGACATGATAA-AATCCAGTCCTT**
Probes in region LCR22E – LCR22F:	
RSPH14 (RTDR1)	**GGTGTGTCATTT-TGACGTCATCCC**
GNAZ	**TCACCATCTGCT-TTCCCGAGTACA**
RSPH14 (RTDR1)	**CTCCTTGGAGCT-TCCCATTAACAT**

RAB36	**AGCTGGATGCTT-GGACGCGCCGCT**
Probes in region LCR22F – LCR22G:	
SMARCB1	**CTTCGGGCAGAA-GCCCGTGAAGTT**
SMARCB1	**CATCAGCACACG-GCTCCCACGGAG**
Probe in region LCR22G – LCR22H:	
SNRPD3	**CCGGTGAGGTAT-ATCGGGGGAAGC**
4q35-qter	
SLC25A4	**CATCAAGATCTT-CAAGTCTGATGG**
KLKB1	**ATGCCCAATACT-GCCAGATGAGGT**
8p23	
PPP1R3B	**ACCGAGCTCCTA-GACAACATTGTG**
MSRA	**GCAACAGAACAG-TCGAACCTTTCC**
GATA4	**TGGATTTTCTCA-GATGCCTTTACA**
9q34.3	
EHMT1	**AAATGCTGCAAA-GCACACTCAGGA**
EHMT1	**GGACCCCGTTGA-TGGAAGCAGCCG**
10p14	
GATA3	**GAGTGCCTCAAG-TACCAGGTGCCC**
GATA3	**AACAGCTCGTTT-AACCCGGCCGCC**
TCEB1P3	**TGTAGACCACAT-GATGGAGATTTG**
CELF2 (CUGBP2)	**GACATTCACTGT-GGAAATTTGGTG**

CELF2 (CUGBP2)	**TCCCCCGGTCAT-GGTCGGAAAAGG**
NEBL	**CTGGGATCCTTT-TCTGTTCACTCA**
17p13.3	
RPH3AL	**AGGCGGAATGTG-ATGGGGAACGGC**
RPH3AL	**GTAGTGGACACT-TGTACGTGCACT**
GEMIN4	**AAACAGTGATAG-ACGTCAGCACAG**
YWHAE	**GCCACAGGAAAC-GACAGGAAGGAG**
22q13.3	
ARSA	**GGAGGATCAGAT-CTCCGCTCGAGA**
SHANK3	**ACCAACTGTGAT-CAGTGAGCTCAG**

On the other hand, subjects with a negative result for 22q11.2DS by FISH, were tested by MLPA assay with the SALSA MLPA P311 - a kit that can detect deletions or duplications on genes related to CHD outside the 22q11 region. The assay follows the manufacturer's recommendations (MRC-Holland, Amsterdam, The Netherlands). Probe sequences are shown in **Table 2.** Molecular analysis was performed by both ABI3130 sequencing and Coffalyser software (MRC-Holland). For each sample analyzed, commercial controls in triplicate were used.

Table 2. P311 probes arranged according to chromosomal location

Region	Partial sequence (24 nt adjacent to ligation site)
GATA4	
Upstream	**CATGCTCAAGAT-AGGCACTGGAGC**
Upstream	**GAGGTTCTTCTT-TAAAATCCATTC**
Exon 1	**TTTCTTCCCTTT-CTTTGCTCCTTC**
Exon 3	**CTCAGTAGATAT-GTTTGACGACTT**

Exon 4	**CTACATGAAGCT-CCACGGGGTACG**
Exon 5	**AAGAACCTGAAT-AAATCTAAGACA**
Exon 6	**CAACTCCAGCAA-CGCCACCACCAG**
Exon 7	**CACAAGGCTATG-CGTCTCCCGTCA**
***CTSB* gene**	**AAGTGTAGCAAG-ATCTGTGAGCCT**
NKX2-5	
Exon 1	**CCGGCCAAGTGT-GCGTCTGCCTTT**
Exon 1	**AGGTGAGGAGGA-AACACAGGCCCC**
Exon 2	**CGCTCCAGCTCA-TAGACCTGCGCC**
Exon 2	**CGGGATTCCGCA-GAGCAACTCGGG**
TBX5	
Exon 1	**GACGTTGGAAGA-AGACCTGGCCTA**
Intron 1	**CTATTCTGGGTA-AGCAGTAAACCC**
Exon 2	**GCCTGACGCAAA-AGACCTGCCCTG**
Exon 3	**AATCAAAGTGTT-TCTCCATGAAAG**
Exon 5	**TCCTTCCAGAAA-CTCAAGCTCACC**
Exon 6	**TACCAGCCTAGA-TTACACATCGTG**
Exon 8	**GTGAGGCAAAAA-GTGGCCTCCAAC**
Exon 8	**CCATTGTACCAA-GAGGAAAGGTGA**
Exon 9	**AAGAAGATTCCT-TCTACCGCTCTA**
Exon 9	**TTTGCTTTGGTT-TTGTCCTGCCTT**
BMP4	
Exon 1	**TGCAGGGACCTA-TGGTGAGCAAGG**
Intron 1	**CGCAGGCCGAAA-GCTGTTCACCGT**
Exon 3	**TGGTAACCGAAT-GCTGATGGTCGT**
Exon 4	**AACATCTGGAGA-ACATCCCAGGGA**
CRELD1	
Exon 4	**CAAGTCAGACTT-CGAGTGCCACCG**
Exon 11	**GCATTCCCCATC-TTAACTGATTTA**
22q11 region (DiGeorge)	

CDC45	**ATGTTCGTGTCC-GATTTCCGCAAA**
GP1BB	**CACAACCGAGCT-GGTGCTGACCGG**
DGCR8	**GACTCAGCGACT-GCACCAGTGGCA**

Results

We divided 20 individuals who presented CHD and facial dysmorphia into two groups: one with ten subjects who have already been diagnosed by FISH and a second group with ten subjects whose 22q11.2DS diagnosis have not been confirmed by the same methodology. We observed that all patients who had been previously diagnosed by FISH were found to have the exact same deletion size, LCRs and genes involved (MLPA P250 kit): a typical 3Mb deletion comprising LCR22A - LCR22D (**Figure 1**). On the other hand, patients who did not have their diagnosis defined by FISH showed different molecular results by MLPA (P311 kit), ranging from normal findings to alterations in the *GATA4* and *NXK2-5* genes.

Both groups of subjects showed similar and distinct cardiological abnormalities. Eight heart defects were found only in the group previously diagnosed by FISH and nine heart defects were found only in the group that was yet to be diagnosed (**Figure 1**). The three most common cardiac abnormalities observed in those diagnosed with 22q11.2DS were atrial septal defects (5/10), interventricular communication (4/10) and right aortic arch (4/10). *GATA*4 exon 1 deletion subject showed an unique cardiac phenotype that was not shared with any other group: double outlet left ventricle, non-restrictive interventricular communication, right ventricular hypoplasia and tricuspid atresia. Also, *GATA4* when deleted or duplicated in different exons (1 and 6, respectively) showed distinct congenital heart defect phenotypes between them.

In addition, subjects presenting no molecular alterations have been observed in the second group (6/10). CHDs in those patients were shared by both the first and second groups, however, we can highlight the following malformations as exclusive to patients without identification of molecular alterations: pulmonary valve stenosis, pentalogy of fallot, mild tricuspid regurgitation.

Discussion

Researchers are aware of the complexity surrounding 22q11.2DS diagnosis. In developing countries, where access to molecular technologies is scarce, a patient's phenotype is one of the most important indicators for the initial diagnosis of a genetic disease, and often the only stage of assessment. Brazil is no different. It was only in 2014 that the Sistema Único de Saúde (SUS) instituted financial incentives for applying molecular methodologies to diagnose patients suspected of having a genetic condition (Ordinance no. 981, of May 21, 2014: https://bvsms.saude.gov.br/bvs/saudelegis/gm/2014/prt0981_21_05_2014.html). However, even today, health services face challenges in accessing these methodologies on a routine basis. Our research group has been studying cases of 22q11.2DS in partnership with health institutions in southern Brazil for more than a decade using different approaches. Here, all cases were selected based on CHD findings and facial dysmorphia as the first indication for 22q11.2DS molecular analysis.

22q11.2DS is characterized by haploinsufficiency resulting from a hemizygous deletion in the region 11.2 on the long arm of chromosome 22, meaning that the gene alleles have no homologic counterparts.[18] The deletion types and sizes show a high degree of variability due to several low-copy-number repeat sequences (LCRs) flanking the deleted region. LCRs are specific DNA regions that are generally 10-300 kilobases (kb) in size and 95-97% similar to each other. As a result, the breakpoints are grouped together in defined regions, increasing the capacity for recombination. This type of recombination is strongly associated with genomic diseases, with approximately 37 regions of the genome already evidenced.[19,20]

It is known that 4 LCRs (A, B, C and D) define this region and due to their substantial sequence similarity, facilitate non-allelic meiotic homologous recombination, resulting in translocation, deletion, or unbalanced duplication.[19] The majority of patients show a 3Mb heterozygous deletion comprising four repeats extending from LCR22A to LCR22D and involving approximately 40 genes.[20] The 1.5 Mb deletion

between the LCR22A and LCR22B regions can be identified in ~10% of affected individuals.[19] Between the 22q11.2 deletion group of our study, all patients presented the same deletion size comprising the following genes: *CLTCL1, HIRA, CDC45-1, GP1BB, TBX1, TXNRD2, DGCR8, ZNF74, KLHL22, MED15, SNAP29, LZTR1*. It means, a typical deletion in LCR22A-LCR22D.

HIRA (Histone cell cycle regulator (OMIM: 600237) gene is located between LCR22A and LCR22B and is the main gene targeted for FISH analysis when investigating this syndrome due to its significant impact in embryonic and cardiac development.[21] Based on this, we wondered why the subjects who had the HIRA deletion (identified by FISH) in our study were shown to have the same size of deletion, LCRs and genes involved when analyzed by MLPA? So, every time we identify the HIRA deletion, can we suggest that the patient has the typical deletion? New studies with this focus will be necessary to try to answer these questions including a significant number of affected patients. We tried to find studies with focus on "How *HIRA* deletion impacts the genes subsequent deletion in 22q region?". We found studies applying different investigation approaches such as whole-genome sequencing[22], MLPA assays[20] and reviews[23,24], however none of them brought a discussion that could answer our questions about *HIRA* or could explain our "coinciding" results.

Other studies not related to 22q11.2DS demonstrated *HIRA* role in other biologic processes. *HIRA* encodes a histone chaperone H3.3 and plays an important role in the epigenetic regulation of gene expression, influencing transcription, genome integrity, cellular senescence, and genome reprogramming.[25] DILG et al. 2016[26] demonstrated by embryonic stem cell study that *HIRA* plays a major role in the cardiogenic mesoderm. Mutant HIRA embryos presented oedema and cardiac malformations such as ventricular septal defect (VSD), atrial septal defect (ASD), thin ventricular wall and constricted pulmonary trunk (PT). These two studies help us confirm that *HIRA* has an important impact in embryonic development that could possibly explain the 22q11.2DS phenotype. However, we continue not understanding if *HIRA* deletion can indicate a typical deletion when using only FISH as a diagnostic tool.

It is known that individuals with the LCR22A - LCR22B deletion have the full spectrum of phenotypes that is also found in individuals carrying typical LCR22A - LCR22D deletions, suggesting that key phenotypes of 22q11DS are mostly due to a decrease in the gene's dosage located within LCR22A - LCR22B region, i.e., where *HIRA* is located. Also, cardiovascular defects seem to be three times more frequent in LCR22A - LCR22B or LCR22A - LCR22D than distal deletions.[24,27] Looking at this, is it possible that the subsequent genes deleted after the LCR22A-LCR22B region cannot interfere with the 22q11.2 deletion phenotype and are just the remaining result of the size of the complete deletion caused by the genomic rearrangement? The commonly deleted regions contain not only *HIRA* but additional genes, including *TBX1*, that is also involved in embryonic development. Patients with hemizygous deletion manifest diverse clinical features, ranging from cardiac malformation and craniofacial and limb anomaly to psychiatric disorders.[28] If we search the literature for studies on each gene, we will see that there is a huge number of findings related to the influence of each of them on the human organism. What is striking is trying to explain how they interact with each other and what interference in the phenotype of such a complex syndrome only one altered gene can cause compared to more genes involved.

Copy number variations, which constitute either gross DNA deletions or duplications, were identified as being critical dosage-sensitive genome for cardiac development. Examples of this include the *TBX1* deletion at 22q11.2, the *GATA4* deletion at 8p23.1, and the *NKX2-5* deletion at 5q35.1.[29] Our CHD results are quite diverse, as is commonly found in literature. The three most common cardiac abnormalities observed in those diagnosed with 22q11.2DS were atrial septal defects (5/10), interventricular communication (4/10) and right aortic arch (4/10). According to literature, VSD, ASD, and TOF are the most common heart defects associated with chromosomal abnormalities.[14,30] Although the number of patients presented in this study is small, our results corroborate the literature as the most CHD finding was ASD. At the same time, TOF was also present in the 22q11.2DS subjects (2/10) but also in *GATA4* duplication and normal

sample. Curiously, VSD was not reported in our cases, but it is important to note that VSD is one of the cardiac malformations that comprises TOF.[14]

In addition, we were able to observe a distinct cardiological profile, especially for patients who were suspected of having 22q11.2DS but were diagnosed with a *GATA4* deletion in exon 1. CHDs observed are shown in **Figure 2**. Also, patients who had *GATA4* deletion in exon 6 shared the cardiac phenotype with those diagnosed with 22q11.2DS.

The patients who did not have a diagnosis defined by neither FISH nor MLPA (6/10) showed congenital heart defects found only among this sample as well as CHDs that were shared with del 22, *GATA4* exon 6 deletion, *NKX2-5* duplication and *GATA4* exon 1 duplication patients (**Figure 3**). Again, *GATA* exon 1 deletion subject showed an unique cardiac phenotype.

CHD candidate genes share common features, but their properties lack statistical significance for definitive categorization.[31] Also, they can provide new knowledge about the essential regulatory molecules and pathways involved in cardiogenesis, complementing, and extending the wealth of information gained from studying heart development in experimental models. Studying spontaneous gene variants in sporadic CHD can reveal novel genes implicated in cardiogenesis while providing detailed information.[32]

Outside of 22q11.2 region, other candidate genes such as *GATA4* and *NKX2-5* have also been proven to be responsible for heart development and diseases.[33] Mutations of cardiac transcription factor genes, such as *GATA4* and *NKX2-5* were identified in genetic linkage studies of large affected families.[34] Studies have investigated the relationship between mutations at the GATA4 level and the appearance of a specific heart defect, mainly in syndromic cardiac septal defects. The main research directions are oriented toward the GATA4 gene and T-box transcription factor.[35] *GATA4* belongs to *GATA* family which consists of 6 structure-conserved transcription factors. Over 100 known mutation sites have been identified within its gene, which are related to the structural heart defect.[36] We have observed three subjects presenting duplication/deletion in *GATA4* and at the same time, two of them shared cardiac findings

(**Figure 2**). SHAKER et al. 2017,[37] identified nonsynonymous sequence variation in exon 1 of *GATA4* in cases of septal defects: isolated VSD and combined VSD and ASD in Egyptian children. WANG et al. 2011,[38] observed two mutations in exon 1 and 6 of *GATA4* in addition to other mutations in patients with VSD and minor anomalies. Other authors have described distincts variants in different *GATA4* exons related to heart defects.[39,40] *GATA4* seems to be indeed an important cardiac regulator gene, no matter which exons alterations are involved and its investigation, and of 22q11.2 deletion syndrome in patients with CHDs constitute an important issue in prenatal diagnosis, contributing to family planning through appropriate genetic counseling and management of extracardiac symptoms.[41]

GATA4 mediates target gene expression by forming complexes with other cardiac transcriptional factors, including *NKX2-5*, another regulator essential for proper cardiogenesis with its expression and functions overlapping with those of *GATA4* during embryogenesis. Moreover, *GATA4* and *NKX2-5* can interact physically and regulate synergistically the expression of multiple important cardiac genes.[42,43] *NKX2-5* is a vertebrate member of the NK-2 class of homeobox genes and is expressed in early heart myocardiocyte progenitors and developing heart, as well as in the pharynx, spleen and stomach.[44] The main heart defects related to *NKX2-5* are conotruncal abnormalities, such as Tetralogy of Fallot.[45,46]

In this study, we found a patient presenting *NKX2-5* duplication associated with interventricular communication, left ventricular systolic dysfunction and patent foramen ovale. However, all those defects were shared with 22q11.2 deletion and/or normal subjects. No studies linking *NKX2-5* duplication and CHD were found in the literature. What we know is that the process of cardiac development is complex and that *NKX2–5* does participate in the protein-protein interaction with other transcription factors. Approximately 50 different mutations in this gene have been identified to date, and only a few have been functionally characterized. The mutant *NKX2-5* factor can regulate a few off-targets downstream to facilitate CHD development.[47] Perhaps in order to better understand the *NKX2-5* influence in cardiogenesis we should look into the failure of cardiac transcription

factor networks resulting from genetic instability in cardiac cells.[48] As *NKX2-5* mutations exhibit common inheritance patterns, screening could also identify family members who may also be at risk.[47]

We've performed a two groups analysis between individuals with the same clinical diagnosis but different molecular results. *HIRA* is a well-known gene on congenital heart defects phenotypes, however further studies on how this gene interacts with others are still needed to elucidate why the same deletion size is often found on 22q11.2DS subjects. *GATA4* also showed to be a key gene in CHD phenotypes. On the other hand, *NKX2-5* duplications are yet to be clarified in the cardiogenesis context. To sum up, molecular diversity in cardiac malformations is a reality and a great challenge since genotype-phenotype correlation is hindered. Therefore, new insights on that matter should be considered: 22q11.2 deletion syndrome should only be linked to the chromosome 22 region, or is there a phenotype variability to be looked at that involves a broader genomic environment?

Data availability statement

All data relevant to the study is included in the article.

Ethics statements

Patient consent for publication

Not applicable.

Ethics approval

This study involves human participants and was approved by REC Nº 2.315.917 and Nº 3.577.284. Participants gave informed consent to participate in the study before taking part.

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Competing Interests

Authors declare not have any competing financial interests in relation to the work described.

Author Contributions

BD designed experiments, performed methodology and wrote the manuscript; DD helped contributed to data extraction and write the manuscript; AB, RM and NM contributed to perform methodology and data extraction. The BG performed the patients' morphological assessment. RR and PG provided feedback on the manuscript.

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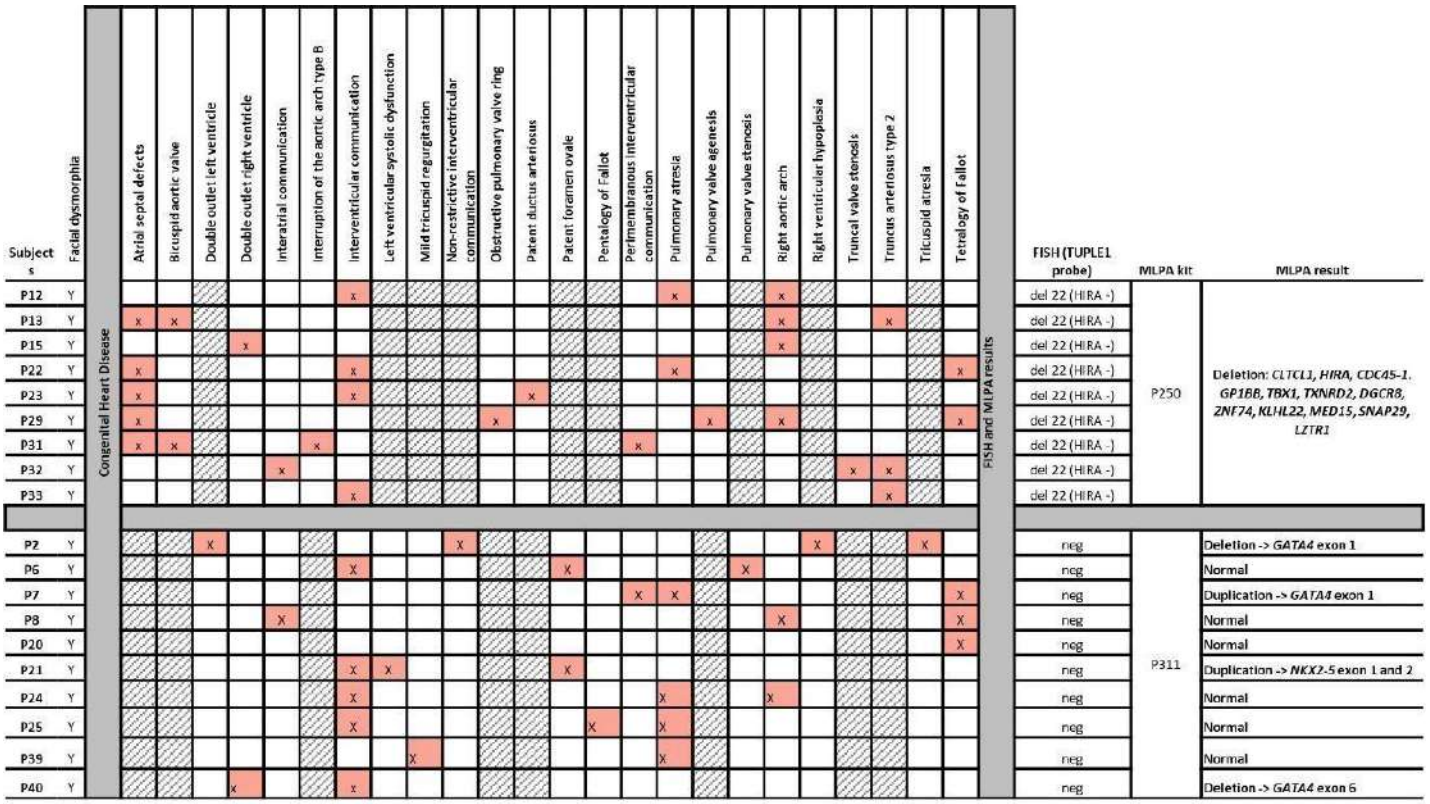
Figure legends:

Figure 1. Heart defects and molecular genetic results of both groups of patients analyzed.

Figure 2. Congenital heart defect findings shared with 22q11.2 deletion, *GATA4* deletion/duplication and *NKX2-5* duplication.
*LVSD was identified only in *NKX2* duplication. PFO was identified just in *NKX2-5* duplication and normal subjects.

Figure 3. Cardiac profile among subjects with no molecular diagnostic and subjects with genetic alterations. Converging and diverging heart defects phenotypes are illustrated according to the molecular alteration (**red:** 22q11.2 deletion; **yellow:** *GATA4* exon 6 deletion; **blue:** *NKX2-5* exon 1 and 2 duplication; **green:** *GATA4* exon 1 duplication). CHD's of subject's without any molecular finding are pictured in **gray.**



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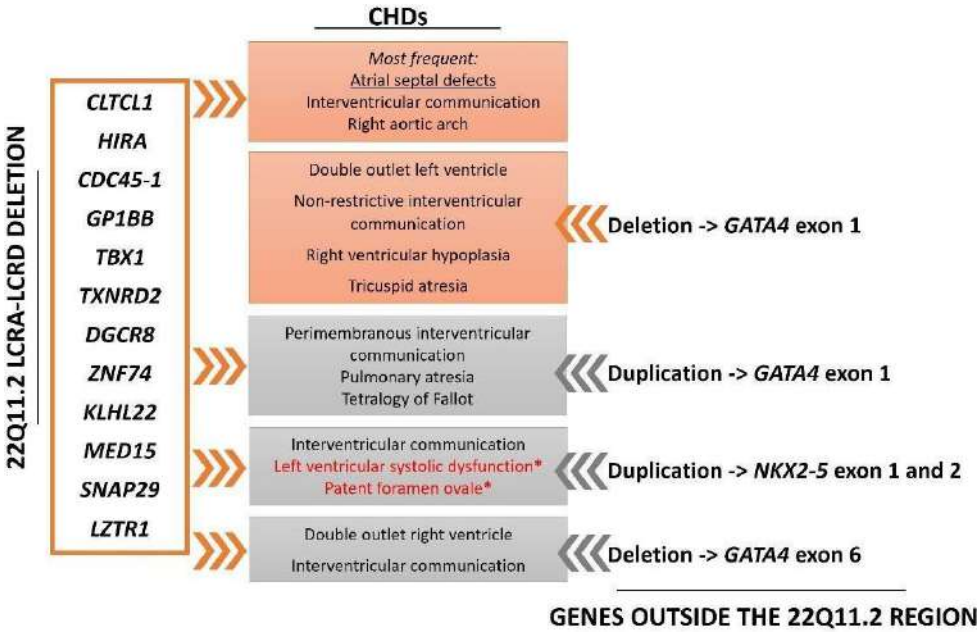

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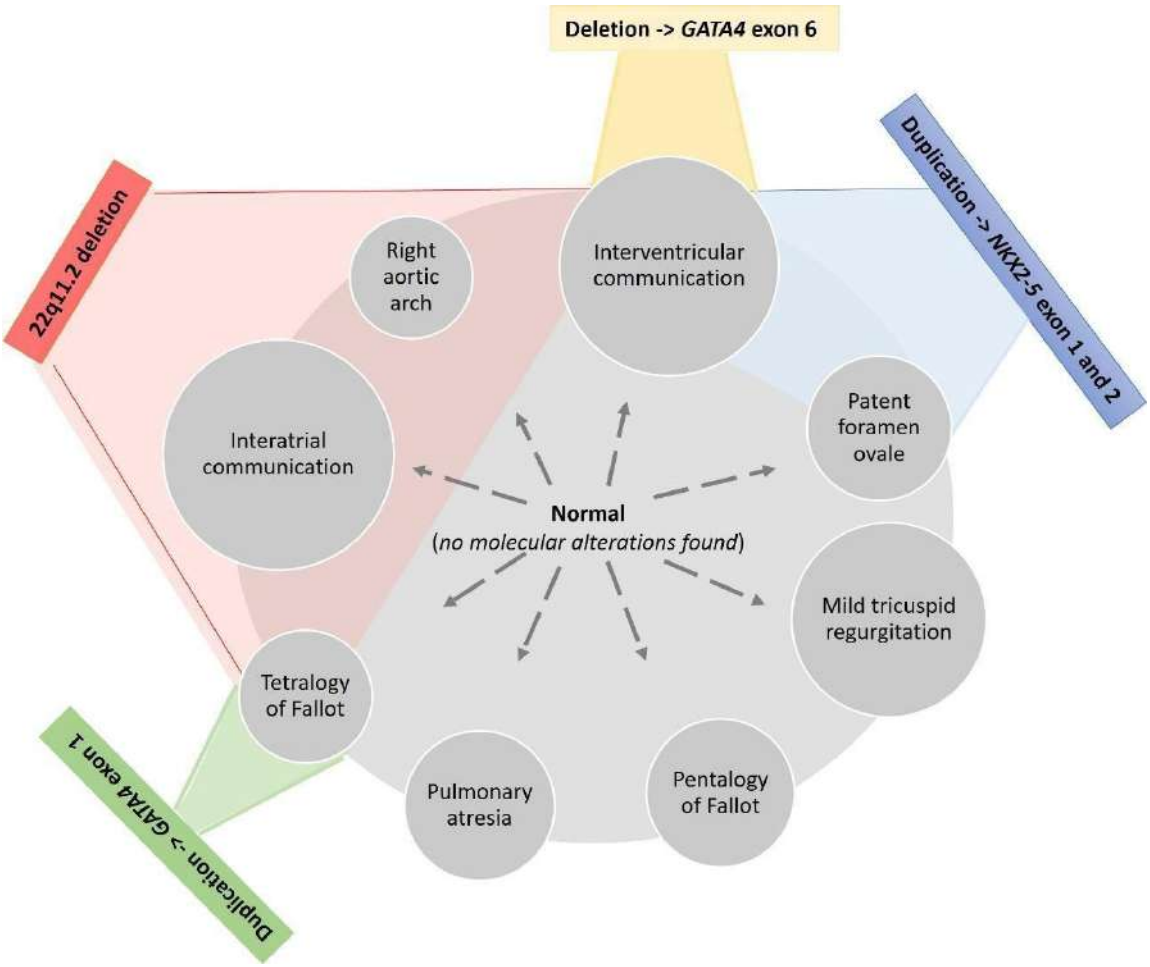

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5. CONCLUSÕES

O *HIRA* é um gene bem conhecido nos fenótipos de defeitos cardíacos congênitos, mas ainda são necessários mais estudos sobre como esse gene interage com outros para elucidar porque o mesmo tamanho de deleção é frequentemente encontrado em indivíduos com 22q11.2DS. Além disso, enfatizamos que estudos com metodologias claras e replicáveis são extremamente importantes para garantir uma investigação adequada e o posterior diagnóstico de indivíduos com 22q11.2DS. Os resultados encontrados na revisão sistemática podem ter vieses analíticos devido à investigação de uma única metodologia molecular na maioria dos indivíduos incluídos.

Trouxemos também a análise de genes não localizados na região 22q11.2. O *GATA4* também demonstrou ser um gene fundamental nos fenótipos de CHD de pacientes que não apresentam a deleção 22q11.2. Por outro lado, as duplicações do *NKX2-5* ainda não foram esclarecidas no contexto da cardiogênese. Em resumo, a diversidade molecular nas malformações cardíacas é uma realidade e um grande desafio, uma vez que a correlação genótipo-fenótipo é difícil. Portanto, novas percepções sobre esse assunto devem ser consideradas: a síndrome de deleção 22q11.2 deve estar ligada apenas à região do cromossomo 22 ou há uma variabilidade fenotípica a ser observada que envolve um ambiente genômico mais amplo?

6. CONSIDERAÇÕES FINAIS

O presente estudo faz parte de um projeto maior intitulado “Avaliação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas.”**,** aprovado pelos Comitês de Ética em Pesquisa (CEP) do HCSA (Parecer N° 2.315.917), UFCSPA (Parecer N° 2.729.168) e do IC-FUC (Parecer No 3.604.989).Deste projeto maior estão sendo desenvolvidos diversos outros trabalhos e projetos.

A realização de testes genéticos específicos possibilita a obtenção de diagnósticos mais precisos, levando a um planejamento terapêutico mais otimizado. Através deste estudo foi possível fornecer acesso as novas tecnologias diagnósticas para as síndromes de microdeleções, bem como a avaliação genética completa para os pacientes. Cada resultado obtido originou um laudo que foi entregue na consulta médica. Além disso, casos que não foram elucidados neste estudo, estão sendo encaminhados para investigação utilizando outras técnicas já realizadas no laboratório de Citogenética da UFCSPA.

7. ANEXOS

7.1. Parecer do Comitê de Ética da UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Investigação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas

Pesquisador: Paulo Ricardo Gazzola Zen

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 86036418.4.0000.5345

Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre

Patrocinador Principal: MINISTERIO DA EDUCACAO

DADOS DO PARECER

Número do Parecer: 2.729.168

Apresentação do Projeto:

Embora as doenças genéticas sejam individualmente raras, sua frequência conjunta faz com que elas representem um importante problema de saúde pública. De todos os neonatos, 2 a 3% têm pelo menos uma anormalidade congênita maior, das quais pelo menos 50% são causadas exclusivamente ou parcialmente por fatores genéticos. As incidências de anomalias cromossômicas e distúrbios monogênicos em neonatos são de aproximadamente 1 em 200 e 1 em 100, respectivamente. A cardiopatia congênita é a alteração congênita mais comum e uma das principais causas de morbidade infantil relacionadas a malformações congênitas. Com incidência de 19 a 75 casos a cada mil nascidos vivos, possui etiologia complexa e multifatorial, com cerca de 80% das cardiopatias congênitas surgindo através da combinação de fatores genéticos e ambientais. Cerca de 20% dos casos podem ser atribuídos a anomalias cromossômicas, síndromes mendelianas, desordens genéticas não sindrômicas ou teratógenos. As doenças genéticas representam um importante problema de saúde pública, pois, além de provocarem uma enorme proporção de mortes infantis,são a causa de grande parte das internações em hospitais pediátricos. Atualmente são poucos os exames genéticos realizados via SUS. O presente projeto visa a realização de uma abordagem clínica e genética em pacientes suspeitos ou portadores de doenças genéticas ou síndromes malformativas. A amostra será composta por pacientes do SUS

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

7.2 Parecer do Comitê de Ética do HCSA

HOSPITAL DA CRIANÇA
SANTO ANTÔNIO

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas

Pesquisador: Paulo Ricardo Gazzola Zen

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 74971917.2.0000.5683

Instituição Proponente: Hospital da Criança Santo Antônio - Santa Casa/RS

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.315.917

Apresentação do Projeto:

Avaliação etiológica de pacientes encaminhados para investigação no serviço de Genética da UFCSPA

Objetivo da Pesquisa:

Objetivo geral

Realizar investigação clínica e genética de pacientes suspeitos ou portadores de doenças genéticas ou síndromes malformativas, atendidos pelo Serviço de Genética Clínica da UFCSPA/HCSA.

Objetivos específicos:

- Investigar anomalias genéticas utilizando técnicas como FISH, MLPA, arrayCGH e sequenciamento/exoma;
- Descrever relatos de caso e séries de casos envolvendo indivíduos portadores de doenças genéticas ou síndromes malformativas em periódicos científicos;
- Constituir um repositório de amostras de DNA de indivíduos suspeitos ou portadores de doenças genéticas ou síndromes malformativas;
- Possibilitar o acesso a novas tecnologias aos pacientes suspeitos ou portadores de doenças

Endereço: Av. Independência,155
Bairro: INDEPENDENCIA **CEP:** 90.035-074
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3214-8997 **Fax:** (51)3214-8997 **E-mail:** cephcsa@santacasa.tche.br

7.3 Parecer do Comitê de Ética do IC-FUC

INSTITUTO DE CARDIOLOGIA DO RS / FUNDAÇÃO UNIVERSITÁRIA DE CARDIOLOGIA/ IC/FUC

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Investigação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas

Pesquisador: Paulo Ricardo Gazzola Zen

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 86036418.4.3001.5333

Instituição Proponente: Instituto de Cardiologia do RS / Fundação Universitária de Cardiologia

Patrocinador Principal: MINISTERIO DA EDUCACAO

DADOS DO PARECER

Número do Parecer: 3.604.989

Apresentação do Projeto:

O presente projeto visa a realização de uma abordagem clínica e genética em pacientes suspeitos ou portadores de doenças genéticas ou síndromes malformativas. atendidos por médicos geneticistas em hospitais públicos.

Objetivo da Pesquisa:

- Realizar investigação genética de pacientes do SUS suspeitos ou portadores de doenças genéticas ou síndromes malformativas, atendidos por médicos geneticistas em hospitais públicos.

Avaliação dos Riscos e Benefícios:

O risco físico aos indivíduos está relacionado com a coleta do sangue. Como as coletas serão realizadas preferencialmente de modo associado com as coletas de rotina do hospital, não haverá risco adicional. O atendimento as possíveis complicações decorrentes da coleta de sangue se dará do mesmo modo rotineiramente realizado no hospital no qual o paciente é atendido, sempre assistido pelo médico geneticista colaborador.

O material coletado será preservado confidencial, sendo que será sempre assegurado o anonimato dos pacientes envolvidos na pesquisa .

Endereço: Av. Princesa Isabel, nº 370 - Centro Cultural Rubem Rodrigues - Ramal: 4136 e Av. Princesa Isabel, nº 395
Bairro: Santana **CEP:** 90.620-000
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3230-3600 **Fax:** (51)3223-2746 **E-mail:** cep.icfuc@cardiologia.org.br

7.4 Registro ComPesq

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Pró-Reitoria de Pesquisa e Pós-Graduação

Comissão de Pesquisa - COMPESQ

ATESTADO DE REGISTRO

Dados do Projeto:

Número: **154/2022**
Título: **Avaliação etiológica de pacientes suspeitos ou portadores de doenças genéticas, ou síndromes malformativas**
Coordenador: **Paulo Ricardo Gazzola Zen**

Vigência: **19/05/2022** a **30/06/2027**

Pesquisadores:

Equipe UFCSPA:

- Paulo Ricardo Gazzola Zen
- Rafael Fabiano Machado Rosa
- Bruna Lixinski Diniz
- Marilu Fiegenbaum

Equipe Externa:

- Juliana Josahkian - UFSM
- Cláudia Fernandes Lorea - UFPEL

Atestamos que o projeto de pesquisa acima identificado foi previamente aprovado pelo CEP e após, foi registrado no Sistema de Registro de Projetos de Pesquisa da UFCSPA. Salientamos que cabe ao CEP toda a avaliação referente às questões éticas do projeto e que qualquer alteração no projeto original deve ser reportada ao CEP que aprovou o projeto. Este atestado não garante a concessão de recursos financeiros por parte da UFCSPA.

Porto Alegre, 23 de junho de 2022

RENATA PADILHA GUEDES
Coordenadora Da Comissão De Pesquisa

7.5 Produção Bibliográfica (2020-2024)

1. Pseudohypoparathyroidism with Ectopic Calcification and 22q11 Deletion Syndrome: A Rare Case (2020).
2. Congenital Heart Defects and Dysmorphic Facial Features in Patients Suspicious of 22q11.2 Deletion Syndrome in Southern Brazil (2020).
3. Microarray-Based Comparative Genomic Hybridization, Multiplex Ligation-Dependent Probe Amplification, and High-Resolution Karyotype for Differential Diagnosis Oculoauriculovertebral Spectrum: A Systematic Review (2020).
4. Candidate genes of oculo-auriculo-vertebral spectrum in 22q region: A systematic review (2020).
5. A child with cat-eye syndrome and oculo-auriculo-vertebral spectrum phenotype: A discussion around molecular cytogenetic findings (2021).
6. Fluorescence in situ hybridization (FISH) as an irreplaceable diagnostic tool for Williams-Beuren syndrome in developing countries: a literature review (2023).
7. Quality of life of type 1 neurofibromatosis patients: a scoping review protocol (2024).
8. Quality of life of adolescent children and young adults with neurofibromatosis type 1: scope review (2024).

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

Bruna Lixinski Diniz[1] Andressa Barreto Glaeser[1] Desirée Deconte[1] Bruna Baierle Guaraná[2] Rafael Fabiano Machado Rosa[2] Paulo Ricardo Gazzola Zen[2]

[1] Graduate Program in Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Brazil
[2] Department of Internal Medicine, Clinical Genetics, UFCSPA and Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Porto Alegre, Brazil

Address for correspondence Paulo Ricardo Gazzola Zen, PhD, Serviço de Genética Clínica, UFCSPA, Rua Sarmento Leite, 245/403 Bairro Centro, Porto Alegre, Rio Grande do Sul 90050-170, Brazil (e-mail: paulozen@ufcspa.edu.br).

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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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is rare.[6,7] We report a girl, diagnosed with 22q11DS, with ectopic calcifications in soft tissues and suspicion of PHP.

Case Description

We report a 2-month and 13 day-old girl with a history of congenital heart disease, presence of ectopic calcifications in soft tissues (periosteal region), and facial dysmorphism. The proband was the first child of young and nonconsanguineous parents, with a healthy maternal half-sister. Pregnancy was uneventful and the prenatal serologies were negative. The mother denied smoking or alcohol intake during pregnancy. The child was born by cesarean section at 39 weeks and 5 days of gestation, weighing 2,630 g, with meconium-stained amniotic fluid and Apgar scores of 8 and 10. The patient evolved with desaturation, requiring orotracheal intubation. She was immediately transferred to the neonatal intensive care unit. Her neonatal period was complicated with several episodes of sepsis that were treated by antibiotic therapy. Echocardiogram showed double outlet right ventricle with severe pulmonary hypoplasia and minimal ductus arteriosus persistence (patent ductus arteriosus [PDA]).

Neonatal screening revealed a subclinical hypothyroidism (thyroid stimulating hormone [TSH] 14.8 uUl/mL [VR 0.85–6.15 uUl/mL] and T4 1.28 mg/dL [VR 0.94–1.44 mg/dL]), hypocalcemia that remained in the following dosages (total calcium [CaT] 8.6 mg/dL; 6.6 mg/dL; 8.3 mg/dL [VR 8.7–10.5 mg/dL]) and calciuria (CaT 11 mg/L; 29 mg/L; 177 mg/L). Creatinine (<0.1 mg/dL) and urea (28 mg/dL) levels were normal. Renal diseases and immunoglobulinopathies were excluded. Sodium (140 mEq/L [VR 132–146 mEq/L]), potassium (4.9 mEq/L [reference value (RV) 3.5–5.5 mEq/L]), magnesium (2.1 mg/dL [RV 1.6–2.4 mg/dL]), ionic calcium (4.8 mg/dL [RV 4.0–4.8 mg/dL]), serum inorganic phosphorus (5.6 mg/dL [VR 3.5–6.6 mg/dL]), glucose (82 mg/dL [VR 70–99 mg/dL]), and 25-hydroxy vitamin D (27 ng/mL, [VR >20 ng/mL]) dosages were normal. Hormonal investigation of parathyroid function (PTH) initially showed values close to the limit of the reference value (PTH 77 pg/mL; 85.2 pg/mL [VR 18–88 pg/mL]). After supplementation with calcium phosphate, cholecalciferol, and calcitriol, PTH levels were elevated (156.8 pg/mL).

Physical examination revealed bone callus in the right wrist. Radiological examinations showed no morphological or structural alterations in the skull and face, cervical spine, dorsal spine, or sacral loin. However, a double cortical contour was observed in the proximal third of both femurs, suggesting cortical hyperostosis or periosteal calcification along the femoral diaphysis. Dysplasia of the left acetabular ridge was also observed, as well as signs of osteopenia in the long bones, upper and lower limbs, and pelvis. Signs of osteopenia were noticed in both hands and wrists. Ectopic calcifications in soft tissues were observed in the distal end of the right forearm, in the wrist bones and in the proximal end of the metacarpal (►**Fig. 1**).

High-resolution karyotype showed a normal female chromosomal constitution (46,XX). Molecular cytogenetic testing by fluorescent in situ hybridization using the commercial Vysis LSI DiGeorge/TUPLE1 (Abbot Molecular) dual color probe set showed a deletion of 22q11.2.

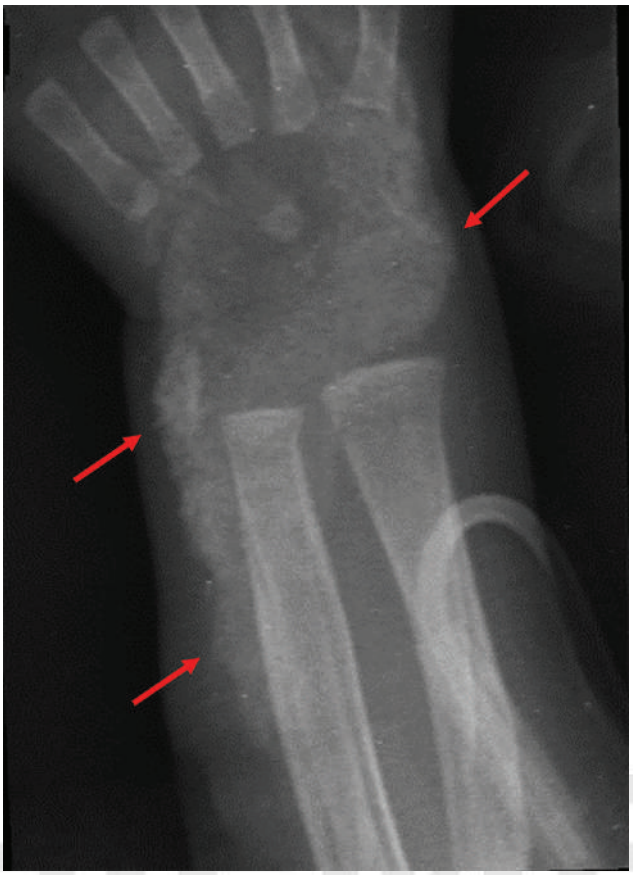

Fig. 1 Ectopic calcification in the right wrist evidenced on radiography.

At 8 months of age, the patient was readmitted for bronchiolitis. Dosages of PTH levels remained elevated (123.7 pg/mL). Pathogenesis evaluation of hypocalcemia revealed repeatedly high or normal levels of PTH, which was consistent with the diagnosis of PHP. The proband was submitted to a correction surgery for her congenital heart disease. While being operated, the proband had an episode of cardiac arrest and remained on mechanical ventilation by tracheostomy, developing a severe neurological condition. She passed away on the same day. Thus, the patient was diagnosed with 22q11DS and with presence of ectopic calcifications that could be associated with PHP.

Discussion

This report aimed to show a newborn girl diagnosed with 22q11DS and suspicion of PHP. The suspicion was raised due to hypocalcemia and increased levels of PTH along with ectopic calcifications in soft tissues.

PHP is a rare condition characterized by resistance or nonresponse to PTH that may have normal and/or increased serum levels.[6] As for the PHP type, biochemical and hormonal values verified in our case corroborate with the results found in other studies where the cases are diagnosed with PHP1a. Also, presence of hyperphosphatemia was not always evidenced (►**Table 1**). PHP1a is also known as AHO and has a variety of clinical findings such as short stature, mental

Table 1 Clinical findings for PHP1a: our case and literature case reports

Study	Patient age	Clinical findings for PHP1a							
		Seizures	Calcemia (N, ↑, ↓)	Hyperphosphatemia	Hypothyroidism	PTH (N, ↑, ↓)	TSH (N, ↑, ↓)	Ectopic calcification	AHO confirmed
Our case	2 mo and 13 d		↓		x	↑	↑	x	
Liu et al[6]	14 y	x	↓	x		N	N	x	
Adachi et al[8]	P1: 13 d P2: 9 d		N			↑	N	x	
			N		x	N	N	x	
Riepe et al[9]	7 mo					↑		x	x
Lubell et al[10]	2 y		N	x	x	↑	↑	x	x
Zung et al[11]	3 y		N			↑	↑	x	x

Abbreviations: ↑, high; ↓, low; AHO, Albright hereditary osteodystrophy; N, normal; P, patient; PHP1a, pseudohypoparathyroidism, type 1a; PTH, parathyroid hormone; TSH, thyroid stimulating hormone.

retardation, short metacarpals and obesity. During childhood, the clinical manifestations of PHP1a are normally subtle and, therefore, tend to be easily ignored.[6] Due to patient's death in the first month, it was not possible to identify dysmorphologies compatible with AHO.

Some individuals diagnosed with PHP1a have resistance to other hormones besides PTH, normally TSH and gonadotropins. Resistance to TSH, clinically indistinguishable from primary hypothyroidism, may become apparent before hypocalcemia and, occasionally, can be diagnosed through neonatal metabolic screening. The resistance to PTH and TSH is a possible explanation to justify the suspicion of PHP1a in our case.[12] Although ectopic calcification may be an early sign of PHP1a, there is no clear description about when it starts to develop. However, patients diagnosed with PHP1a and presence of subcutaneous calcification in the neonatal period have been reported.[8,9]

Association between 22q11DS and PHP in children is rare, mainly due to the variety of clinical features.[6,7] Abnormal parathyroid function was the first hormonal disorder related in 22q11DS: from a severe neonatal hypocalcemia to subclinical PTH failure. 22q11DS has an estimated prevalence of 1 per 4,500 live births and it is the most common autosomal deletion syndrome. In total, 30 to 50 genes are located in the deleted region of chromosome 22q11.2, which includes several transcription factors, that regulate thymus and parathyroid development.[6,7] The limited number of reports associating PHP and 22q11DS is mainly due to the location of the Gs-a protein on chromosome 20. However, Craigen raised the hypothesis that a more extensive deletion of chromosome 22 may lead to PHP due to the haploinsufficiency of protein G. This haploinsufficiency would be a consequence of a Gs-alpha protein locus adjacent to the critical region of chromosome 22. Heterozygous mutations in the GNAS gene are the main cause of Gsa protein-reduced activity.[9] Liu et al described a patient diagnosed with 22q11DS that had a wide phenotypic spectrum with multiple dysmorphisms. The authors suggested, that when an individual is diagnosed with PHP, 22q11DS should be considered. It is not impossible that 22q11DS occurs simultaneously with PHP.[6]

Different age groups, diagnosed with PHP have been reported (►**Table 1**). Therefore, it is important to note that the investigation for PHP, which may or may not be associated with 22q11DS, should be considered in all patients with hypocalcemia, independently of their age. PHP may be underdiagnosed in patients with 22q11DS since hormonal (hyper/hypothyroidism) and mineral (hypocalcemia) disorders are common in these individuals.[6] However, ectopic calcifications are usually evidenced in PHP.[3] Considering that the 22q11 region includes several transcription factors that regulate thymus and parathyroid development, we suggest that individuals diagnosed with 22q11DS and with bone dysmorphisms should be considered for parathyroid abnormal function investigation. Therefore, PHP could be a new clinical finding associated with 22q11 deletion.

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Conflict of Interest
None declared.

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

Bruna Lixinski Diniz[1] Andressa Schneiders Santos[2] Andressa Barreto Glaeser[1] Bruna Baierle Guaraná[3] Cláudia Fernandes Lorea[4] Juliana Alves Josahkian[5] Janaína Huber[6] Rafael Fabiano Machado Rosa[1,3] Paulo Ricardo Gazzola Zen[1,3]

[1] Department of Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil
[2] Department of Biomedicine, UFCSPA, Porto Alegre, RS, Brazil
[3] Department of Internal Medicine, Clinical Genetics, UFCSPA and Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Porto Alegre, RS, Brazil
[4] Child and Adolescent Health Care Unit, Hospital Escola da Universidade Federal de Pelotas (HE-UFPEL), Pelotas, RS, Brazil
[5] Department of Clinical Medicine, Hospital Universitário de Santa Maria (HU-SM), Santa Maria, RS, Brazil
[6] Department of Congenital and Pediatric Heart Disease, Instituto de Cardiologia/Fundacão Universitária de Cardiologia, Porto Alegre, RS, Brazil

Address for correspondence Paulo Ricardo Gazzola Zen, PhD, Serviço de Genética Clínica/UFCSPA, Rua Sarmento Leite, 245/403–Bairro Centro, Porto Alegre, RS 90050-170, Brasil (e-mail: paulozen@ufcspa.edu.br).

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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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CHD is a condition characterized by structural and functional abnormalities of the heart and great vessels, which originate during cardiac embryogenesis.[5] It has an incidence of 19 to 75 cases per thousand live births. CHD has a complex and multifactorial etiology, with approximately 80% of CHDs being a combination of genetic and environmental factors.[6] Aneuploidies and microdeletions of chromosomal regions are associated with the development of CHD,[7] but how this chromosomal imbalance alters cardiogenesis is unclear and probably much more complex than a gene dosage effect. The majority of CHD occurs as isolated malformations while 25 to 30% are associated with extracardiac abnormalities and are often found in association with known genetic syndromes.[8]

Cardiac malformation caused by genetic or chromosomal abnormalities, such as Down and velocardiofacial syndrome, is found approximately 20% of cases".[9] In the general population, 22q11.2 deletion is one of the most common detectable causes of several clinical conditions. A large number of individuals with CHD have 22q11.2 deletion, such as individuals with interruption of the aortic arch type B (52%), truncus arteriosus (34%), tetralogy of Fallot (ToF) (16%), and ventricular septal defects (5–10%).[10]

G-band karyotype is considered the gold standard for chromosomal diseases analysis such as aneuploidy, trisomy, or monosomies, including large structural rearrangements (> 5–10 Mb). However, visualization of small submicroscopic alterations, such as microdeletions, is unable to be performed by this technique.[11] Fluorescent *in situ* hybridization (FISH) was the first methodology applied in cytogenetic microdeletion research,[12] and is currently one of the main techniques for clinical diagnosis as well as for microdeletion and microduplication analysis.[13] Through hybridization probing using doubly labeled fluorescent probes, the identification of aneuploidies and chromosomal rearrangements can be obtained in 48 hours.[14]

Although genetic diseases are individually rare, their frequency as a group is an important field in medical care. Early diagnosis is extremely important and directly impacts on infant morbidity and mortality when associated with congenital malformations, especially in the neonatal period. Identification of chromosomal abnormalities is important in prenatal follow-up and in early diagnosis, contributing to family planning through adequate genetic counseling.

In Brazil, the unified health system (UHS) offers every Brazilian citizen full, universal, and free access to health services. Considered one of the largest and best public health systems in the world, the UHS benefits approximately 180 million Brazilians and provides medical assistance from simple outpatient procedures to highly complex care. However, molecular genetic tests are not routinely provided by this system, neglecting many individuals who have some genetic disease. Thus, research works, such as this one, are essential to help in the diagnosis of patients who need molecular investigation.

Despite the phenotypic variability of 22q11.2DS, CHD has become the main finding for clinical suspicion along with the presence or absence of facial and vertebral dysmorphic findings.[15–18] 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.

Materials and Methods

This was a prospective study with convenience sampling developed at the cytogenetics laboratory/Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) from January 2017 to December 2019. The sample consisted of patients cared for by the UHS presenting clinical findings suggestive of 22q11.2DS. The patients were followed up at the Hospital da Criança Santo Antônio, Hospital Escola of Universidadede Pelotas, Hospital Universitário de Santa Maria and Instituto de Cardiologia do Rio Grande do Sul. After genetic evaluation, a blood sample was collected in a heparin tube and then sent to the cytogenetics laboratory/UFCSPA for cell culture and analysis by FISH.

Genetic evaluation consisted of a review of clinical data and medical history such as echocardiography, followed by a dysmorphological physical examination. Then, a peripheral blood sample was collected from all participants in a heparin tube for cell culture, which was based on Moorhead et al[19] protocol. Molecular cytogenetic testing by FISH was performed using the commercial Vysis LSI DiGeorge/TUPLE1 (Abbott Molecular) region dual-color probe, which identifies deletions of band 22q11.2.This probe mixture contains the OrangeSpectrum TUPLE 1 (HIRA) probe (30 regions without TUPLE 1, D22S553, D22S609, and D22S942 coding) and the GreenSpectrum LSI arylsulfatase A (ARSA) control probe that maps the telomeric end of 22q (22q13.3). The protocol suggested by the manufacturer was used to perform this methodology on fixed cell suspension samples. The analysis was performed under an Axio Imager Z2 Zeiss fluorescence microscope (Göttingen, Germany). In each case, 10 metaphase and 200 complete interphase nuclei were analyzed for respective hybridization signals of the probes. The pictures were captured using a high-resolution digital camera coupled to the fluorescence microscope and processed using Isis Fluorescence Imaging System software. The described analysis, with the count of fluorescent signals per metaphase and per interphase nucleus, were performed in the laboratory standard form.

Research ethics committee of all participant hospitals and UFCSPA approved the present study. Only patients whose parents consented to participate were included in the study.

Results

Blood samples from 47 individuals aged 3 days to 14 years with clinical findings suggestive of 22q11.2DS were referred to the cytogenetics laboratory. The final sample consisted of 46 individuals (23 females and 23 males). One patient was excluded due the absence of cellular growth.

In total, three group patients with profiles suspicious for 22q11.2DS were referred for analysis: individuals with only dysmorphic features ($n = 4$), individuals with isolated heart disease ($n = 7$), and individuals with both dysmorphia and CHD ($n = 35$). Of all individuals analyzed, 56.52% (26/46) had karyotype results: 88.46% (23/26) with normal chromosomal

Table1 Results of cytogenetic analysis according to the profiles of suspected individuals

Groups	Normal karyotype ($n = 23$)	FISH results	Abnormal karyotype ($n = 3$)	FISH results	Without karyotype ($n = 20$)	FISH results
Only dysmorphisms ($n = 4$)	3	del22 (0)	0	del22 (0)	1	del22 (0)
Isolated heart disease($n = 7$)	1	del22 (0)	0	del22 (0)	6	del22 (0)
CHD + dysmorphisms($n = 35$)	19	del22 (4)	3	del22 (0)	13	del22 (3)

Abbreviations: CHD, congenital heart disease; FISH, fluorescent *in situ* hybridization.

constitution (46.XX/46.XY) and 11.53% (3/26) with altered chromosomal constitution. In addition, 46.47% (20/46) had no karyotype result. Alterations identified in the three altered karyotypes were 46,XX,del(16) (q12.1q22); 47, XX, + mar and 46,XY,t(6;8) (q23.3;q22.1). FISH was performed in 46 patients with clinical findings of 22q11DS, including those with altered karyotype results. In the overall sample, 15.21% (7/46) showed a 22q11.2 deletion, consistent with the clinical diagnosis of 22q11.2DS. Among the three suspect groups' profiles, only patients with dysmorphic features associated with CHD had the deletion. (►**Table 1**).

Dysmorphia and Cardiac Alterations Analysis of Patients with 22q11.2 Deletion

The main alterations found in patients with 22q11.2 deletion were dysmorphic facial features (100%); skeletal abnormalities (57.14%); breast abnormalities (28.57%); skin abnormalities (14.28%); neurological alterations (14.28%); endocrine alterations (42.85%), and CHD (100%) (►**Table 2**).

Considering the types of alterations, some dysmorphisms and cardiac defects were identified exclusively in patients with 22q11 deletion, such as small anterior fontanel (1/7), ectopic calcifications in soft tissue (1/7), café au lait spots (1/7), pseudohypoparahyroidism (PHP) (1/7), obstructive pulmonary valve ring (1/7), truncus arteriosus (1/7), and bicuspid aortic valve (2/7) (►**Tables 2** and **3**).

Unexpected Results

The three altered karyotypes identified by FISH did not show a 22q11 deletion. However, idic (22q11.2) chromosome was identified in the individual with karyotype 47,XX, + mar. In addition, one of the patients without a karyotype result showed a 22q13 (LSI ARSA × 1) deletion by FISH. The FISH analyses of both patients are presented in ►**Fig. 1**.

Discussion

22q11.2DS is known for the heterogeneity of clinical findings and the difficulty of establishing a reliable analysis based only on phenotype. In Brazil, the diagnosis is still a clinical one due to the difficulty of performing genetic tests, especially in UHS, which if performed could help in better follow-up and treatment of these individuals. The main medical centers, when less experienced, are not exempt from underdiagnosing patients with 22q11.2DS.[20] The overall prevalence of clinical alterations detected may differ according to the age of the patient and the specialist's experience. Currently, 180 clinical features and symptoms, such as physical and behavioral abnormalities, have already been described. However, typical findings are not found in all cases,[21] corroborating the clinical findings of this study.

In our study, we classified the patients with clinical suspicion of 22q11.2DS referred for molecular analysis into three clinical profiles. Of these, only patients with dysmorphic features associated with CHD had a 22q11.2 deletion (7/35). This result is compatible with literature. 22q11.2 deletion is more frequent in patients with CHD associated with other clinical findings (80–90%) than in patients with isolated conotruncal heart disease (29%).[22] The interindividual variability of the 22q11.2 deletion phenotype is characteristic, once individuals with significant clinical expression of the syndrome can be found, as well as in moderately affected individuals. The precise phenotypic evaluation of patients with deletion demonstrates that facial abnormalities—severely or slightly expressed—are detectable in all cases,[23] which corroborates with our findings. All individuals with 22q11.2 deletion besides heart defects showed facial dysmorphic features that ranged from skull, nose, eyes, and ears alterations (►**Fig. 2**). Among the facial abnormalities found, the small anterior fontanel was exclusively seen in deleted patients ($n = 1$, ►**Table 2**).

In our sample, 91.3% of the patients had CHD and in 16.7%, the malformation was isolated. All individuals with 22q11.2 deletion showed extracardiac alterations and CHD (15.21%). Other studies found similar results. Fokstuen et al[15] investigated the incidence of 22q11.2 deletion in a series of patients with extracardiac alterations with CHD and isolated heart defects. All patients with the deletion presented with major or minor additional extracardiac anomalies. No deletion was detected in the group of isolated cardiac malformations. Wozniak et al[16] also analyzed children with CHD (conotruncal and nonconotruncal) who presented with at least one additional characteristic of 22q11.2DS. The deletion was detected in 14.94% of the patients examined and after CHD, an abnormal facial finding was the most evidenced clinical feature in the study (84.51%). Halder et al[17] also performed a study to determine the prevalence and capacity of clinical criteria to predict 22q11 deletion. In total, only

Table 2 Clinical findings observed in patients with 22q11 deletion x patients without 22q11 deletion

	Individuals diagnosed with 22q11.2 deletion								*Without 22q11.2 deletion*
	P12	P13	P15	P22	P23	P29	P31	*n*	*n*
Age	2 mo	5 d	3 mo	16 d	14 y	5 d	6 mo		
Face dysmorphia									
Skull									
Large anterior fontanel			+				+	2	7
Small anterior fontanel		+						1	0
Nose									
Nasal bridge, wide		+	+		+			3	11
Nasal bridge, depressed							+	1	2
Tubular nose						+		1	1
Nares, anteverted		+						1	1
Eyes									
Palpebral fissure	+	+						2	4
Epicanthal fold					+			1	2
Ears									
Low ear implantation			+					1	8
Microtia	+							1	1
Overfolded superior helix	+	+		+			+	4	9
Skeletal abnormalities									
Ectopic calcification in soft tissue			+					1	0
Sacral anomalies		+						1	12
Short neck		+	+			+		3	6
Clinodactyly of the fifth fingers		+						1	10
Hypoplastic phalanges		+						1	1
Hypoplastic nails				+				1	2
Breast abnormalities									
Teletelia		+	+					2	3
Inverted nipple		+	+					2	1
Skin abnormalities									
Café au lait spots							+	1	0
Neurological alterations									
Delayed psychomotor development					+			1	3
Endocrine alterations									
Hypocalcemia	+	+	+					3	1
Pseudohypoparathyroidism			+					1	0
CHD	+	+	+	+	+	+	+	7	35

Abbreviations: CHD, congenital heart disease; d, day; mo, month; n, number of patients; P, patient; y, year.

6.16% of individuals had the deletion and none of the cases with isolated heart defects was positive for 22q11.2DS. The most frequent alterations found in these individuals were also dysmorphic facial features. The differences in prevalence of patients with the deletion who have CHD and facial dysmorphic features can be explained by the sample size.

When comparing our results with other studies, it can be seen that screening for 22q11.2 microdeletion in cases of cardiac malformations associated with extracardiac manifestations, in the form of facial dysmorphism, should be considered. It is also known that it is difficult to carry out an efficient dysmorphological assessment in newborns or in infants in the intensive care unit (ICU). These characteristics are often subtle or less evident, making CHD one of the main clinical suspicions for 22q11DS in these individuals. In these situations, it is important to observe if the patient does not

Table 3 Heart defects observed in patients with 22q11 deletion x patients without 22q11 deletion

	Individuals diagnosed with DS22q11								Without DS22q11 diagnosis
CHD	P12	P13	P15	P22	P23	P29	P31	*n*	*n*
Pulmonary valve agenesis						+		1	1
Right aortic arch	+	+	+			+		4	5
Pulmonary atresia	+			+				2	3
Atrial septal defects		+		+	+	+	+	5	27
Interventricular communication	+			+	+			3	19
Double outlet right ventricle			+					1	5
Interruption of the aortic arch type B							+	1	1
Obstructive pulmonary valve ring						+		1	0
Patent ductus arteriosus					+			1	14
Tetra logia of Fallot				+		+		1	12
Truncus arteriosus		+						1	0
Bicuspid aortic valve		+					+	2	0

Abbreviations: CHD, congenital heart disease; n, number of patients; P, patient.

have any other alterations compatible with the syndrome since isolated heart disease has not been demonstrated to be a good parameter for molecular analysis referral.

Conotruncal heart defects are often found in these individuals. ToF is the most described alteration,[24] but interruption of the aortic arch type B, truncus arteriosus, and ventricular septal defects are also frequently observed.[10] We analyzed the types of heart defects present in patients with 22q11.2 deletion and verified if there were any exclusive alteration in these individuals. In total, 12 heart defects were observed (►**Table 3**). Obstructive pulmonary valve ring, truncus arteriosus, and bicuspid aortic valve were present only in patients with the deletion.

Study of a specific "cardiac phenotype" in patients with 22q11.2 deletion shows that a particular cardiac anatomy can be identified.[18] In fact, patients with conotruncal heart defect often have additional heart defects that may lead to the suspicion of the presence of the 22q11 deletion.[23] In our sample, the most frequent alterations in this individuals were atrial septal defect (ASD) (5/7), followed by right aortic arch (4/7). However, these findings were also observed in the nondeleted individuals associated with other heart defects. Coincidentally, the three exclusive alterations in the deleted individuals were associated with an ASD and right aortic arch. Aortic arch alterations are observed in 85% of 22q11.2DS cases, and when there is an association with other heart defects, it is one of the main reasons to be referred for a 22q11DS investigation.[7] Association between interruption of the aortic arch type B, persistent truncus arteriosus, ToF, and ventricular septal defect with ASD have also been described in patients with 22q11.2DS.[25]

Other clinical features were also seen exclusively in the deleted patients, such as ectopic calcification in soft tissue ($n = 1$), café au lait spots ($n = 1$), and PHP ($n = 1$). Ectopic calcification was a consequence of PHP in only one patient of this study, a rare case of association of PHP with 22q11.2 deletion.[26]

FISH is the most widely used genetic diagnostic procedure for 22q11.2 deletion analysis.[27–29] However, commercially available FISH probes cannot detect atypical deletions.[30] In total, seven deleted individuals (15.21%) were confirmed, but it is important to take into account that although the rest of the individuals have clinical signs of the syndrome and have no detectable deletion, other causes may be responsible for the phenotype. Mutations in genes associated with heart disease, such as *TBX1* (located in the deleted region of chromosome 22),[31] *GATA4* (8p23.1),[32] and *DGS2* (10p13–14),[33] are other possible causes.

Others diagnostic methods can also be used to identify 22q11.2 deletion and determine microdeletion size and chromosomal breakpoints, such as high-resolution comparative genomic hybridization, multiplex ligation dependent probe amplification (MLPA), short tandem repeats, as well as quantitative polymerase chain reaction (qPCR).[34] Kuo et al[35] described a case of a fetus with CHD diagnosed by ultrasonography (singleton fetus with heart defects including overriding aorta, small pulmonary artery, and ventricular septal defect) associated with extracardiac defects, and referred for molecular analysis for 22q11.2 deletion. MLPA, a-CGH, and FISH techniques confirmed the deletion. Conventional cytogenetic analysis revealed a normal male karyotype. Prenatal identification of CHD is beginning to play an important role, since early diagnosis is crucial for the treatment and follow-up of these individuals, improving the life quality and reducing premature deaths.[36]

In our study, 22q11.2 deletion was not detected by karyotype analysis in any case, reinforcing the idea that this technique is ineffective for micro deletion research. However, karyotype examination is useful to detect other chromosomal alterations that may or may not be related to clinical findings that resemble 22q11.2DS. The three individuals with altered karyotype were referred for 22q11.2 deletion molecular cytogenetic analysis, as they had clinical findings compatible with 22q11.2DS. However, no deletion was detected by FISH.

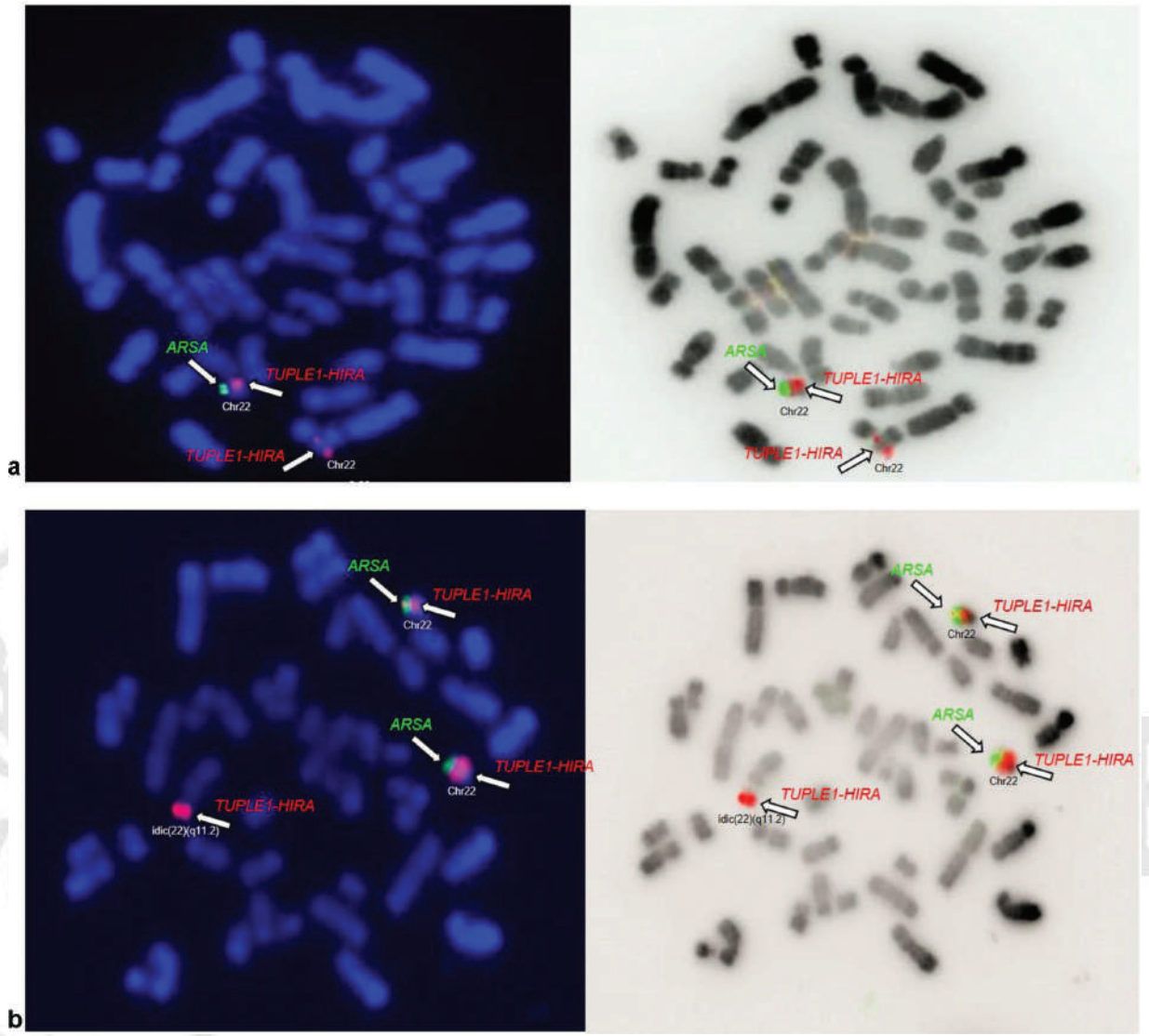

Fig. 1 FISH analysis with/without contrast: red signal, probe TUPLE1/HIRA specific to the 22q11.2 region and green signal, control probe arylsulfatase A specific to the 22q13 region. The arrows indicates the signs of hybridization. (**A**) FISH analysis in a patient with del 22q13. The normal chromosome 22 shows both red and green (marker) hybridization signal, while the deleted one shows only the green signal (marker). (**B**) FISH analysis in a patient with 47,XX, +mar. The normal chromosome 22 shows both red and green (marker) hybridization signal. Here, we can identify that an extra signal from the red probe TUPLE1/HIRA hybridized in the marker identified in the karyotype (+mar), concluding that this marker is idic (22)(q11.2). FISH, fluorescent *in situ* hybridization.

Patients with altered karyotypes (46,XX,del(16)(q12.1q22), 46,XY,t(6;8)(q23.3;q22.1), 47,XX, + mar) had heart defects and dysmorphic features that were also observed in our deleted patients (interventricular communication, ASD, patent ductus arteriosus, right aortic arch, double outlet right ventricle, sacral anomalies, large anterior fontanel, wide nose, and low ear implantation). We have not found any description in the literature of 16q12.1q22 deletion cases, as well as t(6;8) (q23.3; q22.1). Interstitial deletions near the 16q region (16q12.2q21[37] and 16q12.2-q13[38]) have already been reported in cases of skeletal abnormalities and cardiac malformations, respectively.

The 47,XX, + mar patient's karyotype revealed the presence of a nondefined chromosome origin isodicentric marker. Through FISH, it was observed that *TUPLE1/HIRA* probe hybridized on the marker chromosome allowing identification of the idic (22q11.2). This alteration may be associated with cat eye syndrome (OMIM 115470), a rare malformation syndrome whose diagnosis is based on the presence of an extra marker chromosome derived from chromosome 22.[39]

FISH also detected a 22q13 deletion in a patient with clinical characteristics similar to 22q11.2DS. This region has been associated with Phelan-McDermid syndrome (OMIM 606232)[40] and metachromatic leukodystrophy (OMIM 250100),[41] but our patient did not have clinical characteristics compatible with these two disorders.

Conclusion

More assertive clinical criteria for molecular testing are crucial for optimizing spending on testing, better diagnostics, treatment, and follow-up of patients. Furthermore, appropriate genetic counseling can be offered to families to aid them with risk of recurrence as well as prenatal care in future pregnancies.

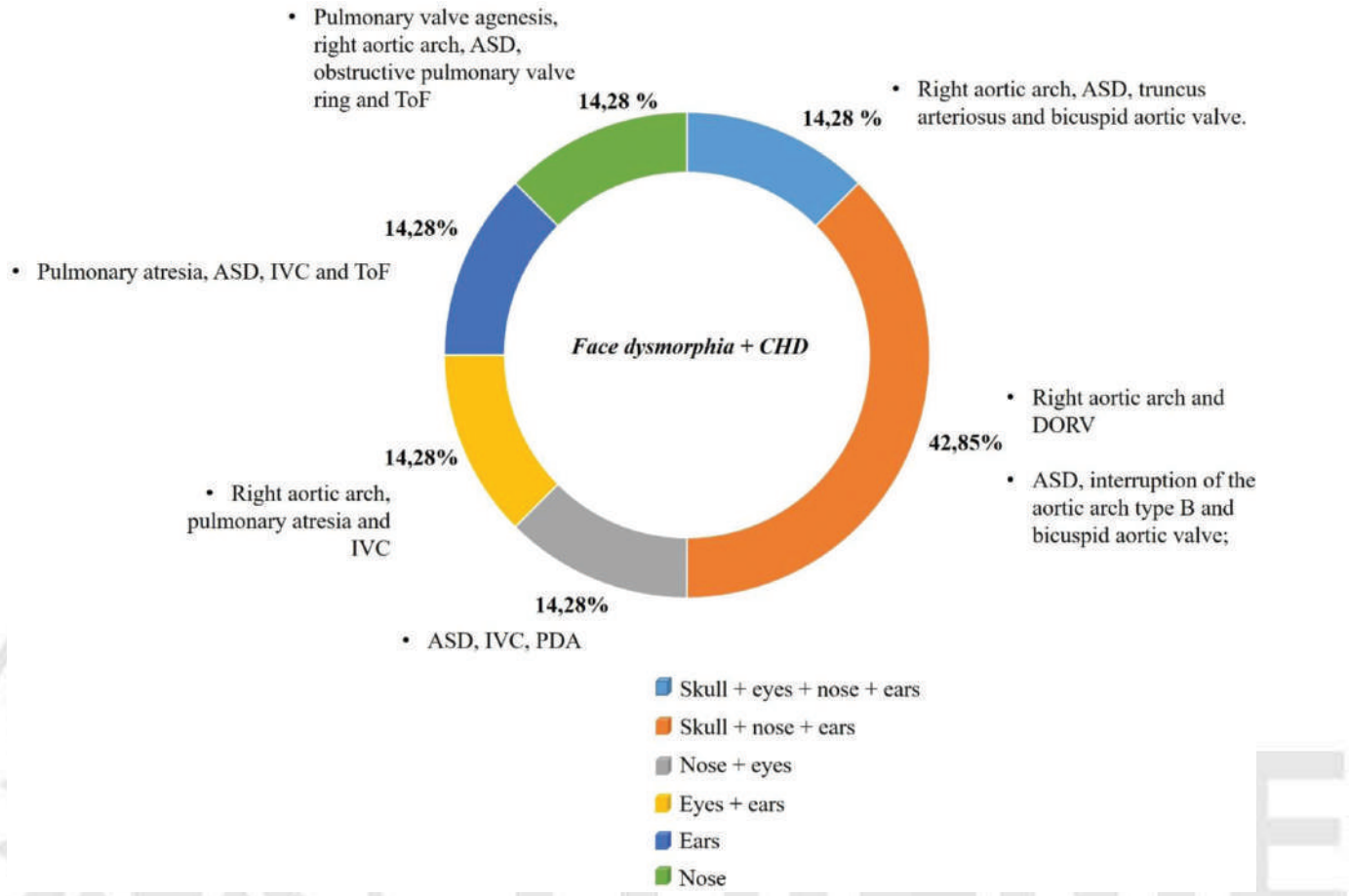

Fig. 2 Percentage of individuals with 22q11 deletion presenting face dysmorphia associated with congenital heart disease.

Facial dysmorphic features (skull, eyes, ear, and nose) associated with CHD proved to be a reliable clinical criterion for referral to undergo molecular analysis. Among heart defects, we suggest that individuals with obstructive pulmonary valve ring, truncus arteriosus, and bicuspid aortic valve associated with ASD and/or right aortic arch should undergo FISH investigation for 22q11.2 deletion. Particularly in newborns, the detection of these cardiac defects is crucial for the early diagnosis of this syndrome, as dysmorphisms associated with 22q11.2DS are often more difficult to evaluate.

Authors' Contributions

B.L.D, A.S.S, A.B.G., and P.R.G.Z performed genetic testing and wrote the manuscript. B.L.D., and P.R.G.Z supervised genetic testing. B.L.D, B.B.G., and R.F.M.R reviewed clinical data and edited the manuscript. A.S.S, A.B.G., B.B.G, C.F.L, and J.A.J reviewed the medical records. B.L.D, P.R.G.Z., and R.F.M.R designed the study, supervised genetic tests, wrote, and edited the manuscript.

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Conflict of Interest

None declared.

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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

Andressa Barreto Glaeser[1] Bruna Lixinski Diniz[1] Desirée Deconte[1] Andressa Schneiders Santos[2] Rafael Fabiano Machado Rosa[3] Paulo Ricardo Gazzola Zen[3]

[1] Graduate Program in Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil
[2] Undergraduate Program in Biomedicine, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil
[3] Department of Internal Medicine, Clinical Genetics, Irmandade Santa Casa de Misericórdia de Porto Alegre, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

Address for correspondence Paulo Ricardo Gazzola Zen, PhD, Serviço de Genética Clínica, Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 245/404, Porto Alegre, RS, CEP: 90050-170, Brazil (e-mail: paulozen@ufcspa.edu.br).

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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

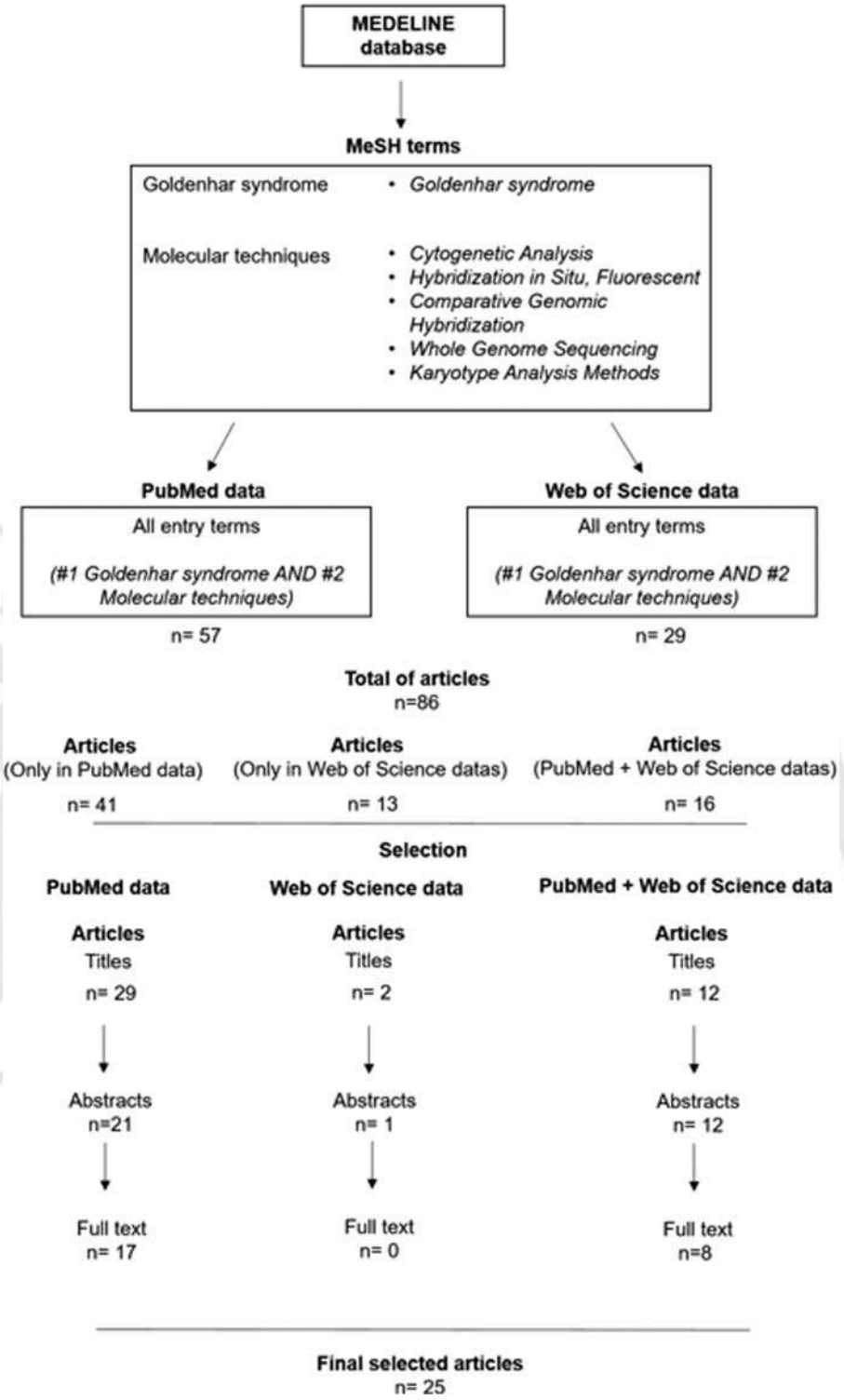

Fig. 1 Flowchart of the study selection process.

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' Contributions

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

Andressa Barreto Glaeser[1] | Andressa Schneiders Santos[2] | Bruna Lixinski Diniz[1] | Desireé Deconte[1] | Rafael Fabiano Machado Rosa[3] | Paulo Ricardo Gazzola Zen[3]

[1]Graduate Program in Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Brazil

[2]Underdegree Program in Biomedicine, UFCSPA, Porto Alegre, Brazil

[3]Department of Internal Medicine, Clinical Genetics, UFCSPA and Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Porto Alegre, Brazil

Correspondence
Paulo Ricardo Gazzola Zen, Serviço de Genética Clínica/UFCSPARua Sarmento Leite, 245/404, Bairro Centro, Porto Alegre 90050-170, RS, Brazil.
Email: paulozen@ufcspa.edu.br

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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.

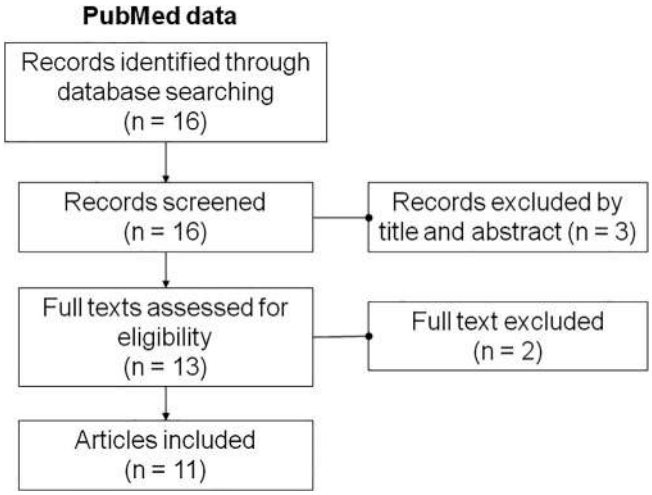

FIGURE 1 Flow diagram of article inclusion

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

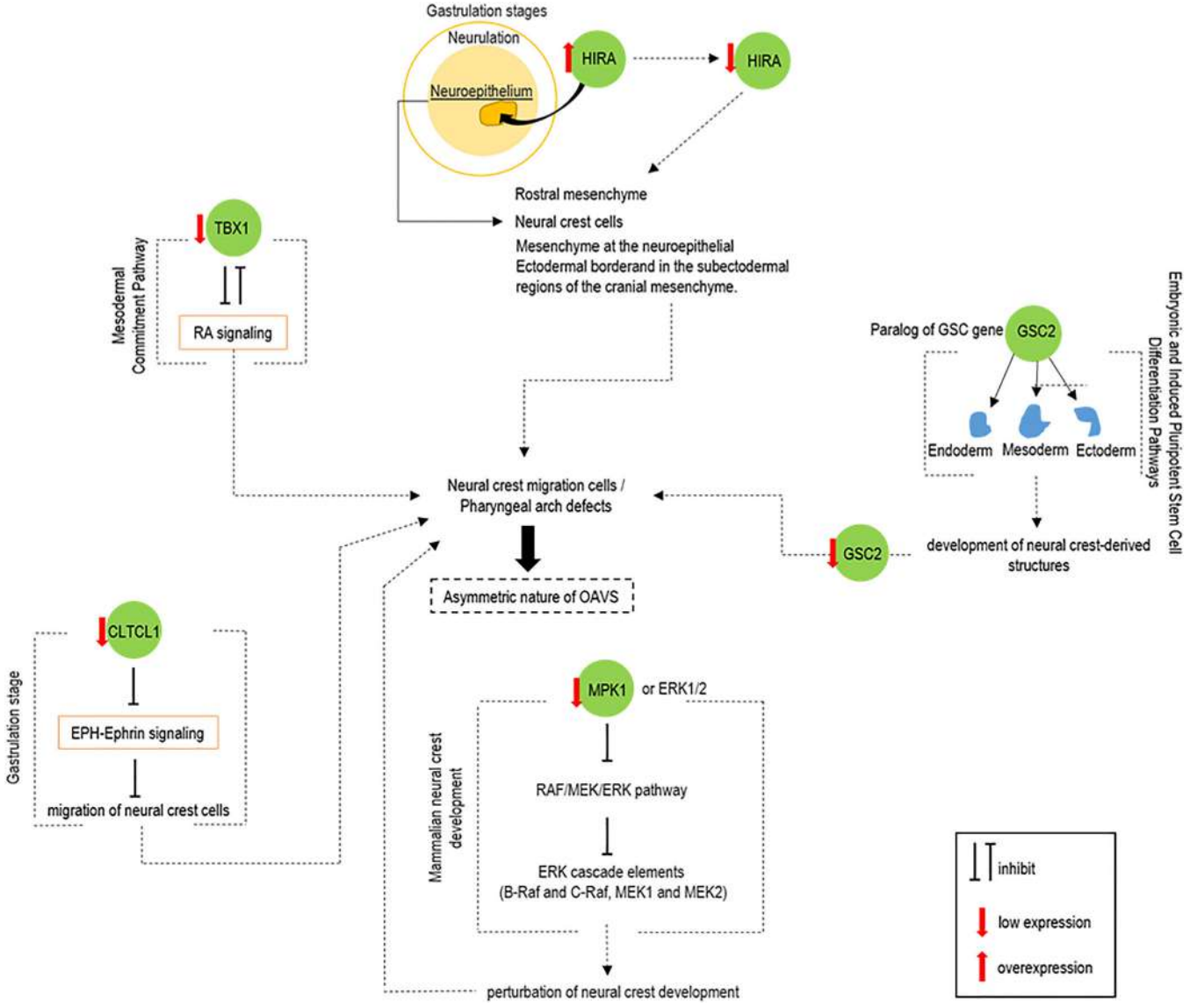

FIGURE 2 Possible pathways involved and mechanisms for oculo-auriculo-vertebral spectrum (OAVS) asymmetry [Color figure can be viewed at wileyonlinelibrary.com]

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.

ORCID

Andressa Barreto Glaeser https://orcid.org/0000-0002-1534-0900
Andressa Schneiders Santos https://orcid.org/0000-0002-7673-3417
Bruna Lixinski Diniz https://orcid.org/0000-0003-1448-0298
Desireé Deconte https://orcid.org/0000-0002-6869-9167
Rafael Fabiano Machado Rosa https://orcid.org/0000-0003-1317-642X
Paulo Ricardo Gazzola Zen https://orcid.org/0000-0002-7628-4877

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A child with cat-eye syndrome and oculo-auriculo-vertebral spectrum phenotype: A discussion around molecular cytogenetic findings

Andressa Barreto Glaeser [a], Bruna Lixinski Diniz [a], Andressa Schneiders Santos [b], Bruna Baierle Guaraná [c], Victória Feitosa Muniz [c], Bianca Soares Carlotto [a], Eduardo Morais Everling [d], Patrícia Yuri Noguchi [d], Aline Ramos Garcia [d], Juliana Miola [d], Mariluce Riegel [e,f], Rafaella Mergener [e], Paulo Ricardo Gazzola Zen [a,g], Rafael Fabiano Machado Rosa [a,g,*]

[a] Graduate Program in Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil
[b] Underdegree Program in Biomedicine, UFCSPA, Porto Alegre, RS, Brazil
[c] Medical Residency, Clinical Genetics, UFCSPA, Porto Alegre, RS, Brazil
[d] Graduation in Medicine, UFCSPA, Porto Alegre, RS, Brazil
[e] Graduate Program in Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[f] Medical Genetics Service, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil
[g] Department of Internal Medicine, Clinical Genetics, UFCSPA and Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA), Porto Alegre, RS, Brazil

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ABSTRACT

Cat eye syndrome (CES) is a rare chromosomal disorder that may be evident at birth. A small supernumerary chromosome is present, frequently has 2 centromeres, is bisatellited, and represents an inv dup(22)(q11) in those affected. It's known that the 22q11 region is associated with disorders involving higher and lower gene dosages. Conditions such as CES, 22q11 microduplication syndrome (Dup22q11) and oculoauriculovertebral spectrum phenotype (OAVS) may share genes belonging to this same region, which is known to have a predisposition to chromosomal rearrangements. The conditions, besides being related to chromosome 22, also share similar phenotypes. Here we have added a molecular evaluation update and results found of the first patient described with CES and OAVS phenotype, trying to explain the potential mechanism involved in the occurrence of this association.

1. Introduction

Cat-eye syndrome (CES) (OMIM #115470), also known as Schmid-Fraccaro syndrome, chromosome 22 partial tetrasomy, or chromosome 22 inversion duplication is a rare genetic condition affecting 1 in 150,000 live births (Sharma et al., 2014). CES is caused by the existence of a small supernumerary marker chromosome derived from the proximal part of the 22q11 chromosome (Melo et al., 2013). Most commonly observed clinical findings in these patients include a classic triad of iris colobomas, preauricular skin tags/pits, and anal atresia (Jedraszak et al., 2013; Mears et al., 1994; Schinzel et al., 1981). However, they can also present multiple malformations, involving eyes, ears, and cardiac, anorectal and urogenital systems, indicating a high possibility of phenotypic variability, which cause is still unknown (Berends et al., 2001). The 22q11 region is highly susceptible to chromosomal rearrangements and is associated with other conditions and phenotypes such as 22q11.2 deletion syndrome (DiGeorge/Velocardiofacial syndrome) (SD22q11.2), 22q11.2 microduplication (Dup22q11) (McDermid and Morrow, 2002; Portnoi, 2009), and oculoauriculovertebral spectrum phenotype (OAVS) (Beleza-Meirelles et al., 2015; Colovati et al., 2015; Digilio et al., 2009; Derbent et al., 2003; Glaeser et al., 2020; Quintero-Rivera and Martinez-Agosto, 2013; Spineli-Silva et al., 2018; Torti et al., 2013).

OAVS (OMIM #164210), which includes Goldenhar syndrome and hemifacial microsomia, is a rare and etiologically heterogeneous phenotype that mainly involves craniofacial malformations caused by first and second branchial arches alterations during embryonic development (Goldenhar, 1952; Gorlin et al., 2001), possibly due to a vascular (Poswillo, 1973) or a neural crest cell migration abnormality (Cohen et al., 1989). This spectrum is clinically wide, but the major

* Corresponding author. Genética Clínica UFCSPA Rua Sarmento Leite, 245/403 – Bairro Centro Porto Alegre, RS, CEP: 90050-170, Brazil.
E-mail address: rosa@ufcspa.edu.br (R.F. Machado Rosa).

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clinical features are ear abnormalities (anotia, microtia, and preauricular skin tags or pits), hemifacial microsomia, ocular defects, and vertebral malformations (Bragagnolo et al., 2018; Rooryck et al., 2010). Most cases are sporadic and etiology is not well understood. Association of OAVS phenotype with environmental factors, family case descriptions, chromosomal abnormalities, and some candidate genes suggest that this condition may have a rather heterogeneous origin (Bragagnolo et al., 2018). From the advent of new technologies, several chromosomal abnormalities have been associated with this spectrum (Rooryck et al., 2010; Beleza-Meireles et al., 2014), including deletions and duplications involving 5q13.2 (Huang et al., 2010), 5p15 (Descartes, 2006; Josifova et al., 2004), 10p14 (Dabir et al., 2006), 22q11.2 (Xu et al., 2008; Digilio et al., 2009; Tan et al., 2011; Quintero-Rivera and Martinez-Agosto, 2013) and 14q23.1 (Gimelli et al., 2013) regions. Among the potential candidate genes, *MYT1* was the first identified by molecular analysis (Lopez et al., 2016). The genes *YPEL1*, *HIC2* and *MPAK1/ERK2* have also been suggested due to their association with inadequate pharyngeal arch development and heart disease (Spinelli-Silva et al., 2018). However, until the moment, no gene has been confirmed to cause OAVS phenotype.

There are few reports in the literature of CES patients presenting OAVS phenotype (Beleza-Meireles et al., 2015; Quintero-Rivera and Martinez-Agosto, 2013; Rosa et al., 2010; Torti et al., 2013) and the mechanisms involved in the development of this clinical presentation are still not well elucidated. Here we have added a molecular evaluation update and results found of the first patient described with CES and OAVS phenotype, made by Rosa et al. (2010), trying to explain the potential mechanism involved in the occurrence of this association.

2. Patient and methods

2.1. Clinical report

A female patient, the first daughter of a healthy and non-consanguineous family, was initially evaluated at 26 days of life. At 4 months of gestation, her mother had syphilis and systemic arterial hypertension diagnosed (drug treatment was performed for both conditions). She worked in tobacco fields during pregnancy.

The child was born by normal delivery, weighing 3,080 g, measuring 50 cm, with a head circumference of 36 cm and Apgar scores of 8 and 10 in the first and fifth minutes, respectively. At birth, it was noted that the patient had craniofacial dysmorphism (Fig. 1), including hemifacial microsomia, ocular hypertelorism, downward slanting palpebral fissures, epicanthic folds, abnormal ears with microtia, preauricular skin tags, in addition to anorectal (imperforate anus with rectal fistula and sacral pit) and neurological (cerebral hypoplasia with ventricular dilation) abnormalities. She also showed neuropsychomotor development delay. No cardiological, ophthalmological or otolaryngological abnormalities were evidenced.

2.2. Genetic diagnostic testing

The GTG-Banding karyotype showed mosaicism: mos 47,XX,+mar [68]/46,XX[32] (Rosa et al., 2010; Silva et al., 2015). Parental karyotyping was normal. The marker chromosome present in the child was identified as an inv dup(22)(pter->q11.2::q11.2->pter) *de novo*, compatible with the CES diagnosis featuring an OAVS phenotype (Rosa et al., 2010).

Silva et al. (2015) conducted a study to investigate the presence of microdeletions in the 22q11.2 and 5p regions in this patient with fluorescent in situ hybridization (FISH) technique using the DNA probes Tel Vysion TM TUPLE 1 and Tel Vysion TM 5p SG (Abbott Molecular Inc., Des Plaines, Illinois, USA); however, no abnormalities were found.

At age 10, the patient underwent a whole genome comparative hybridization analysis by microarrays (array-CGH). It was performed using an 8 × 60K whole genome platform (design 021924, Agilent Technologies, Santa Clara, California, USA), with an average length of 40 kb between probes. The experiments were performed according to the manufacturer's protocol.

3. Results

Whole-genome array-CGH identified an increase of ~1.5 Mb involving the 22q11.1q11.21(17.153.988-18.641.468) region. This patient's breakpoints correspond to CES critical region (*CECR*), and include 21 RefSeq genes: *XKR3, HSFY1P1, GAB4, CECR7, IL17*RA, *CECR6, CECR5, CECR4, CECR1, CECR2, SLC25A18, ATP6V1E1, BCL2L13, BID, MIR3198, MICAL3, MIR648, FLJ41941, PEX26, TUBA8* e *USP18*. This report presents a complete molecular research of a patient with CES overlapping with the OAVS phenotype.

4. Discussion

The 22q11 region is associated with disorders involving higher and lower gene dosages. Conditions such as CES, Dup22q11 and OAVS may share genes belonging to this same region, which is known to have a predisposition to chromosomal rearrangements. Although 22q11.2 deletions and duplications are reciprocal rearrangements and predicted to occur at the same frequency, the number of duplication cases reported in the literature is considerably less and this is likely due to their milder clinical effect (Portnoi, 2009). Further to the associations with the 22q11 microdeletion, it has already been described that some genes found in this region, when duplicated, can also influence craniofacial alterations, a characteristic that occurs in both CES and OAVS, and

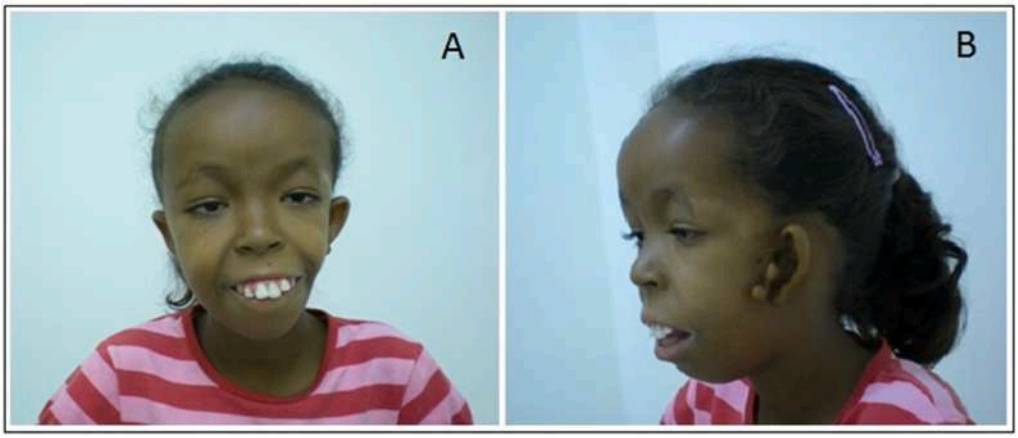
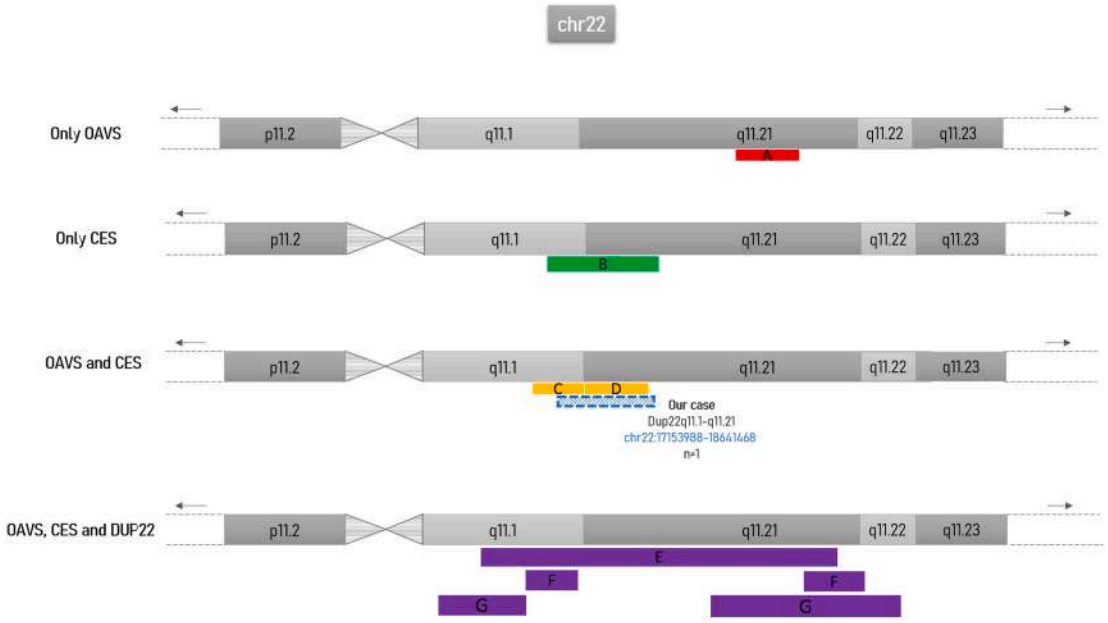


Fig. 1. Craniofacial features evidenced in the patient at age 10: hemifacial microsomia (A), preauricular skin tags and abnormal left ear (after microtia surgery).

Dup22q11 (Beleza-Meireles et al., 2015; Glaeser et al., 2020).

According to studies that described individuals with clinical findings of CES/OAVS and/or Dup22q11, it is possible to observe that molecular alterations start from the region chr22:15908677, and may extend to the region chr22:21734594 (Beleza-Meireles et al., 2015; Quintero-Rivera and Martinez-Agosto, 2013; Torti et al., 2013). Fig. 2 shows studies that described the regions found in CES, OAVS and/or Dup22q11 and our case. There is common involvement of genes *IL17*RA, *CECR1, CECR2, ATP6V1E1, BID, MICAL3, PEX26, TUBA8* and *USP18* with previously published cases (Beleza-Meireles et al., 2015; Knijnenburg et al., 2012; Quintero-Rivera and Martinez-Agosto, 2013; Torti et al., 2013).

There is no description of a candidate gene for the CES phenotype as well as the OAVS. However, it is known that homologous recombination events between 22q11 breakpoints during meiosis imply rearrangements at CES (Emanuel and Shaikh, 2001) and U-type exchange, between the sister chromatid and homologous chromosomes can also induce invdup formation (22) at CES (Van Dyke et al., 1977). Alterations such as these may originate phenotypes similar to those found in conditions such as OAVS and Dup22q11, with craniofacial dysmorphy being one of the most described clinical manifestations. It is known that the



A	Beleza-Meireles et al., 2015	Dup22q11.21	chr22: 20402618- 20659614	n=1	RIMBP3
B	Knijnenburg et al., 2012	Dup22q11.1-q11.21	chr22: 17799398 - 18397897	n=2	CECR2, SLC25A18, ATP6V1E1, BCL2L13, BID, all completely and MICAL3 partially
C	Beleza-Meireles et al., 2015	Dup22q11.1	chr22: 17068186-17290334	n=2	CCT8L2 (partial), FABP5P11, TPTEP1, SLC25A15P5, PARP4P3, ANKRD62P1-PARP4P3, ANKRD62P1, VWFP1, XKR3(partial)
D	Quintero-Rivera and Martinez-Agosto, 2013	Dup22q11.2	chr22:17381569-18618107	n=1	CECR1, CECR2, CECR3, CECR5, CECR6, CECR7, GAB4, AS1, IL17RA, ATP6V1E1, BCL2L13, BID, MIR3198-1, MIR648, FLJ41941, PEX26, TUBA8, MICAL3, USP18, G6T3P.
E	Beleza-Meireles et al., 2015	Dup22q11.21	chr22: 16495650-21616784	n=1	many including TBX1
F	Beleza-Meireles et al., 2015	Dup22q11.1 + Dup22q11.21	chr22: 17068186-17290334 + chr22:21468352-21722313	n=1	CCT8L2 (partial), FABP5P11, TPTEP1, SLC25A15P5, PARP4P3, ANKRD62P1-PARP4P3, ANKRD62P1, VWFP1, XKR3(partial) + POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13, FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6
G	Torti et al. 2013	Dup 22q11.1 + Del 22q11.2	chr22: 15908677-17021,290 + chr22: 20128774-21734594	n=1	IL17RA, CECR1, CECR2, SLC25A18, ATP6V1E1, BID, MICAL3, PEX26, TUBA8, and USP18 + HIC2, RIMBP3B, RIMBP3C, UBE2L3, SDF2L1, MIR130B, PPIL2, YPEL1, MAPK1, TOP3B, VPREB1, PRAME, GGTLC2, and RTDR1
Our case	Glaeser et al. 2021	Dup22q11.1-q11.21	chr22:17153988-18641468	n=1	XKR3, HSFY1P1, GAB4, CECR7, IL17RA, CECR6, CECR5, CECR4, CECR1, CECR2, SLC25A18, ATP6V1E1, BCL2L13, BID, MIR3198, MICAL3, MIR648, FLJ41941, PEX26, TUBA8, USP18.

Fig. 2. Location of molecular findings by chromosomal region described in CES, OAVS and/or Dup22q11 and in our patient. A: Dup22q11.21 (*RIMBP3*). B: Dup22q11.1-q11-21 (*CECR2*, *SLC25A18*, *ATP6V1E1, BCL2L13, BID,* all completely and *MICAL2* partially). C: Dup22q11.1 (*CCT8L2* (partial), *FABP5P11, TPTEP1, SLC25A15P5, PARP4P3, ANKRD62P1-PARP4P3, ANKRD62P1, VWFP1, XKR3* (partial)). D: Dup22q11.2 *(CECR1, CECR2, CECR3, CECR6, CECR7, GAB4, AS1, IL17*RA, *ATP6V1E1, BCL2L12, BID, MIR3198-1, MIR648, FLJ41941, PEX26, TUBA8, MICAL2, USP18, G6T3P)*. E: Dup22q11.21 (many including *TBX1*). F: Dup22q11.1 (*CCT8L2* (partial), *FABP5P11*, *TPTEP1*, *SLC25A15P5*, *PARP4P3*, *ANKRD62P1-PARP4P3*, *ANKRD62P1*, *VWFP1*, *XKR3* (partial)) + Dup22q11.21 (*POM121L7*, *GGT2*, *BCRP2*, *KB-1592A4.15*, *KB-2A4.13*, *FAM230B*, *KB-1592A4.A4*, *KB-1183D5.9*, *POM121L8P*, *BCRP6*). G: Dup22q11.1 (*IL17*RA, *CECR1*, *CECR2*, *SLC25A18*, *ATP6V1E1*, *BID*, *MICAL3*, *PEX25*, *TUBA8*, *USP18*) + Del 22q11.2 (*HIC2*, *RIMBP3B*, *RIMBP3C*, *UBE2L3*, *SDF2L1*, *MIR130B*, *PPIL2*, *YPEL1*, *MAPK1*, *TOP3B*, *VPREB1*, *PRAME*, *GGTLC2*, *RTDR1*). Our case: Dup22q11.1-q11.21 (*XKR3*, *HSFY1P1*, *GAB4*, *CECR7*, *IL17*RA, *CECR6*, *CECR5*, *CECR4*, *CECR1*, *CECR2*, *SLC25A18*, *ATP6V1E1*, *BCL2L13*, *BID*, *MIR3198*, *MICAL3*, *MIR648*, *FLJ41941*, *PEX26*, *TUBA8*, *USP18*) (Beleza-Meireles et al., 2015; Knijnenburg et al., 2012; Quintero-Rivera and Martinez-Agosto, 2013; Torti et al., 2013).

abnormal neural crest development is one of the main causes of craniofacial alterations (Schilling and Le Pabic, 2014), in which several pathways may be involved. Depending on the gene expression in each pathway, especially those responsible for neurulation and cardiogenesis, embryonic development may be affected.

In total, fourteen genes have already been identified in the critical region of the CES, located in a proximal 22q11 region, measuring about 2–2.5 Mb. Regarding the genes involved in the CES critical region, the CECR loci were proposed as probable candidates (Bridgland et al., 2003; Footz et al., 2001; Quintero-Rivera and Martinez-Agosto, 2013; Riazi et al., 2000). *CECR1* and *CECR2* genes were considered related to the duplication phenotype in the CECR loci (McDermid and Morrow, 2002).

CECR1 or *ADA2* (Adenosine Deaminase 2) (OMIM *607575) is homologous to adenosine deaminase growth factors (*ADGF*) and is expressed in human embryos in the outflow tract and in the heart atrium, as well as in the ganglion of cranial nerves VII and VIII, suggesting its potential involvement in cardiac and facial defects observed at CES (Riazi et al., 2000). *CECR1* has a domain of adenosine deaminase (ADA) that exerts its function in part through the maintenance of the extracellular adenosine level (Akalal and Nagle, 2001), which is responsible for cellular proliferation and migration, besides mediating a variety of physiological effects, such as vasodilation (Dubey et al., 1996).

CECR2 (OMIM *607576) encodes a supposed transcriptional co-activator involved in the neurulation and remodeling of chromatin. This action happens through the CECR2-containing remodeling factor (CERF) complex, which is expressed in the central nervous system during the posterior development of the eye and brain, demonstrating patterns of expression in embryogenesis in neural tissues and inferring a potential role in neurogenesis and malformations involving the neural tube (Banting et al., 2005; Quintero-Rivera and Martinez-Agosto, 2013). *CECR2* mutations trigger deregulation of mesenchymal or ectodermal transcription factors, neurogenesis and inner ear development (Fairbridge et al., 2010; Dawe et al., 2011; Quintero-Rivera and Martinez-Agosto, 2013).

In regards to CECR loci, our patient has alteration not only in *CECR1* and *CECR2* genes but also in *CECR6*, *CECR5* and *CECR4*. The predominance of craniofacial alterations in the patient, with findings such as hemifacial microsomia, abnormal ears with microtia and preauricular skin tags, that belong to the clinical spectrum of OAVS, could be explained mainly by the involvement of these genes. However, descriptions of this loci in the literature are associated only with the CES, and there is still no explanation about the overlap with the OAVS phenotype. In a recent review by Glaeser et al. (2020), some candidate genes were highlighted for association with OAVS and craniofacial dysmorphia, but the CECR loci did not show relevant results in the study. Thus, it is not possible to exclude that other genes located within 22q11.1-q11.21 region may play additional roles in phenotype and its variability (Quintero-Rivera and Martinez-Agosto, 2013). It is probable that craniofacial dysmorphia and the phenotypic overlap of OAVS with CES and SD22q11.2/Dup22 could be the result of epigenetic alterations from mutations in their respective causative genes, or in the case of hemifacial microsomia, from teratogenic factors. In addition, the description of facial asymmetry in genetic abnormalities with a mosaic etiology, including mosaic trisomy 22, suggests that a subset of cases may be due to somatic mosaicism. However, this would only explain the asymmetry. It is also noteworthy that the chromosomal rearrangement present by our patient overlapped with that of pseudo-TORCH syndrome 2 (PTORCH2), an autosomal recessive condition associated with pathogenic variants in the *USP18* gene (OMIM # 617397), that is located at 22q11.21 region. However, its clinical presentation differs from that observed in our patient and CES, and include findings such as microcephaly, brain malformations, intracranial calcifications, seizures, respiratory insufficiency, liver dysfunction, and often thrombocytopenia (Meuwissen et al., 2016). However, all these potential etiologies are still awaiting further experimental research to demonstrate a true association (Quintero-Rivera and Martinez-Agosto, 2013).

*IL17*RA (OMIM * 605461) is known for its role in the immune system, being transcribed in the type A receptor of interleukin-17 in T lymphocytes. An important paralogue is the *IL17RD* gene, which is part of the interleukin 17 receptor complex, with great performance in signaling the fibroblast growth factor, inhibiting or stimulating mitosis through MAPK/ERK signaling, which is involved in the development of the neural crest. Glaeser et al. (2020) suggested that alterations in the MAPK/ERK pathway in the mammalian neural crest development would cause a disturbance of neural crest development and consequently craniofacial malformations in individuals with OAVS. Based on the sequence and functional similarities, *IL17*RA appears to be a human homologous of the zebrafish *Sef* gene and is likely to play critical roles in endothelial or epithelial functions such as proliferation, migration and angiogenesis (Yang et al., 2003). These characteristics suggest that *IL17*RA may play a key role in the development of the OAVS phenotype found in our patient with CES.

ATP6V1E1 (OMIM *108746) mutations were reported in studies with individuals clinically diagnosed with OAVS, but with molecular findings overlapping the critical region of CES and dup22q11 (Quintero-Rivera and Martinez-Agosto, 2013; Torti et al., 2013). It is known that over-expression of this gene can cause anal atresia, preauricular skin tags or pits, growth hormone deficiency and unilateral renal agenesis (Knijnenburg et al., 2012). Tasse et al. (2005) describe that the preauricular skin tags, besides the factors that favor exacerbated cell growth, are characteristics of clinical diagnosis of OAVS. *ATP6V1E1* also plays a role in RET signaling pathway, which also includes RAF/MAP kinase cascade pathway, responsible for regulating processes such as proliferation, differentiation, survival, senescence and cell motility in response to growth factors, hormones and cytokines. Therefore, this gene may be collaborating for the formation of the OAVS phenotype associated with CES.

BID (OMIM *601997) encodes a cell death agonist and regulates apoptosis (GeneCards), fundamental from embryonic development. This gene was described in a 22q11.2 region duplication in an individual with CES, distal to the critical CES region (Footz et al., 1998). Its role in inducing premature cell death could influence organ development or overall growth. In addition, it acts on the ERK signaling pathway, involved in the development of the neural crest (Parada et al., 2015; Dinsmore and Soriano, 2018) and is important in the asymmetric nature of the OAVS and some characteristics of CES. *TUBA8* (OMIM *605742) is also involved in the same pathway, besides EphB-EphrinB Signaling, and encodes a member of the family of proteins that form cytoskeleton regulating microtubules. It can play a role in the regulation of growth, proliferation and cellular migration, as in development and maintenance of the central nervous system (Romaniello et al., 2015).

Based on its amino acid sequence, *USP18* (OMIM *607057) is a member of the USP family, which is responsible for removing ubiquitin or Ubl proteins from its conjugated substrates (Honke et al., 2016). In mammalian cells, many proteins are modified by ubiquitination, an important process for different vital events, such as the cell cycle, besides cell differentiation and proliferation (King et al., 1996; Zhu et al., 1996). Ubiquitination plays a central role in the regulation of transport processes as endocytosis and protein turnover (Rotin and Staub, 2011). In particular, the NEDD4-2 (E3 ubiquitin-protein ligase NEDD4, also known as neural precursor cell expressed developmentally down-regulated protein 4) is an enzyme that in humans is encoded by the *NEDD4* gene (Kumar et al., 1997). *NEDD4* plays an important function in neuronal development and is responsible for the dendritic formation in neurons, forming a signaling complex. *USP1*8 has already been described in patients clinically diagnosed with OAVS presenting a duplication overlapping in the critical region of CES (Torti et al., 2013) and Dup22q11 (Beleza-Meireles et al., 2015). The important role in cell ubiquitination may be the key to understanding how this gene would be involved in the occurrence of malformations, especially in the cranial neural crest. There are still no studies describing an association of *USP18*

with the phenotype of CES/OAVS and/or Dup22q11, although the pseudo-TORCH syndrome 2, which occurs due to the deficiency of this gene, is located at 22q11.21 region (Meuwissen et al., 2016), as previously pointed. Thus, more research is still needed to understand their involvement with the cases of malformations.

MICAL3 (OMIM * 608882) does not seem to be involved in important pathways for the phenotypes described; however, its paralog, the *MICAL2* gene, encodes a regulating protein of the SRF signaling pathway. This pathway is important during embryo development and is associated with the formation of the mesoderm and is crucial for skeletal muscle growth (Knöll and Beck, 2011). The activation of the SRF pathway can occur through the MAP kinase, an additional component that may be acting on the phenotypes involved. Torti et al. (2013) also described a duplication of this gene in the CES critical region in a patient with OAVS; however, there was no association with this phenotype.

PEX26 (OMIM *608666) has an interaction with the *PEX1* gene, responsible for encoding a member of the AAA protein family, a large group of ATPases associated with many cellular activities (Matsumoto et al., 2003). It is strongly related to peroxisome biogenesis disorders (PBD), a group of conditions caused by a partial or generalized defect in peroxysomal biogenesis, such as Zellweger syndrome (ZS) (Zellweger et al., 1988). The ZS has some clinical features similar to OAVS and CES, such as craniofacial dysmorphism and brain and eye malformations (Zellweger et al., 1988); however, *PEX2*6 has not yet been reported as a candidate for OAVS phenotype, or CES. The other genes duplicated in our patient were reviewed in the literature, but we have not found possible associations with the phenotype described.

These findings corroborate the hypothesis that genes in this duplicate region, particularly those involved in neurogenesis and neural crest development, may play important roles in the etiology and pathogenesis of OAVS and CES, mainly influencing facial asymmetry. MAPK/ERK pathway seems to be commonly involved in neural crest maldevelopment through mutations in *IL17*RA, *ATP6V1E1*, *BID* and *MICAL3*, involving the phenotype overlap of the two conditions. Ubiquitination associated with the *USP18* gene also seems to be a key factor in understanding the phenotypes described.

To our knowledge, this study contributes to the investigation of the genotype-phenotype relation of these two rare conditions that may be associated, highlighting the importance of differential diagnosis in individuals with CES and/or OAVS.

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Author statement

The authors attest that they have obtained informed consent for publication of the images.

CRediT authorship contribution statement

Andressa Barreto Glaeser: Conceptualization, Methodology, Investigation, Writing – original draft, Project administration. **Bruna Lixinski Diniz:** Investigation, Writing – original draft, Writing – review & editing, Visualization. **Andressa Schneiders Santos:** Investigation, Writing – original draft, Writing – review & editing. **Bruna Baierle Guaraná:** Writing – original draft, Writing – review & editing. **Victória Feitosa Muniz:** Writing – original draft, Writing – review & editing. **Bianca Soares Carlotto:** Investigation, Writing – original draft, Writing – review & editing. **Eduardo Morais Everling:** Investigation. **Patrícia Yuri Noguchi:** Investigation. **Aline Ramos Garcia:** Investigation. **Juliana Miola:** Investigation. **Mariluce Riegel:** Investigation, Funding acquisition, Supervision. **Rafaella Mergener:** Investigation. **Paulo Ricardo Gazzola Zen:** Funding acquisition, Supervision, Writing – review & editing. **Rafael Fabiano Machado Rosa:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

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Fluorescence in situ hybridization (FISH) as an irreplaceable diagnostic tool for Williams-Beuren syndrome in developing countries: a literature review

Hibridização *in situ* fluorescente (FISH) como ferramenta diagnóstica insubstituível para a síndrome de Williams-Beuren em países em desenvolvimento: uma revisão de literatura

Bianca Soares Carlotto[a], Desirée Deconte[a], Bruna Lixinski Diniz[a], Priscila Ramires da Silva[a], Paulo Ricardo Gazzola Zen[a,b], André Anjos da Silva[c,*]

ABSTRACT

Objective: The aim of this study was to sum up and characterize all Williams-Beuren syndrome cases diagnosed by fluorescence in situ hybridization (FISH) since its implementation, as well as to discuss FISH as a cost-effective methodology in developing countries.

Data source: From January 1986 to January 2022, articles were selected using the databases in PubMed (Medline) and SciELO. The following terms were used: Williams syndrome and In Situ Hybridization, Fluorescence. Inclusion criteria included Williams-Beuren syndrome cases diagnosed by FISH with a stratified phenotype of each patient. Only studies written in English, Spanish, and Portuguese were included. Studies with overlapping syndromes or genetic conditions were excluded.

Data synthesis: After screening, 64 articles were included. A total of 205 individuals with Williams-Beuren syndrome diagnosed by FISH were included and further analyzed. Cardiovascular malformations were the most frequent finding (85.4%). Supravalvular aortic stenosis (62.4%) and pulmonary stenosis (30.7%) were the main cardiac alterations described.

Conclusions: Our literature review reinforces that cardiac features may be the key to early diagnosis in Williams-Beuren syndrome patients. In addition, FISH may be the best diagnostic tool for developing nations that have limited access to new technologic resources.

Keywords: Williams syndrome; Williams-Beuren syndrome; Fluorescence in situ hybridization; Literature review.

RESUMO

Objetivo: Caracterizar todos os casos de síndrome de Williams-Beuren (SWB) diagnosticados por hibridização ***in situ*** fluorescente (FISH) desde sua implementação, assim como discutir a relação custo-benefício da metodologia de FISH em países em desenvolvimento.

Fontes de dados: Entre janeiro de 1986 e janeiro de 2022 foi realizada uma busca nas bases de dados PubMed (Medical Literature Analysis and Retrieval System Online — Medline) e Scientific Electronic Library Online (SciELO) usando os seguintes termos: síndrome de Williams e hibridização *in situ* fluorescente. O critério de inclusão utilizado foi conter a descrição detalhada de caso(s) de SWB por FISH. Apenas estudos escritos em inglês, espanhol e português foram incluídos. Trabalhos que apresentavam sobreposição de síndromes/condições genéticas foram excluídos.

Síntese dos dados: Após os processos de inclusão, 64 artigos e 205 indivíduos com SWB diagnosticados por meio do método de FISH foram incluídos. O achado mais frequente entre os indivíduos foi a presença de algum tipo de malformação cardíaca (85,4%). A estenose aórtica supravalvar (62,4%) e a estenose pulmonar (30,7%) foram as alterações cardíacas mais descritas. A maioria dos estudos era proveniente dos continentes Europa, Ásia e América do Norte.

Conclusões: A presente revisão de literatura reitera que as malformações cardíacas podem ser a chave para o diagnóstico precoce em pacientes com SWB. Ainda, a técnica de FISH parece ser a melhor ferramenta de diagnóstico para os países em desenvolvimento, cujo acesso às novas tecnologias ainda é escasso.

Palavras-chave: Síndrome de Williams; Síndrome de Williams-Beuren; Hibridização in situ fluorescente; Revisão de literatura.

Corresponding author. E-mail: andreanjos@unisinos.br (A. A. Silva)
[a]Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil.
[b]Irmandade da Santa Casa de Misericórdia de Porto Alegre, Porto Alegre, RS, Brazil.
[c]Universidade do Vale do Rio dos Sinos, São Leopoldo, RS, Brazil.
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INTRODUCTION

Williams-Beuren syndrome (WBS) (OMIM #194050) is a rare developmental disorder with an autosomal dominant trait and numerous clinical findings. WBS is considered a contiguous gene syndrome caused by a microdeletion on chromosome 7q11.23 that ranges in size from 1.5 to 1.8 Mb and encompasses approximately 28 genes.[1,2] The prevalence of the syndrome is estimated to be 1 per 7,500 live births.[3] Distinctive facial features (long philtrum, epicanthal folds, broad forehead, bitemporal narrowness, periorbital fullness, stellate and/or lacy iris pattern, short nose with a bulbous nasal tip, wide mouth, full lips, and mild micrognathia), developmental and intellectual delay, an overly sociable personality, cardiovascular diseases, and idiopathic hypercalcemia comprise the overall WBS phenotype.[4,5]

Fluorescence in situ hybridization (FISH) analysis is considered the gold standard test for precise molecular diagnosis of microdeletion syndromes, such as WBS. Although new technologies such as array-CGH and multiplex ligation-dependent probe amplification (MLPA) outdated FISH, mostly in diagnosing atypical deletion cases, FISH is still a valuable and cost-effective tool to confirm WBS clinical suspicion.[2,6,7]

The area of rare diseases faces a lot of major obstacles with regard to gaining a deep understanding of each syndrome in order to improve patient care. In developing countries, some of these hurdles include difficulty in obtaining a timely and precise diagnosis, shortage of specialized healthcare workers, lack of research, and resource constraint.[8] In Brazil, for example, the Universal Health Service, a large and well-established public healthcare system, does not offer molecular and genetic tests on a daily basis, which directly affects the diagnosis and management of patients with genetic rare diseases.[9] Therefore, an ultimate molecular diagnosis of patients with rare genetic diseases is extremely important. A proper and precise diagnosis aids in the access of proper resources, avoids additional molecular investigation, decreases prognostic uncertainty, allows genetic counseling, and provides psychosocial benefits to both the patient and the family.[10]

The aim of this literature review was to sum up and characterize all WBS cases diagnosed by FISH since its implementation as well as to discuss FISH as a cost-effective methodology in developing countries.

METHOD

This literature review was designed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses guidelines.[11] The literature search was conducted using PubMed (Medline) and SciELO. Mesh and DECS descriptors were used to index articles with the following terms: Williams syndrome and In Situ Hybridization, Fluorescence. The exact search terms for PubMed/MESH terms were "Syndrome, Williams OR Contiguous Gene Syndrome, Williams OR Supravalvar Aortic Stenosis Syndrome OR Williams-Beuren Syndrome OR Syndrome, Williams-Beuren OR Williams Beuren Syndrome OR Beuren Syndrome OR Syndrome, Beuren OR Hypercalcemia-Supravalvar Aortic Stenosis OR Aortic Stenoses, Hypercalcemia-Supravalvar OR Aortic Stenosis, Hypercalcemia-Supravalvar OR Hypercalcemia Supravalvar Aortic Stenosis OR Hypercalcemia-Supravalvar Aortic Stenoses OR Stenoses, Hypercalcemia-Supravalvar Aortic OR Stenosis, Hypercalcemia-Supravalvar Aortic OR Chromosome 7q11.23 Deletion Syndrome OR Williams Contiguous Gene Syndrome) AND (FISH OR Hybridization in Situ, Fluorescent OR FISH Technique OR FISH Techniques OR Technique, FISH OR Techniques, FISH OR Fluorescent in Situ Hybridization OR FISH Technic OR FISH Technics OR Technic, FISH OR Technics, FISH OR Hybridization in Situ, Fluorescence OR In Situ Hybridization, Fluorescent," and for SciELO/DECS, terms were "Williams Syndrome" AND "In Situ Hybridization, Fluorescence."

Included articles were selected in a two-step analysis: title and abstract screening, followed by a full-text read. Authors were categorized into two pairs for independent screening and further discussion of potential disagreements (A.S./B.C. and D.D/P.S.). If the disagreement remained, a "senior reviewer" (B.D.) decided if the study would be included or excluded. Inclusion criteria for the first step were as follows: have a case or cases of WBS as well as any indication of FISH performance. In the second step, the inclusion criteria included WBS cases diagnosed by FISH with a stratified phenotype for each patient. Only studies from January 1986 to January 2022 written in English, Spanish, and Portuguese were included. Studies with overlapping syndromes or genetic conditions were excluded.

Publication metadata were extracted using a data extraction template that was created and modified according to all the studies reviewed. The publication details were captured and summarized in a tabular format developed by the authors of this review. The data extracted from all articles were as follows: article ID, total of cases, case stratification, auditory, behavioral, calcium, cardiovascular, cognitive, connective tissue, dental, endocrine, facial features, gastrointestinal, genitourinary, growth, hematology, integument, musculoskeletal, neurologic, ocular and visual, respiratory, tumor, "typical face," sample type, gender, age at diagnosis with FISH, diagnosis with other molecular techniques beyond FISH, FISH probes, and authors' countries.

RESULTS

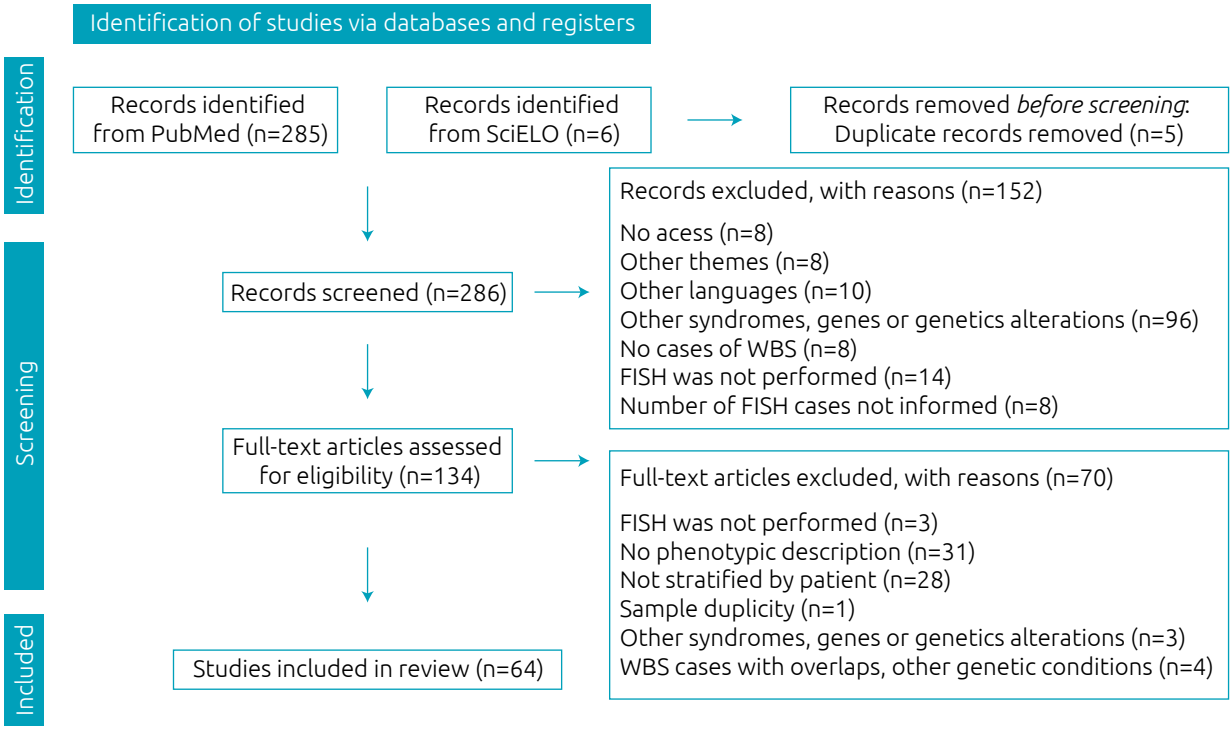
After screening, 64 articles were included. A flow diagram of the literature search is depicted in Figure 1. A total of 205 individuals diagnosed with WBS by FISH were included and further analyzed. Demographic analysis showed that 48.5% (66/136) were female and 51.5% (70/136) male. Age at diagnosis ranged from 3 weeks to 37.75 years, with an average of 9.4 years and a median of 6.4 years. Clinical features were evaluated using the Guidance for Clinician in Rendering Pediatric Care.[5] Cardiovascular malformations were the most frequent finding (85.4%), followed by neurological alterations (59.1%), cognitive delay (49.8%), facial dysmorphisms (48.3%), and behavioral changes (46.3%). Clinical findings are described in Table 1. A significant percentage of the patients (40.5%) did not have a clear description of their facial dysmorphisms, terms such as "typical face" or "elfin face" were used instead.

Fluorescence in situ hybridization

Among the included studies, 53.1% did not report the probe(s) used for WBS diagnosis. The described probes are shown in Table 2.[12,13] FISH analysis with more than one probe was performed in seven articles.[14-20] In these studies, a variation in the deletion length was observed among patients since

Table 1. Patients' clinical findings by systems.

Main clinical features	Frequency (%)
Cardiovascular	85.4
Neurologic	59.1
Cognitive	49.8
Facial features	48.3
Behavioral	46.3
Growth	35.6
Connective tissue	29.8
Gastrointestinal	26.3
Integument	24.9
Dental	23.9
Auditory	20.0
Ocular and visual	18.5
Calcium	18.1
Musculoskeletal	13.7
Genitourinary	13.2
Respiratory	7.8
Endocrine	2.9
Hematology	2.9
Tumor	2.9



FISH: fluorescent in situ hybridization; WBS: Williams-Beuren syndrome.

Figure 1. Flow diagram.

different probe sets were used. Other molecular and cytogenetics technologies were used in order to aid in the diagnosis of WBS. Microarray (9.3%) and microsatellite (6.3%) analysis were the most frequent techniques performed alongside FISH. MLPA, PCR, QMPSF, qPCR, and Southern analysis were also performed, and each of them comprised 4.4% of the included studies.

Where, in theworld, are fluorescence in situ hybridization and Williams-Beuren syndrome studies from?

An overview of the authors' locations is described by continents in Table 3. Europe, Asia, and North America comprised the highest percentage of studies included in this review. On the contrary, Latin America and Africa had the lowest numbers, with 4.7 and 3.1% of the studies, respectively. Among the countries, the United States (14.1%), Italy (12.5%), Japan (9.4%), and the United Kingdom (7.8%) were highlighted.

DISCUSSION

Initially, WBS diagnoses were made purely based on the clinical features observed in the patients. The observed phenotype should meet the descriptions provided by Williams et al.[21] and Beuren et al.[22] WBS was primarily described as a syndrome

Table 3. Overview of the authors' locations by continents.

Continents	Frequency (%)
Africa/Europe	3.1
Asia	26.6
Asia/North America	1.6
Eurasia	3.1
Europe	37.5
Europe/Eurasia	1.6
Europe/North America	6.3
Latin America	4.7
North America	15.6

Table 2. FISH probes, BACs, cosmids, PACs, and YACs described and used by all included articles.

FISH probes	Genes	Diagnosed cases (n)
Commercial FISH probes		
WSCR probe (ONCOR, Gaithersburg, MD)	*ELN*	92
Q Biogene, currently MP Biomedicals, probe number CP5155-DC		1
Vysis LSI ELN Kit (Vysis; Abbott Laboratories, Abbott Park, IL)	*ELN+LIMK1*	29
MD Williams-Beuren Kreatech probe	*ELN+LIMK1+CYLN2*	1
Cytocell Williams-Beuren region probe	*LIMK1+EIF4H+RFC2+CYLN2+GTF2IRD1+TBL2+BAZ1B*	3
BACs/Cosmids/PACs/YACs		
cELN-272 and cELN-11D	*ELN*	9
Elastin cosmid		1
Cosmid P5155		1
ELN cosmid 82C and the *ELN/ LIMK1* cosmid 34B FISH probes	*ELN+LIMK1*	1
Cosmid probes	*ELN+LIMK1+STX1A*	4
CTB-8H17	*FKBP6+FZD9+WSTF**	1
BACs 1008H17, 592D8, P195H06, 1148G03, 054H15; cosmids 182B11, 183E1	*FKBP6+FZD9+ELN+STX1A+GTF2IRD1+CYLN2+GTF2I*	1
Probes B315H11 and CITB51J22	*FZD9+BAZ1B+TBL2+LIMK1+RFC2*†	1
BACs 1008H17, 315H11, 592D8, 155B1, 363B4; cosmids 12915, 82c2, 34b3, 152a8, 128d2, 102f12, 135f3, 82b11, 209c11, 47d1, 160g4, 183e1; PACs 632N4, 391G2, 195H6	*ELN+LIMK1+FZD9+FKBP6+BAZ1B+BCL7B+TBL2+ WBSCR14+STX1A+CLDN3+EIF4H+HSPCO46+RFC2+CYLN2*	3

*Genes referenced according to Korenberg et al.[12]; †Genes referenced according to van Hagen et al.[13].
FISH: fluorescent in situ hybridization; WBS: Williams-Beuren syndrome; BAC: bacterial artificial chromosomes; PAC: P1-derived artificial chromosomes; YAC: yeast artificial chromosomes.

characterized by supravalvular aortic stenosis (SVAS), intellectual disability, facial dysmorphism, dental anomalies, and peripheral pulmonary artery stenosis. Further studies described additional dysmorphic features but without a proper etiology explanation.[23-25] A WBS score (diagnostic index) was developed by Preus[26] in order to assess syndrome features and provide patients' diagnoses.

The term "elfin facies" was described in the 1970s to characterize all recurrent facial dysmorphisms found in WBS individuals.[24,27] WBS facial phenotype is distinguished by a broad forehead, medial eyebrow flare, periorbital fullness, strabismus, stellate iris pattern, flat nasal bridge, malar flattening, full cheeks and lips, a long and smooth philtrum, a rather pointed chin, and a wide mouth.[28] In our review, 40.5% of the included patients were described as having an "elfin face" or a "typical face." The choice of a general description instead of detailed information regarding facial dysmorphisms in WBS patients may hinder a genuine clinical diagnosis of the syndrome. Since 1986, the use of generic terms to report WBS facial features has been discouraged.[28] In our review, studies that provided a detailed description of patients' dysmorphisms showed that the most prevalent features were full lips and a long philtrum (28.3%), followed by periorbital fullness (27.3%), wide mouth (26.3%), full cheeks (25.4%), and broad nasal tip (22.9%). A WBS patient's clinical evaluation is extremely relevant in order to provide a clear and precise diagnosis. Heterogeneity between patients' facial dysmorphism is also broadly described in the literature.[2] The term "elfin face," whose definition is based on a mythological and abstract figure, does not reflect the variety of facial features already described throughout WBS individuals. Therefore, the use of generic terms as part of the syndrome spectrum should be discouraged. Hence, we strongly recommend the use of standardized nomenclature to describe the facial phenotype of WBS patients.

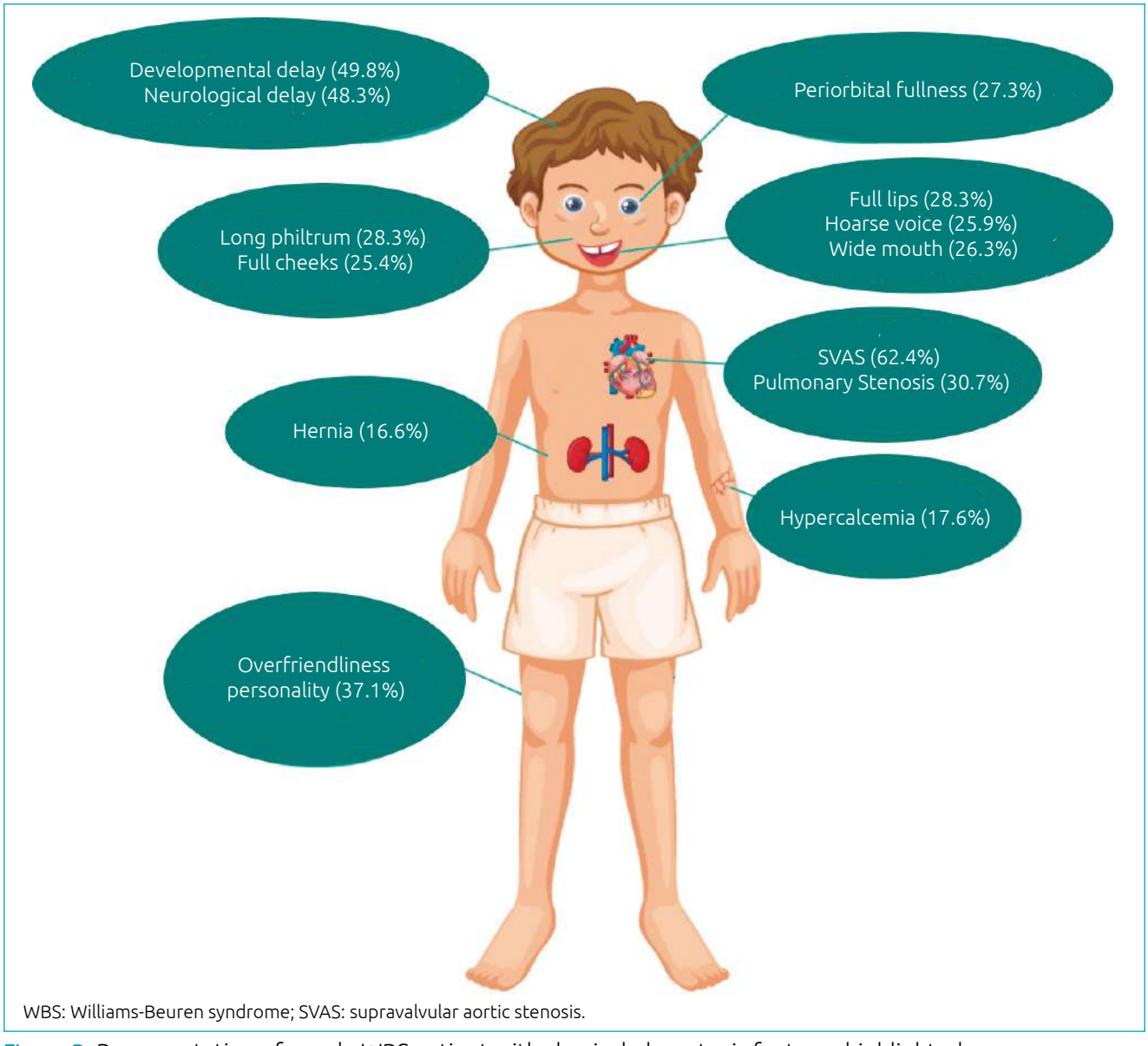
Cardiovascular alterations (80%) are the most frequent features observed in WBS children and are also the major causes of infant morbidity and mortality within the syndrome.[5] SVAS (75%) is considered the main cardiac finding observed in WBS patients, followed by pulmonary artery stenosis (50%).[5,29] In our review, SVAS (62.4%) and pulmonary stenosis (30.7%) were the main cardiac alterations described. The high percentage of cardiovascular malformation among WBS patients points out the value of a detailed cardiovascular screening in an early clinical diagnosis of the syndrome. Although heart features are already known and often described within the syndrome spectrum, WBS patients' diagnoses are still delayed (>1 year, on average).[29] WBS neonatal diagnosis is challenging since some classical features include a friendly personality and facial dysmorphisms that are usually observed only days or months after birth. WBS clinical phenotype is also heterogeneous, and features tend to develop over time, which hinders a proper early clinical diagnosis.[30-32] However, the main congenital heart diseases (CHDs) described in WBS can be screened and diagnosed through routine ultrasonography during the first trimester of pregnancy when performed by expert ultrasonographists.[33] Therefore, we suggest that patients suspicious of WBS should go through a careful examination when looking for cardiovascular findings. As opposed to facial dysmorphisms that are observed over time, congenital heart diseases can be diagnosed early with the aid of prenatal ultrasound. Hence, in order to provide an early diagnosis for WBS patients, CHDs may be the golden key.

Developmental delay (90%) is often observed in WBS individuals. Therefore, referral to early intervention programs such as special education and vocational training is crucial to improve physical, speech, and nutrition features as well as social integration among patients.[4,5] Intellectual delay ranging from light to moderate (75%) and a unique cognitive and behavioral profile are other features frequently described in WBS individuals.[5,34,35] WBS children present an over-friendliness personality characterized by an intense drive for social interaction, a desire to form affectionate bonds, and an increased feeling of empathy. Therefore, an early intervention that aims to enhance social interactions and improve social skills is needed to ease the social inclusion of teenagers and adults with WBS.[35] In our review, developmental delay was found in 49.8% of the patients. Intellectual delay and an over-friendliness personality were described in 48.3 and 37.1% of the individuals, respectively. An overall view of all phenotypic features is shown in Table 1 and Figure 2.

FISH was first performed by Pinkel et al.[36] and Pinkel et al.[37] This cytogenetic technique provides a rapid, precise, and reliable molecular analysis to confirm the suspicion of a clinical diagnosis. FISH is considered the gold standard method for chromosome microdeletion syndromes diagnosis.[4,6,38,39] The WBS diagnosis rate of FISH is over 90% of the cases.[39] WBS molecular etiology was described in the 1990s, the same decade that FISH was implemented as a diagnostic tool for WBS individuals.[40-42] In 1993, Ewart et al.[1] found that the molecular cause of WBS was a microdeletion at chromosome 7q11.23 after observing a elastin gene (*ELN*) hemizygosity by FISH. In our review, peripheral blood was the most commonly collected sample. Surprisingly, we found a case of postmortem diagnosis performed by FISH using both formalin-fixed tissues and paraffin-embedded sections from the kidney. Literature shows that buccal swabs can also be used as samples for FISH microdeletion analysis.[43] Therefore, FISH proved to be an extremely versatile technique when it comes to sample types that can be used as a DNA source.

ELN, *LIMK1*, and *CYLN2* were the main genes designed within FISH probes used in the included studies. *ELN* encodes a structural protein that composes a diversity of body tissues and is the major gene associated with WBS. Therefore, *ELN* deletion can cause some connective tissue abnormalities such as cardiovascular diseases, in particular SVAS in WBS individuals.[1,4,44] *LIMK1* deletion is associated with constructive visuospatial cognition abnormalities as well as neurological features in WBS patients.[45-47] On the contrary, *CYLN2* is a gene associated with cerebellar malformation and neurological impairment that can lead to hippocampal dysfunction and a delay in the development of motor skills.[46,48] Some studies performed other molecular methodologies alongside FISH that allowed a large number of genes to be identified as deleted as well as involved in WBS phenotype. However, the majority of these studies were conducted in developed countries. Therefore, FISH may be the better choice for developing nations that are still lacking in new technologies.

Comparative genomic hybridization (CGH) was first performed by Kallioniemi et al.[49] in order to analyze solid tumor cytogenetics. Nowadays, this technology is widely used to detect chromosome copy number variation.[50] The major advantage of genome-wide array platforms over FISH is the ability to screen for microdeletions and/or duplications throughout the genome that could detect not only WBS but also other syndromes at an earlier age.[50] Gilbert-Dussardier et al.[51] identified a novel microsatellite DNA marker (D7S1870) as a new diagnostic tool for hemizygosity detection in individuals suspected of



WBS: Williams-Beuren syndrome; SVAS: supravalvular aortic stenosis.

Figure 2. Representation of a male WBS patient with classical phenotypic features highlighted.

WBS. Both CGH and microsatellites became valuable methodologies in the investigation of deletion length and characterization of microdeletion syndromes. In addition, MLPA was later added to the pool of molecular technologies that aid researchers in the diagnosis of WBS patientes.[52] In this review, included studies dated from 1993 to 2018 (Figure 3)[1,36,37,49,51,52]. Although CGH, microsatellites, and MLPA were implemented in 1992, 1995, and 2002, respectively, FISH was still performed throughout the years.

Fluorescence in situ hybridization X comparative genomic hybridization cost-effective analysis in developing countries

In the field of genetic rare diseases, molecular diagnosis is essential in order to elucidate the etiology of these conditions as well as provide a genotype-phenotype correlation for uncommon clinical outcomes.[10] Nowadays, different and newer technologies are available to aid molecular investigation and further diagnosis. However, former standard methodologies, such as FISH, are still considered the gold standard for the detection of rare conditions, mainly in developing countries where financial support is limited and affordable technologies are preferred.[2,6,7,53,54,55]

FISH probes covering the *ELN* gene detect the majority of the deletion in children clinically diagnosed with WBS.[39,53,54] Nickerson et al.[39] showed that more than 90% of the patients were hemizygous for the elastin gene, while Souza et al.[53] verified that 83% of the children clinically diagnosed with WBS had the same deletion. Moreover, Ramírez-Velazco et al.[54] analyzed patients clinically diagnosed with WBS and identified that 66% of them had the 7q11.23 deletion detected by FISH. The study also performed CGH analysis in 23 cases where all FISH results were confirmed (18 deletions and 5 negatives). The additional information provided by CGH was the deletion sizes, which enables the patient's classification into typical and atypical deletions.[54] Both studies were conducted in developing countries (Brazil and Mexico), and the conclusion that FISH is the most feasible, effective, and economical approach in those nations was unanimous.[53,54]

In addition, a Brazilian study estimated the techniques' budget in the United States and Brazil and revealed that, on average, US$600 is needed to perform both FISH and CGH analysis in the United States, while in Brazil, FISH analysis would cost US$800 and CGH analysis US$1200.[56] Additionally, CONITEC, a committee that advises the Brazilian Ministry of Health, estimated the financial impact of molecular technologies

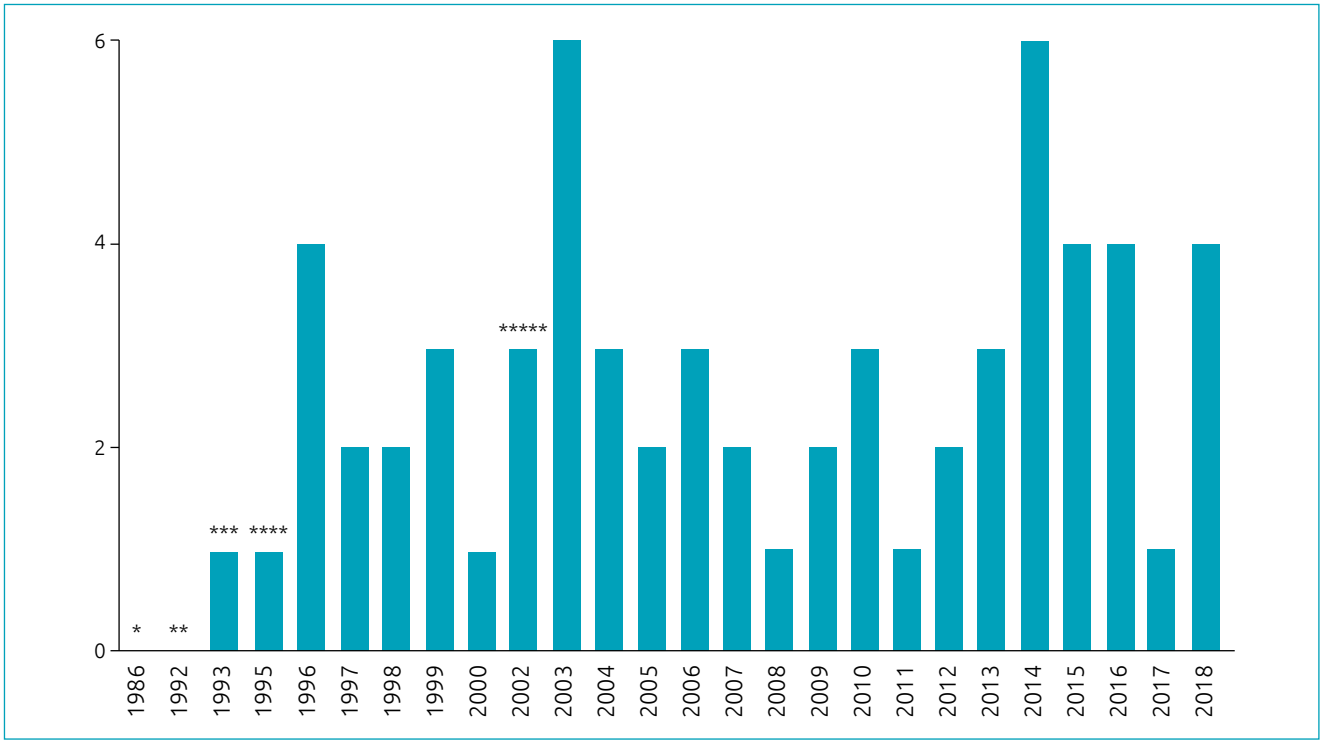


Figure 3. Included studies by year of publication. * FISH's performed for the first time by Pinkel et al.[36,37]; ** CGH's performed for the first time by Kallioniemi et al.[49]; *** Ewart et al.[1] showed that WBS is caused by a microdeletion at chromosome 7q11.23 through FISH; **** a novel microsatellite DNA marker for WBS patients[51]; ***** MLPA is performed for the first time by Schouten et al.[52]. FISH: fluorescent in situ hybridization; WBS: Williams-Beuren syndrome; MLPA: multiplex ligation-dependent probe amplification; CGH: comparative genomic hybridization.

in the country and evaluated the cost of FISH and CGH analysis at R$400 and R$2.000, respectively.[57] Therefore, since all newer technologies are expensive, mainly in countries without proper research support, their use is recommended when FISH is negative in order to investigate atypical deletions.[55] The lack of medical genetic services, health facility limitations, and healthcare access restrictions are also hardships faced by developing nations.[58] Consequently, a proper evaluation by the government is needed in order to seek better healthcare services as well as improved research outcomes, mainly for patients with rare diseases such as WBS.[8]

Although our results are significant, some limitations of this review would be the restriction to access some potential studies that could contribute to our data. To include more articles published around the world, the inclusion of additional databases, such as Embase and Biblioteca Virtual em Saúde, would be interesting.

It is known that the clinical diagnosis alone is insufficient in order to give a proper treatment and follow-up for WBS patients. WBS has a heterogeneity of features described, and a lot of these characteristics are only observed after months or years of life. Therefore, the combination of a successful diagnostic rate for WBS individuals by FISH and a proper cardiac screening (mainly SVAS) may be the key for an early and precise diagnosis in neonatal patients with WBS. An early diagnosis is crucial since these individuals need a multiprofessional healthcare team in order to lessen and soften further complications as well as prevent secondary complications.[4,5] In addition, FISH is a atemporal technology that seems to be irreplaceable for WBS diagnosis, mainly in countries that lack newer methodologies.

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Conflict of interests

The authors declare there is no conflict of interest.

Authors' contribution

Study design: Carlotto BS, Silva AA. *Data collection:* Carlotto BS, Deconte D, Silva PR, Silva AA, Diniz BL. *Data analysis:* Carlotto BS, Deconte D, Silva PR, Silva AA, Diniz BL. *Manuscript writing:* Carlotto BS, Deconte D, Diniz BL. *Manuscript revision:* Carlotto BS, Deconte D, Diniz BL, Silva PR, Zen PRG, Silva AA. *Study supervision:* Zen PRG, Silva AA.

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REVIEW PROTOCOL

Quality of life of type 1 neurofibromatosis patients: a scoping review protocol

Qualidade de vida de portadores de neurofibromatose tipo 1: um protocolo de revisão de escopo

Lucas Paulo de Souza[1]
ORCID:0000-0003-0935-1117

Bruna Lixinski Diniz[1]
ORCID: 0000-0003-1448-0298

Paulo Ricardo Gazzola Zen[1]
ORCID: 0000-0002-7628-4877

[1]Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil

Editors:
Ana Carla Dantas Cavalcanti
ORCID: 0000-0003-3531-4694

Paula Vanessa Peclat Flores
ORCID: 0000-0002-9726-5229

Corresponding author:
Lucas Paulo de Souza
E-mail: lucaspdesouza1995@gmail.com

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ABSTRACT

Objective: To map concepts, findings, and limitations related to quality of life in children, adolescents, and young adults with neurofibromatosis type 1. **Method:** This is a scoping review protocol based on Joanna Briggs Institute (JBI) guidelines. Data searches will be conducted on PubMed/MEDLINE, EMBASE, Web of Science, Lilacs, CINAHL, Open Grey, and Google Scholar. The retrieved manuscripts will be organized using the Rayyan tool for duplicate identification and removal. Subsequently, the articles and other materials will be processed in the same tool for screening and selecting eligible studies by two independent researchers, and this entire process will be described in a flowchart adapted from the PRISMA-ScR checklist. As appropriate, data extracted from eligible manuscripts will be presented in tables, figures, and flowcharts. The data will be discussed and correlated to identify potential strengths and limitations related to the research topic.
Descriptors: Quality of Life; Neurofibromatosis 1; Review.

RESUMO

Objetivo: Mapear conceitos, achados e limitações acerca da qualidade de vida de crianças, adolescentes e adultos jovens portadores de neurofibromatose tipo 1. **Método:** Trata-se de um protocolo de revisão de escopo baseado nas diretrizes do Joanna Briggs Institute (JBI). A busca de dados será realizada nas plataformas PubMed/MEDLINE, EMBASE, Web of Science, Lilacs, CINAHL, Open Grey e Google Scholar. Os manuscritos encontrados serão organizados através da ferramenta Rayyan para identificação e exclusão de duplicatas. Na sequência, os artigos e demais materiais seguirão na mesma ferramenta para triagem e seleção de estudos elegíveis por dois pesquisadores independentes, sendo esse processo todo descrito em um fluxograma adaptado do Checklist PRISMA-ScR. Os dados extraídos dos manuscritos elegíveis serão apresentados em tabelas, quadros e fluxogramas, conforme pertinente. Os dados serão discutidos e inter-relacionados, com a finalidade de identificar potencialidades e limitações acerca do tema de pesquisa.
Descritores: Qualidade de Vida; Neurofibromatose 1; Revisão.

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a multisystem phacomatosis with an autosomal dominant inheritance pattern. Multiple café-au-lait macules, freckles, multiple neurofibromas, learning disabilities, behavioral problems, and other complications of varying severity and complexity characterize it. The expression of the disease and its various complications varies even among individuals within the same family. Advances in molecular biological analysis and imaging techniques have not only helped to elucidate the etiological and clinical characteristics of NF1 but have also provided better prospects for therapeutic intervention for those affected by the disease[1,2].

According to the United Kingdom Neurofibromatosis Association Clinical Advisory Board, the incidence of NF1 is estimated to be from 1 in 2,500

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to 1 in 3,000, with no evidence of the predominance of the disease in specific populations or sexes. However, studies indicate a symmetric distribution between cases resulting from genetic transmission (i.e., when one of the parents is an NF1 carrier) and cases resulting from new genetic mutations[1-3]. As a result, each child of an NF1 carrier has a 50% chance of inheriting the disease-causing variant, with an almost 100% chance of manifestation. Consequently, a child who inherits an NF1-causing variant is expected to develop some phenotypic features of NF1, which may vary even within the same family[2].

Regardless of how the disease is acquired, the diagnosis is predominantly clinical[1-3], based on criteria established by the National Institutes of Health in 1988 and still in use today[1-3]. Suspicion of NF1 arises when the patient presents with any clinical manifestations outlined in Figure 1[1,2].

- ✓ 6 or more café-au-lait macules (>5 mm in children or >15 mm in adults)
- ✓ ≥ 2 cutaneous and/or subcutaneous neurofibromas OR 1 plexiform neurofibroma
- ✓ Axillary or inguinal freckling
- ✓ Optic glioma
- ✓ ≥ 2 Lisch nodules or ≥ choroidal abnormalities
- ✓ Presence of bone dysplasia
- ✓ First-degree relative diagnosed with NF1

Source: Adapted from Ferner et al., 2007; Friedman, 2022.

Figure 1 – Clinical criteria for diagnosing NF1. Porto Alegre, RS, Brazil, 2023

The diagnosis of NF1 is made when a patient has two or more features described in the suggestive findings[1,2] (Figure 1). A negative molecular test for NF1 does not necessarily exclude the diagnosis of the disease, as some patients have the above clinical features without a detectable NF1 variant. In addition, many of the clinical features of NF1 increase in frequency with age. Adults diagnosed with NF1 may not have been diagnosed in early childhood because these features were absent[1-4].

Currently, there is no cure for NF1, but patients must undergo multidisciplinary/professional follow-up at least once a year or more frequently in case of complications or new manifestations. Cutaneous neurofibromas can be surgically removed if they cause pain, interfere with daily life, or are aesthetically disfiguring. This approach is considered the gold standard for neurofibroma management.

Monitoring the neuropsychomotor development of infants is essential, as is tracking their educational progress to quickly identify and address issues such as attention-deficit/hyperactivity disorder, which is common in NF1 patients. Mental health problems are also common in NF1 carriers, caused by both the clinical manifestations of the disease (e.g., multiple neurofibromas) and the complex and unpredictable prognosis of the disease. These issues represent a health risk because they directly affect their quality of life (QoL).

According to the World Health Organization, QoL is "an individual's perception of his or her position in life in the context of the culture and value systems in which he or she lives and concerning his or her goals, expectations, standards, and concerns"[5]. In the field of health and its subsets, the growing interest in the concept of QoL has influenced not only public health policies but also care practices and protocols. This is because the process of health and illness is complex, dynamic, and multifactorial[6].

Numerous instruments have been developed to measure QoL in the population, of which the Pediatric Quality of Life Inventory™ (PedsQL™) is particularly prominent in pediatrics. Developed by Varni and colleagues in 1999[7], the PedsQL™ aims to provide a comprehensive tool for assessing QoL in children and adolescents. The questionnaire, known as the PedsQL™ Generic Core Scores, is a broad instrument[7]. With the discovery of new diseases and advances in health care, it has had to be adapted to different pathologies, including NF1, to allow more refined analyses of QoL in pediatric patients.

Because of the disease's complexity and potential impact throughout a patient's life, anyone diagnosed with NF1 requires continuous multidisciplinary follow-up to identify, treat, and/or monitor complications as early as possible. This often results in the patient and their support network (parents, family members, caregivers, etc.) making frequent and recurrent visits to different healthcare services and levels. The context of being a carrier of a complex, incurable disease with self-limited treatment can affect a patient's life and lead to a reduction in their quality of life. Therefore, this study aims to review the concepts and findings related to QoL in children, adolescents, and young adults

with NF1 and to identify limitations associated with this topic in the current literature.

METHOD

This is a protocol for a scoping review (SR) of the literature. SRs are commonly used to outline the key concepts that support and/or guide a particular field of research and clarify working definitions and even conceptual boundaries of a particular topic[8]. SR studies are gaining traction in the global scientific literature, and the contribution of this type of review to mapping relevant and current research is undeniable. It can significantly benefit professionals in their clinical practice and researchers in generating new research on a topic[9].

The current protocol is structured according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines[10]. PRISMA-P, originally a checklist to guide researchers in formulating a systematic review protocol, was adopted to develop this scoping review protocol to ensure a higher level of methodological rigor.

Protocol and registration

The SR will be conducted according to the Joanna Briggs Institute (JBI) guidelines[8], and the results will be structured according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) checklist[11]. This protocol has been registered on the Open Science Framework (OSF) platform under osf.io/vcqdx[12].

Research question

The PCC mnemonic was used to formulate the research question, where P refers to population (children, adolescents, and young adults), C refers to concept (QoL), and C refers to context (being a carrier of NF1). Thus, the guiding research question is: How has the QoL of children, adolescents, and young adults with NF1 been addressed in Brazil and around the world?

Inclusion criteria

Population

Studies involving children, adolescents, and young adults aged 5 to 25 years with NF1 will be included.

Study concept

Studies must focus on the analysis of quality of life, regardless of how it is approached, treated, or analyzed.

Context

This review will be set in the context of NF1 carriers.

Types of evidence sources

As proposed by the JBI, a SR has a broader scope of research with less restrictive criteria. Following the JBI protocol, data from multiple sources of evidence with diverse study designs will be used. The broad nature of SR questions helps gather evidence from diverse and heterogeneous sources[8].

Search strategy

Based on the PCC mnemonic, search strategies will be constructed using the *Descritores em Ciência da Saúde* (DeCS)/Medical Subjects Headings (MeSH) Portuguese, English, and Spanish terms. An example of the systematization of the search strategy can be seen in Figure 2.

Eligibility criteria

The selected databases are PubMed/MEDLINE, EMBASE, Web of Science, Lilacs, and CINAHL. Open Grey and Google Scholar databases will be used. Studies published in Portuguese, English, and Spanish will be considered eligible for gray literature searches. The timeframe for inclusion will be from January 2018 to June 2023, with the possibility of extending the timeframe based on the availability of studies at the time of the search, supported by a clear rationale in the final manuscript. Included sources will include fully published articles, preprints, online handbooks, theses, and dissertations. If necessary, attempts will be made to contact the primary author to request the full manuscript/document for those unavailable during the initial search.

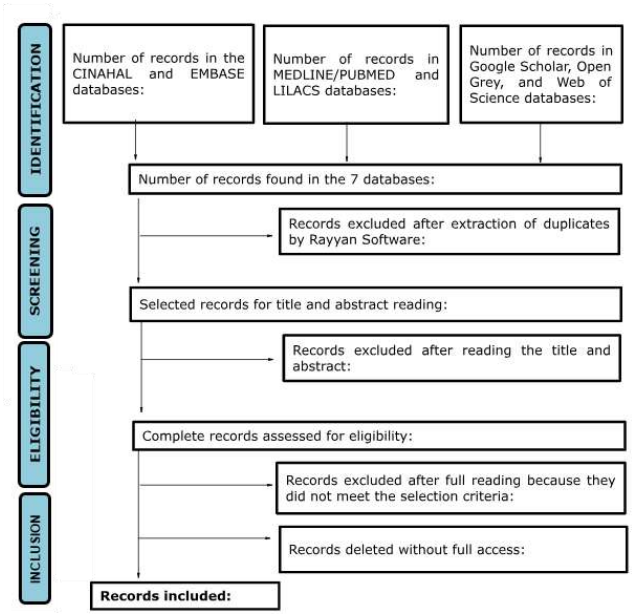
Study selection

After searching the databases, eligible studies will be selected systematically, in pairs, independently, and blinded, as recommended by JBI[8], using the Rayyan tool. In case of disagreements, a third reviewer will make the final decision. The study inclusion process will be organized following the PRISMA checklist[13] and presented in the "results" section of the final manuscript. The checklist has been adapted for

Objective	Mapping concepts, findings, and limitations regarding the quality of life of children, adolescents, and young adults with neurofibromatosis type 1.		
	P (Population)	C (Concept)	C (Context)
Extraction	Children, teenagers, and young adults	QoL	Be a NF1 holder
Combination	"Child", "Child Health", "Adolescent", "Adolescent Health", "Young Adult"	"Indicators of Quality of Life", "Quality of Life"	"Neurofibromatosis 1", "Neurofibromatosis Type 1", "Neurofibromatosis Type I", "Genes, Neurofibromatosis 1"
Construction	Child OR Child Health OR Adolescent OR Adolescent Health OR Young Adult	Indicators of Quality of Life OR Quality of Life	Neurofibromatosis 1 OR Neurofibromatosis Type 1 OR Neurofibromatosis Type I
Application	Child OR Child Health OR Adolescent OR Adolescent Health OR Young Adult AND Indicators of Quality of Life OR Quality of Life AND Neurofibromatosis 1 OR Neurofibromatosis Type 1 OR Neurofibromatosis Type I		

Figure 2 – Systematization of the search strategy. Porto Alegre, RS, Brazil, 2023

this review and is provided in Figure 3.



Source: Adapted from Page et al., 2021.
Figure 3 – Flowchart for the selection of eligible studies. Porto Alegre, RS, Brazil, 2023

First, the retrieved studies are analyzed using the Rayyan tool to identify duplicates, which are recorded and removed. After eliminating duplicate manuscripts, the systematic process of selecting eligible studies using the Rayyan tool will continue.

The first step in selecting eligible studies will be to read the title and abstract of all materials found to identify those that meet the inclusion and exclusion criteria. This step will be performed by a researcher trained according to the standards of this protocol. The selection will be carried out using the Rayyan tool, and in case of uncertainty, the material will automatically proceed to the second selection stage.

The second stage of selecting eligible studies, also within the Rayyan tool, will involve reading the full manuscripts of the materials screened in the previous stage. This blind selection phase will be performed by two different researchers, with a third reviewer to decide on the inclusion or exclusion of studies in case of disagreement. Manuscripts that meet the objectives and inclusion criteria will be included, and data will be extracted. At this stage, manuscripts unavailable for full reading will be excluded.

Data extraction

The synthesis of data from the selected manuscripts is presented to provide readers with a robust, up-to-date, and systematic overview of QoL in children, adolescents, and young adults with NF1. To achieve this, a synoptic table has been developed containing the authors' names, year of publication, aims or proposal of the manuscript, methodology, main results, and conclusions. The discussion of the results will be interrelated in a narrative manner, aiming to clarify the theoretical and methodological approaches of the research topic. Figure 4 illustrates the model of the synoptic table that will be used to synthesize the results.

Author	Journal	Year of publication	Aims	Methodology	Findings	Conclusions or concluding remarks

Source: Adapted from Peters et al., 2020.
Figure 4 – Data extraction summary table template. Porto Alegre, RS, Brazil, 2023

Data presentation
The data from the articles and other documents found will be extracted, synthesized, and organized into tables, figures, and flowcharts according to the relevance and nature of the findings. In addition, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method will be applied to the included manuscripts to assess the quality of evidence, which will be classified into four levels: high, moderate, low, or very low[14]. A discussion will then be presented that relates the findings to this SR's objective and guiding question. This will identify the potential strengths and limitations of the topic.

CONFLICT OF INTERESTS
The authors have declared that there is no conflict of interests.

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AUTHORSHIP CONTRIBUTIONS
Project design: Souza LP de, Diniz BL, Zen PRG
Data collection: Souza LP de, Diniz BL, Zen PRG
Data analysis and interpretation: Souza LP de, Diniz BL, Zen PRG
Writing and/or critical review of the intellectual content: Souza LP de, Diniz BL, Zen PRG
Final approval of the version to be published: Souza LP de, Diniz BL, Zen PRG
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Ciência

A1 QUALIS CAPES

Artigos

0167/2024 - QUALIDADE DE VIDA DE CRIANÇAS ADOLESCENTES E ADULTOS JOVENS COM NEUROFIBROMATOSE TIPO 1: REVISÃO DE ESCOPO

QUALITY OF LIFE OF ADOLESCENT CHILDREN AND YOUNG ADULTS WITH NEUROFIBROMATOSIS TYPE 1: SCOPE REVIEW

Autor:

• Lucas Paulo de Souza - Souza, L. P. - <lucaspdesouza1995@gmail.com>
ORCID: https://orcid.org/0000-0003-0935-1117

Coautor(es):

• Bruna Lixinski Diniz - Diniz, B. L. - <bruldiniz@gmail.com>
ORCID: https://orcid.org/0000-0003-1448-0298

• João Gabriel Toledo Medeiros - Medeiros, J. G. T. - <joaogt@ufcspa.edu.br>
ORCID: https://orcid.org/0000-0002-2789-9189

• Giovani Basso da Silva - Silva, G. B. - <gbasso70@gmail.com>
ORCID: https://orcid.org/0000-0002-3108-445X

• Paulo Ricardo Gazzola Zen - Zen, P. R.G. - <paulozen@ufcspa.edu.br>
ORCID: https://orcid.org/0000-0002-7628-4877

Resumo:

Introdução: A Neurofibromatose Tipo 1 é uma facomatose que apresenta um padrão de herança autossômica dominante. A expressão da doença, bem como as suas complicações, é variável. Objetivo: Mapear os conceitos e achados acerca da qualidade de vida de crianças, adolescentes e jovens adultos com Neurofibromatose Tipo 1 e identificar as limitações acerca do tema na literatura atual. Método: Trata-se de uma de revisão da literatura do tipo Scooping Review estruturada de acordo com o Preferred Reporting Items for Systematic review and Meta-Analysis Protocols. As bases de dados consultadas foram PubMed/MEDLINE, EMBASE, Web of Science, Lilacs, CINAHL, Open Grey e Google Scholar. Os estudos foram selecionados e analisados quanto a sua qualidade, utilizando o questionário "Checklist for Analytical Cross Sectional Studies". Resultados: 39 registros foram selecionados para avaliação de elegibilidade por dois pesquisadores distintos; destes, oito foram incluídos na revisão por estarem de acordo com os critérios de inclusão estabelecidos. Conclusão: A Neurofibromatose Tipo 1 é uma doença complexa com ampla comorbidade, o que compromete diretamente a qualidade de vida de seus indivíduos. Estudos de intervenção para uma melhor abordagem dessa dimensão se fazem necessários, a fim de melhorar a qualidade de vida.

Palavras-chave:

Qualidade de Vida; Neurofibromatose 1; Pediatria; Genética Médica; Revisão

Abstract:

Introduction: Neurofibromatosis Type 1 is a phakomatosis that presents an autosomal dominant inheritance pattern. The expression of the disease, as well as its complications, is variable. Objective: To map the concepts and findings about the quality of life of children, adolescents and young adults with Type 1 Neurofibromatosis and identify the limitations on the topic in current literature. Method: This is a Scooping Review literature review structured in accordance with the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols. The databases consulted were PubMed/MEDLINE, EMBASE, Web of Science, Lilacs, CINAHL, Open Gray and Google Scholar. The studies were ed and analyzed for their quality, using the "Checklist for Analytical Cross-Sectional Studies" questionnaire. Results: 39 records were ed for eligibility assessment by two different researchers; Of these, eight were included in the review because they met the established inclusion criteria. Conclusion: Neurofibromatosis Type 1 is a complex disease with extensive comorbidity, which directly compromises the quality of life of individuals. Intervention studies to better approach this dimension are necessary in order to improve quality of life.

Keywords:

Quality of Life; Neurofibromatosis 1; Pediatrics; Genetics, Medical; Review

Conteúdo:

INTRODUÇÃO

A Neurofibromatose Tipo 1 (NF1) é uma facomatose sistêmica que apresenta um padrão de herança autossômica dominante. A doença é caracterizada por múltiplas manchas café-com-leite (MCCL), máculas lentiginosas nas dobras cutâneas – popularmente chamado de sardas –, neurofibromas, dificuldade de aprendizagem, entre outras complicações com gravidade e complexidade variadas1. A expressão da doença, bem como as suas diversas complicações, é variável, inclusive em indivíduos dentro de uma mesma família1,2.

Dados apresentados pelo United Kingdom Neurofibromatosis Association Clinical Advisory Board estimam que a incidência da NF1 esteja entre 1:2.500 a 1:3.000, sem diferença estatística em relação ao predomínio da doença sobre determinadas populações e sexo. Em relação à herança genética da doença, cada filho de um indivíduo com NF1 tem 50% de chance de herdar a variante causadora da doença, sendo sua manifestação próxima de 100%. Sendo assim, uma criança que herda uma variante causadora de NF1 irá desenvolver algumas características fenotípicas dela, que podem variar dentro de uma mesma família2.

Indiferentemente da forma através da qual a enfermidade é adquirida, por mutação nova ou herdada, seu diagnóstico é predominantemente clínico, tendo como bases critérios diagnósticos bem estabelecidos, através de um consenso internacional de 20211. Com isso, uma anamnese e exame físico adequados tendem a ser suficientes para o diagnóstico da NF1. Contudo, exames complementares se fazem necessários não só para avaliar o grau de comprometimento do indivíduo, mas também para o diagnóstico precoce e o acompanhamento das complicações inerentes a doença1,2.

Ademais, os critérios de diagnóstico clínico têm baixa sensibilidade em crianças, uma vez que os sinais da NF1 aparecem progressivamente ao longo do tempo. Sendo assim, para aumentar a taxa de diagnóstico na faixa etária abaixo de sete anos e nos indivíduos que apresentam apenas MCCL e sardas nas dobras cutâneas, com ou sem histórico familiar de NF1 e nenhum outro critério clínico, o diagnóstico através de exame molecular pode ser considerado tanto para auxiliar na identificação de uma doença diferencial quanto para confirmar a NF11.

De acordo com o último consenso, suspeita-se de NF1 quando o indivíduo apresenta as manifestações clínicas1 dispostas nos tópicos a seguir:

• Os critérios diagnósticos (A) compreendem um indivíduo que não tem um dos pais diagnosticados com NF1 se dois ou mais dos seguintes achados estiverem presentes:

? 6 ou mais MCCL com mais de 5mm de diâmetro em indivíduos pré-púbere ou >15mm em indivíduos pós-púberes;

? máculas lentiginosas na região axilar ou inguinal;

? ? 2 neurofibromas de qualquer tipo, OU 1 neurofibroma plexiforme (NFp);

? Glioma de via óptica;

? ? 2 nódulos de Lisch ou ? anormalidades coroidais;

? Lesão óssea distinta, como displasia esfenoidal, arqueamento ântero-lateral da tíbia ou pseudoartrose de um osso longo;

? Variante patogênica heterozigótica da NF1 com uma fração alélica variante de 50% em tecido aparentemente normal, como glóbulos brancos.

• Um filho de um dos pais que atende aos critérios diagnósticos especificados em A merece um diagnóstico de NF1 se um ou mais dos critérios em A estiverem presentes.

Hodiernamente, sabe-se que um teste molecular negativo para NF1 não exclui o diagnóstico da doença, tendo em vista que alguns indivíduos apresentam suas manifestações características, mas não uma variante patogênica da NF1 detectável. Outro ponto é que muitas das características clínicas da doença aumentam com a idade do indivíduo, fazendo com que alguns sejam diagnosticados quando adultos1-3.

O acompanhamento multiprofissional é necessário aos que convivem com NF1, tendo em vista que a enfermidade não tem uma cura. As consultas periódicas, com uma frequência a ser definida conforme a gravidade dos achados em cada caso, são necessárias para a identificação e a intervenção precoce de complicações que possam surgir no percurso da doença.

Ainda que perspectivas de tratamento estejam acompanhando os avanços da ciência, o considerado padrão ouro para a retirada de neurofibromas ainda é através de procedimento cirúrgico, sendo este recomendado quando os neurofibromas causam dor, afetam a vida diária do indivíduo e/ou trazem prejuízos estéticos para ele. Contudo, indivíduos com NFp contam com um novo medicamento

cujo princípio ativo é o Selumetinib. Trata-se de um medicamento que atua como inibidor da metiletilcetona (MEK), bloqueando certas proteínas envolvidas no crescimento de células tumorais. Foi feito um ensaio clínico de fase 2 com crianças que apresentavam NFp inoperável, as quais receberam o medicamento; após 1 ano, constatou-se melhora significativa no tamanho do tumor, bem como uma redução da dor local e de outras queixas e sintomas associados4. O medicamento em questão é aprovado pela Agência Nacional de Saúde (ANVISA), porém ainda não está incorporado ao rol do Sistema Único de Saúde (SUS) brasileiro.

Outro ponto fundamental no acompanhamento de quem vive com NF1 é a vigilância do seu desenvolvimento neuropsicomotor, bem como educacional, para que possíveis desvios também sejam precocemente identificados e intervindos. Problemas psicológicos (tais como depressão e ansiedade) também são frequentemente encontrados nas pessoas com NF1, os quais são causados tanto pelas manifestações clínicas da NF1 (p.ex., múltiplos neurofibromas) quanto pelo prognóstico complexo e imprevisível da enfermidade. Com isso, tais achados representam um risco para a saúde, visto que afetam diretamente a sua qualidade de vida (QV).

O conceito de QV é antigo, apresentado pela Organização Mundial de Saúde em meados de 1998 e designando a "percepção do indivíduo de sua posição na vida no contexto da cultura e do sistema de valores nos quais ele vive, considerando seus objetivos, expectativas, padrões e preocupações" 5. No atendimento multiprofissional em saúde e suas variantes, o interesse no conceito de QV vem aumentando desde então, dada a influência não só das políticas de atenção em saúde, mas também das práticas e dos protocolos assistenciais, visto que o processo saúde e doença, assim como o manejo da QV, são complexos, dinâmicos e multifatoriais 6.

Uma revisão sistemática7 sobre QV em pessoas com NF1 e Neurofibromatose Tipo 2 (NF2) evidenciou uma menor QV nas pessoas com algum tipo de neurofibromatose do que nos indivíduos controle, dado reforçado pela metanálise realizada, sendo que os adultos com neurofibromatose (NF) apresentavam uma menor QV quando comparado aos infantes. Conclui-se, a partir disso, que a NF impacta o bem-estar da pessoa, visto que pode afetar o funcionamento físico, emocional e cognitivo do quem convive com a doença. Os autores chamam a atenção pelo baixo número de pesquisas com crianças que vivem com NF7. Isso evidencia a necessidade de estimular mais e novas pesquisas na temática. Além disso, a incorporação de tratamentos já validados, como nos casos de NFp, tanto no sistema privado quanto no SUS, poderia auxiliar quem vive com a doença a ter uma maior QV4.

Tendo em vista a complexidade da doença e suas repercussões, todo indivíduo que convive com NF1 deverá seguir com um acompanhamento multiprofissional contínuo, com a finalidade de identificar e intervir precocemente as possíveis complicações advindas da doença. Com isso, tanto quem vive com NF1 quanto sua rede de apoio acessarão os mais diversos serviços de saúde, muitas vezes de maneira frequente e recorrente. Todo esse contexto de saúde-doença, assim como o sintoma de viver com uma enfermidade complexa, incurável e com tratamento autolimitado, traz certas inferências na vida, causando redução da sua QV6-8. Sendo assim, o objetivo desta revisão de escopo (scope review: SR) é mapear os conceitos e achados acerca da QV de crianças, adolescentes e adultos jovens com NF1 e identificar as limitações acerca do tema na literatura atual.

MÉTODO

Trata-se de uma de revisão da literatura do tipo SR. As SR são usadas para delinear os principais conceitos que sustentam e norteiam um determinado campo de pesquisa, assim como esclarecer as definições de trabalho e limites conceituais de um determinado tópico9. Os estudos de SR vem ganhando espaço na literatura, tendo alto potencial para contribuir para fortalecer tanto os profissionais em suas práticas clínicas quanto os pesquisadores na produção de novas pesquisas10.

A presente SR está estruturada de acordo com o Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P)11. O PRISMA-P é um checklist para guiar a formulação de um protocolo de revisão sistemática, em que seus conceitos gerais podem ser adotados para a elaboração de uma revisão de escopo, a fim de garantir um maior rigor metodológico.

Protocolo e registro

A SR foi conduzida de acordo com as orientações do Joanna Briggs Institute (JBI)9; posteriormente, os resultados foram estruturados de acordo com o check-list Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR)12. O protocolo desta SR foi registrado na plataforma Open Science Framework sob registro osf.io/vcqdx13 e publicado na revista Online Brazilian Journal of Nursing8.

Pergunta de Pesquisa

Na formulação da questão de pesquisa, utilizou-se o mnemônico PCC, onde P se refere à População (crianças, adolescentes e adultos jovens), C se refere ao Conceito (QV) e C se refere ao Contexto (Ter NF1). A questão norteadora da pesquisa foi: Como a QV de crianças, adolescentes e adultos jovens com NF1 vem sendo trabalhadas no Brasil e no mundo?

Critérios de Inclusão

População

Serão incluídos estudos realizados com crianças, adolescentes e jovens adultos, com faixa etária dos cinco aos 25 anos de idade e com NF1.

Conceito

Os estudos que abordem como tema central a análise da QV, independentemente de como esta é abordada, tratada e analisada.

Contexto

Esta revisão está contextualizada com indivíduos que têm NF1.

Tipos de fonte de evidência

De acordo com a metodologia JBI, uma SR possui um escopo de pesquisa amplo e com critérios menos restritivos. Seguindo o protocolo, foram utilizados dados de diversas fontes de evidência, com os mais variados desenhos de estudo9.

Estratégia de busca

A partir do mnemônico PCC, as estratégias de busca foram construídas através dos Decs/Mesh Terms nos idiomas português, inglês e espanhol. A estratégia de busca está sistematizada no quadro 1.

Quadro1

Critérios de elegibilidade

As bases de onde os dados foram coletados foram PubMed/MEDLINE, EMBASE, Web of Science, Lilacs e CINAHL. Na busca da literatura cinzenta, utilizou-se as bases de dados Open Grey e Google Scholar. Foram considerados elegíveis os estudos nos idiomas português, inglês e espanhol publicados no período de janeiro de 2018 a junho de 2023, mês em que os estudos foram coletados. Cabe apontar que, na base de dados PubMed, a busca pelos metadados levou em consideração a data de inserção nela. Com isso, um artigo publicado em 2017 e inserido nas bases de dados em 2018 foi encontrado no momento da busca e por isso entrou na pesquisa. Estão incluídos nesta SR artigos completos publicados, preprint, manuais on-line, dissertações e teses.

Seleção dos estudos

Após a busca nas bases de dados, todas as etapas de seleção dos estudos elegíveis ocorreram de forma sistemática, aos pares, de forma independente e cegada, conforme recomendação do JBI9, através da ferramenta Rayyan. As divergências encontradas foram solucionadas por um terceiro revisor. O processo de inclusão dos estudos foi sistematizado e ocorreu de acordo com o Checklist PRISMA14, sendo apresentado na Figura 1.

Fig.1

Na primeira etapa, os estudos encontrados passaram por uma análise para identificação de duplicatas, as quais foram registradas e excluídas. A segunda etapa de seleção de estudos elegíveis seguiu com a leitura do título e resumo de todos os materiais encontrados, a fim de identificar aqueles que estão de acordo com os critérios de inclusão e exclusão. Foi realizada por um pesquisador treinado com as normas deste protocolo. A seleção foi conduzida pela ferramenta Rayyan e, em caso de dúvidas, o manuscrito foi automaticamente incluído para a terceira etapa de seleção.

A terceira etapa de seleção de estudos elegíveis consistiu na leitura na íntegra de todos os manuscritos triados na fase anterior. Foi conduzida de forma cega por dois pesquisadores distintos, sendo que um terceiro revisor teve a tarefa de decidir manter ou excluir os estudos que geraram divergências. Os manuscritos que atenderam ao objetivo e aos critérios de inclusão tiveram seus dados extraídos.

A síntese dos dados referente aos manuscritos selecionados está apresentada de forma a proporcionar ao leitor uma visão robusta, atualizada e sistematizada sobre a QV de crianças, adolescentes e jovens adultos com NF1. Para tanto, foi elaborado um quadro sinóptico (Quadro 2). A discussão dos resultados encontrados está interrelacionada de forma narrativa, com o intuito de elucidar os enfoques teóricos e metodológicos do tema de pesquisa.

Os estudos foram selecionados e analisados quanto a sua qualidade utilizando o questionário Checklist for Analytical Cross Sectional Studies elaborado pela JBI15. Em cada estudo, foram avaliados os sujeitos e ambientes, bem como se a exposição foi medida de forma válida e confiável, se os critérios e medições da condição foram corretos, se identificaram-se fatores de confusão, se informaram estratégias para lidar com esses fatores, se os resultados foram medidos de forma válida e confiável e se foi usada análise estatística apropriada. Estudos que apresentassem acima de 90% de presença desses itens foram considerados de alta qualidade16. Para gerar o gráfico da avaliação da qualidade e risco de viés, utilizou-se a ferramenta Robvis Online17, adaptando a avaliação da qualidade de acordo com as recomendações da JBI15,16.

RESULTADOS

Ao todo, 39 registros foram selecionados para avaliação de elegibilidade por dois pesquisadores distintos, atingindo uma taxa de concordância de decisão de 89,7% (n=35 registros) e tendo 10,3% (n=4 registros) conflito de decisão. Os manuscritos em conflito foram encaminhados para decisão por um terceiro avaliador, que optou pela exclusão de 75% (n= 3 registros) destes. Ao final, 20,5% (n= 8 registros) foram incluídos por estarem de acordo com
as normas desta RS. Os artigos incluídos podem ser consultados no Quadro 2.

Quadro 2

O Quadro 3 apresenta um gráfico de sinaleira com a síntese da avaliação da qualidade da evidência de cada um dos estudos selecionados. Apenas um estudo24 (12,5%) não apresentou informações suficientes para que todas as perguntas do checklist da JBI fossem respondidas, não atingindo, portanto, qualidade elevada de evidência.

Quadro 3. Síntese da avaliação da qualidade da evidência dos estudos selecionados.

Quadro 3

A Figura 2 representa uma síntese geral da avaliação da qualidade de evidência de todos os artigos selecionados.

Figura 2 – Gráfico de barras ponderadas com a síntese da avaliação geral da qualidade da evidência dos manuscritos selecionados no estudo.

Fig.2

DISCUSSÃO

Um olhar sobre a QV de crianças, adolescentes e adultos jovens saudáveis versus aos seus pares com NF1

O Pediatric Quality of Life InventoryTM (PedsQLTM) Generic Scores é um questionário para avaliação geral da QV de crianças, adolescentes e adultos jovens26,27. Ele possui três versões, sendo elas: cinco aos sete anos, oito aos 13 anos, 14 aos 17 anos e 18 aos 25 anos, com modelo para a autoavaliação do indivíduo e o relato dos pais. Possui asserções que são dispostas em quatro subescalas (saúde física, estado emocional, vida social e atividades escolares) em escala do tipo Likert (0 = nunca, 1 = quase nunca, 2 = algumas

vezes, 3 = muitas vezes, 4 = quase sempre). Para a análise, os valores são transformados em escores (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), os quais geram médias. De acordo com os autores, quanto maior a média (mais prox. 100), maior é a QV. Os valores são apresentados por sua média e desvio padrão26,27.

Cipolletta e cols., na Itália18, aplicaram o PedsQLTM Generic Scores em 120 crianças, adolescentes e jovens adultos, sendo 60 destes com NF1 e 60 saudáveis. No estudo de Cipolletta e cols.18, constatou-se que, em todos os domínios do PedsQLTM Generic Scores, os indivíduos que vivem com NF1 alcançaram pontuações mais baixas quando comparados à população do estudo sem NF1. Em termos de pontuações, os participantes com NF1 e saudáveis pontuaram, respectivamente: domínio saúde física (76.12±15.11, 86.20±10.78), domínio saúde emocional (72.00±18.55, 79.90±14.83), domínio vida social (78.58±20.26, 92.67±10.43), subescala atividades escolares (69.38±17.01, 79.33±17.09).

Especificidades da QV na NF1

A NF1, por se tratar de uma doença com múltiplas implicações, acaba interferindo na QV de seus indivíduos afetados. Questionários genéricos como o PedsQLTM Generic Scores26,27 acabam não sendo suficientes para a magnitude do problema, fazendo com que pesquisadores desenvolvessem versões específicas para NF1.

O PedsQLTM Neurofibromatosis Module (PedsQLTM NF) foi desenvolvido com o objetivo de entregar um questionário específico tanto para a análise da QV de crianças, adolescentes e jovens adultos NF1 quanto para a análise da percepção dos pais sobre a QV do filho com NF120,21. O PedsQLTM NF já foi traduzido transculturalmente para alguns países, dentre eles o Brasil25.

Outros questionários que também podem ser utilizados para mensurar a QV de quem vive com NF1 são os instrumentos Burden of Neurofibromatosis (BoN)28 e o Impact of NF1 on Quality of Life (INF1-QOL)29. Ambos foram desenvolvidos para pessoas adultas que vivem com NF1, com o objetivo de mensurar o impacto da doença28 para quem convive com ela e como medida de QV para ensaios clínicos e intervenções terapeuticas29.

O papel da família na QV de seus filhos com NF1

Evidencia-se a importância de estudos que investiguem a percepção dos pais frente à QV de seus filhos com NF120. Análises comparativas do autorrelato do indivíduo com NF1e da percepção de seu responsável podem ser úteis ao se traçar estratégias de enfrentamento da doença, com intervenções específicas para o aumento da sua QV. Envolver os pais em intervenções pode ser benéfico não só sobre a QV de seus filhos, mas também sobre sua própria QV20, 21, 23.

Comunicação e Letramento em Saúde como preditores para a redução da QV na NF1

Letramento em saúde (LS) é a capacidade do indivíduo de obter, processar e compreender as informações e os serviços básicos de saúde. Isso se faz importante para a tomada de decisão tanto sobre sua saúde quanto sobre os cuidados médicos que necessita ou irá necessitar. Para tanto, o LS compreende os determinantes individuais e sistêmicos que envolvem a habilidade de comunicação entre o paciente e os profissionais de saúde, assim como aspectos culturais de cada indivíduo, as complexidades do sistema de saúde no qual este está inserido, entre outros30.

Uma pesquisa com 305 crianças, adolescentes e jovens adultos com NF1 utilizando o PedsQLTM Generic Scores e o PedsQLTM NF evidenciou menores escores de QV em crianças dos cinco aos sete anos no domínio "dificuldades de fala"; para as faixas etárias dos oito aos 12 anos e 18 aos 25 anos, ambas no "domínio comunicação em saúde"22. Nesse sentido, problemas de aprendizagem também foram elencados como fatores relacionados na redução da QV em escolares com NF124. Há, na literatura, materiais desenvolvidos pensados para a educação em saúde de indivíduos que vivem com NF131,32, e investir em sua disseminação pode ser um fator auxiliar para o LS desta população com consequente aumento da sua QV.

Considerações sobre a QV de indivíduos que vivem com NF1

A QV de crianças, adolescentes e adultos jovens com NF1 é reduzida18-24, e entender a complexidade da doença sobre o indivíduo afetado é necessário. Uma pesquisa17 aponta que a presença das malformações ligadas à doença e os problemas cognitivos associados não poderiam causar, por si só, uma redução na QV. A pesquisa evidenciou que a presença de ansiedade e sintomas depressivos em relação à doença apresentaram uma maior relação com a redução da QV. Os sintomas de distorção de autoimagem também foram mais prevalentes nos indivíduos com NF118 quando comparado ao grupo controle, indicando uma preocupação excessiva de jovens com NF1 sobre distorções da sua autoimagem. Quando convidados a fazer um desenho de si mesmo, aqueles que tinham NF1 davam maior ênfase aos sinais da doença do que em outras características corporais próprias.

Manifestações como neurofibromas, glioma de via óptica e lesões ósseas, como displasia esfenoidal, arqueamento ântero-lateral da tíbia ou pseudoartrose de um osso longo, também podem impactar a QV dos indivíduos com NF1. Por exemplo, o neurofibroma, quando localizado no sistema nervoso central, pode ocasionar manifestações mais graves4,18.

Estudos sugerem uma intervenção nos mais diferentes aspectos da vida desses indivíduos, tais como escola/faculdade/trabalho, e os comportamentos adaptativos e a vida social. Há uma importância geral de novos estudos que explorem a complexa interação de fatores interpessoais que podem impactar a QV18,19, para que se possa não só entender o impacto da doença na QV das pessoas, mas também estudar e validar intervenções que melhorem a QV de quem vive com NF133.

Neste sentido, um estudo americano buscou avaliar a preparação para a transição para a vida adulta e o impacto da NF1 na vida do adulto jovem. A maioria dos participantes relataram que a doença teve um impacto significativo em todos os fatores de sua vida, incluindo educação, carreira profissional, relacionamentos e planejamento familiar. O estudo demonstrou entre os indivíduos com NF1 uma diminuição da prontidão para a transição para a vida adulta, exercendo um impacto negativo na QV do adulto jovem33. Ainda, o desenvolvimento de neurofibromas cutâneos entre a adolescência e a idade adulta muitas vezes gera desfiguração e desconforto progressivo, o que é um fator para a redução da QV, principalmente quando os neurofibromas cutâneos estão localizados na face34.

Outro ponto encontrado como preditor para a redução da QV em indivíduos que vivem com NF1 é a dor. Estes apresentaram pontuações menores na subescala dor do PedsQLTM NF, indicando um ponto causador de redução de QV19, 20, 23. Estudos utilizando o PedsQLTM NF podem auxiliar em melhor entendimento da natureza multidimensional de sintomas específicos da NF1, tais como a dor e seus problemas de funcionamento cognitivo que afetam a QV19-21, 24.

Um ensaio clínico de fase 24 que testou o Selumetinib em crianças com NFp constatou não só uma melhora do aspecto e tamanho do

tumor, mas também uma melhora na QV dos participantes. Com isso, investir em tratamentos para a doença melhoram não só os sinais e sintomas dela, mas também trazem maior QV aos indivíduos, visto que adquirem maior autonomia ao se deparar com melhora dos sintomas da doença e dos aspectos da aparência física que muitas vezes são os que mais impactam a QV4,18,34.

Lai et al19 evidenciam, em seu estudo, além da dor, o prurido como fator importante na relação social com seus pares. Além disso, chama atenção que indivíduos diagnosticados antes dos cinco anos de idade relatavam menos sintomas (como dor e prurido) e tinham pontuações maiores em todas as subescalas da avaliação da QV, exceto no relacionamento social. Nesse mesmo estudo, indivíduos com NFp apresentaram menores escores para QV19.

O bem-estar de indivíduos com NF1 deve ser alvo de pesquisas de intervenção direcionadas para melhorar a saúde e no aumento da QV. Pesquisadores7, 19, 21-24 afirmam que a utilização de escalas voltadas para pessoas com NF1 podem ser imperativas na identificação de indivíduos com necessidades maiores de intervenções especificas para a melhora da sua QV, ao individualizar o "cuidado centrado na pessoa". Também cabe ressaltar que são necessárias estratégias para a prevenção das possíveis complicações relacionadas a NF1, e que o investimento de recursos e tempo são essenciais para auxiliar os indivíduos que vivem com NF1 a alcançar uma maior QV7.

Como limitação do estudo, elenca-se que o acesso a artigos de outros idiomas poderia trazer maior representatividade sobre o olhar de diferentes culturas de pessoas que vivem com NF1. Outra limitação foi o baixo número de estudos da temática nos últimos cinco anos.

CONCLUSÃO

Com essa SR, conclui-se que a NF1 afeta a QV dos indivíduos acometidos, provavelmente por ser uma doença complexa e com manifestações variáveis. São necessários mais estudos envolvendo a nossa realidade e a de outros países para um melhor entendimento de sua dimensão, assim como para o desenvolvimento de pesquisas de intervenção voltadas para a melhora da QV desses indivíduos.

Outrossim, estudos sobre o LS dessa população também são necessários, com o intuito de identificar suas lacunas de conhecimento e melhorar sua comunicação em saúde, haja vista que são um público que frequentemente acessa os mais variados serviços e níveis de saúde. Emponderá-los sobre sua doença pode trazer maior autonomia de decisão para o seu tratamento, além de maior conhecimento sobre sua doença, ações que podem ajudar na manutenção da QV, permitindo que se expressem e melhor compreendam a dimensão de ser uma pessoa que vive com NF1.

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