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CARDIOMIOPATIA HIPERTRÓFICA: AVALIAÇÃO GENÉTICO-MOLECULAR, RASTREAMENTO DE FAMILIARES E PAPEL DOS MICRORNAS COMO BIOMARCADORES

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Tese apresentada como requisito parcial à obtenção do título de doutor em Cardiologia e Ciências Cardiovasculares pelo Programa de Pós-graduação em Ciências da Saúde: Cardiologia e Ciências Cardiovasculares da Faculdade de Medicina da Universidade Federal do Rio Grande do Sul.

Orientador: Profa. Andreia Biolo

Co-orientador: Profa. Beatriz Piva e Mattos

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DEDICATÓRIA

A Deus, que me deu a vida

Aos meus pais, que foram os primeiros professores

À minha esposa Camila, companheira inestimável e médica exemplar

E aos docentes, retentores de grande responsabilidade e motivo de inspiração

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EPÍGRAFE

"At the time of open-heart operation, however, no localized stenotic area was found and the obstruction must be attributed to systolic narrowing of the ventricular outflow tract resulting from massive muscular hypertrophy, the cause of which is obscure."

(Andrew G. Morrow and Eugene Braunwald, Circulation 1959).

RESUMO

A cardiomiopatia hipertrófica (CMH) é a doença cardiovascular de origem genética mais prevalente. Embora a avaliação de familiares em primeiro grau para CMH seja recomendada por diretrizes internacionais através de testes genéticos, estes podem não estar disponíveis ou serem negativos. Estratégias de rastreamento de CMH baseadas em critérios clínicos ainda não foram suficientemente testadas nesta população. Além disso, é desconhecido o papel de microRNAs como biomarcadores para a doença com esta finalidade. Foram avaliados 23 probandos com CMH e 58 familiares em primeiro grau. O rastreamento clínico identificou 10 (17%) familiares acometidos pela CMH. Marcadores clínicos como sintomas relacionados à CMH, história familiar de morte súbita, sopro sistólico e eletrocardiograma (ECG) anormal demonstraram boa acurácia para detecção da doença. Contudo, ECG anormal isoladamente apresentou a melhor performance. Critérios modificados de ECG para o rastreamento de CMH entre os familiares foram capazes de elevar a acurácia da estratégia empregada. Uma revisão sistemática foi realizada para identificar microRNAs diferencialmente expressos na CMH e sua correlação fenotípica. Identificou-se 87 microRNAs em um total de 329 indivíduos estudados. Os microRNAs na CMH apresentaram padrão de *up-regulation* em sua maioria. Entre os analisados, evidenciaram maior correlação fenotípico o mir-21 e mais marcadamente o mir-29a. O rastreamento clínico de familiares em primeiro grau de pacientes com CMH é factível e apresenta boa acurácia, principalmente quando baseado no ECG. Os microRNAs mir-21 e mir-29a apresentam marcada expressão na CMH e poderão ser candidatos a biomarcadores para reconhecimento da doença em fase clínica e pré-clínica.

Palavras-chave: cardiomiopatia hipertrófica, avaliação genética, rastreamento familiar, microRNA

ABSTRACT

Hypertrophic cardiomyopathy (HCM) is the most common cardiovascular genetic disorder. Although the first-degree relatives evaluation for detecting HCM based on genetic testing is recommended by international guidelines, they may not be available or be negative. The HCM screening strategies based on clinical criteria are still not sufficiently tested in this population. Moreover, the role of microRNAs as biomarkers in HCM for this purpose is still unknown. Twenty-three probands with HCM were evaluated and 58 first-degree relatives. The clinical screening was able to identify 10 (17%) relatives with HCM. Clinical markers such as HCM related symptoms, family history of sudden cardiac death, heart murmur, and abnormal electrocardiogram (ECG) showed good accuracy for detecting the disease. However, an abnormal ECG alone presented with the highest performance. A modified abnormal ECG model was able to improve the accuracy of the applied strategy. A systematic review was performed for identifying differentially expressed microRNAs and their phenotypic expression. A total of 87 microRNAs in 329 studied individuals were identified. MicroRNAs in HCM showed a pattern of up-regulation in most cases. Among several microRNAs, mir-21 and mir-29a showed the greatest phenotypic correlation, with the most consistent findings for the latter. HCM clinical screening of first-degree relatives is feasible and showed good accuracy, mainly when an ECG strategy was applied. The microRNAs mir-21 and mir-29a showed pronounced expression in HCM and may be good candidates as biomarkers to recognize the disease in clinical and pre-clinical stages.

Key-words: Hypertrophic cardiomyopathy, genetic evaluation, family screening, microRNA

LISTA DE ABREVIATURAS

CMH – Cardiomiopatia hipertrófica

ECG - Eletrocardiograma

ICFEP – Insuficiência cardíaca com fração de ejeção preservada

NGS – *Next Generation Sequencing*

RNM – Ressonância magnética cardíaca

VE – Ventrículo esquerdo

VUS – Variante de significado incerto

WES – *Whole exome sequencing*

WGS -*Whole genome sequencing*

SUMÁRIO

1) INTRODUÇÃO

A cardiomiopatia hipertrófica (CMH) é a doença cardiovascular de origem genética mais prevalente [1]. Caracteriza-se pela presença de hipertrofia ventricular esquerda assimétrica na ausência de dilatação da câmara ou outras causas [2,3]**.** Demonstra distribuição universal, com incidência semelhante entre os sexos e etnias [4,5]. A exteriorização clinica apresenta padrão bimodal, com pico entre 13-19 anos e após os 40 anos [2–5]. Nas últimas décadas, particular interesse tem sido dado a doença por ser a principal causa de morte súbita em jovens e atletas [6].

A CMH apresenta padrão de herança autossômica dominante com mutações identificadas majoritariamente em genes sarcoméricos [1,7]. Mais de 2.500 mutações foram identificadas, principalmente nos genes da cadeia pesada da beta-miosina cardíaca (*MYH7*) e da proteína C de ligação à miosina (*MYBPC3*) [8,9]. Nova técnica, denominada de sequenciamento de nova geração através da aplicação de painel multigênico (*Next Generation Sequencing* - NGS), permite a codificação de um grande número de genes com menor custo e de forma mais rápida [10]. Mutações são identificadas em cerca de 60-70% dos indivíduos submetidos a genotipagem naqueles com formas familiares bem definidas e em cerca de 30-40% nos casos esporádicos [9,11,12]. Devido à maior disponibilização destes testes e à penetrância incompleta das mutações, os carreadores sem o fenótipo representam um importante grupo sobre os quais persistem interrogações. Os fatores relacionados à expressão fenotípica nos indivíduos assintomáticos carreadores de mutação ainda são desconhecidos [1,9,13].

Os microRNAs, moléculas com função de regulação gênica, tem a sua expressão associada a doenças cardiovasculares e podem estar implicadas na patogenia da CMH [14]. Diversos microRNAs foram identificados com ênfase no miR-21 e miR29a, os quais evidenciaram relação com hipertrofia ventricular esquerda e fibrose miocárdica detectada à ressonância magnética

(RNM) [15,16]. Ainda é desconhecido qual a função destas moléculas no processo de expressão fenotípica em fase pré-clínica e seu papel como biomarcador na prática assistencial.

2) REVISÃO DA LITERATURA

2.1) Conceituação da cardiomiopatia hipertrófica e aspectos demográficos

A cardiomiopatia hipertrófica (CMH) representa a doença cardiovascular de origem genética mais prevalente estimada em 1 para cada 200 indivíduos [1]. O fenótipo caracteriza-se por hipertrofia ventricular esquerda predominantemente assimétrica, identificada na ausência de dilatação da câmara e de outras causas [2,3]. O caráter complexo desta enfermidade é justificado pela marcada heterogeneidade genética e expressiva variação fenotípica [8,17]. A distribuição geográfica é universal com acometimento de diversos grupos étnicos. Devido ao padrão de herança mendeliana, é esperada distribuição semelhante entre os gêneros; contudo, as coortes internacionais registram predomínio no sexo masculino [4,5]. Dados recentes demonstram que pacientes da raça negra apresentam maior probabilidade de diagnóstico em idade precoce e maior limitação funcional [18]. A doença exterioriza-se geralmente na adolescência, embora possa ter expressão clínica tardia, na fase adulta após 40-60 anos [4,5,12,19]. Ainda que a morte súbita tenha se tornado infrequente na atualidade pela ampla aplicação de medidas preventivas, constitui a principal causa de óbito cardiovascular em jovens < 25 anos e atletas com CMH [6].

2.2) Histopatologia

O substrato histopatológico característico da CMH é constituído por hipertrofia miocárdica, desorganização celular, fibrose e doença da microcirculação. Estes estão implicados diretamente no desenvolvimento de isquemia, arritmias e disfunção diastólica do ventrículo esquerdo (VE) [17]. A desorganização celular apresenta distribuição assimétrica no miocárdio, podendo envolver de 10% a 90% de um segmento isolado. Sua correlação com o grau de hipertrofia é baixa e apresenta menor frequência em indivíduos com obstrução na via-de-saída do

VE [20,21]. Por outro lado, a fibrose reparativa relaciona-se em maior grau com massa e espessura parietal máxima do VE. Apresenta-se mais incidente em indivíduos mais velhos, levando a hipótese de que se trata de processo secundário aos demais mecanismos fisiopatológicos [20]. A doença da microcirculação, achado histológico característico da CMH, representa um dos fatores determinantes da presença de isquemia miocárdica [20,22]. Quando avaliada através de RNM cardíaca, identifica-se reduzida perfusão que se correlaciona com o grau de hipertrofia [23]. Outros fatores, no entanto, também associam-se ao desequilíbrio entre oferta e demanda de oxigênio como desorganização celular, a hipertrofia dos miócitos, a fibrose intersticial e a redução da densidade capilar [22].

As mutação sarcoméricas presentes na CMH desencadeiam processos de hipercontratilidade e hipertrofia miocárdica associados ao aumento na produção de colágeno [24,25]. Estudo recente em modelo animal utilizou tecido miocárdio com CMH que foi decelularizado mantendo apenas a matriz extracelular original. Este tecido foi semeado com células tronco pluripotentes derivadas de miócitos saudáveis. Desta forma, produziu-se um tecido que retinha a matriz extracelular original, mas com miócitos normais. Verificou-se que estes miócitos normais desenvolveram hipercontratilidade e alteração de relaxamento, sugerindo que o remodelamento extracelular apresenta papel fundamental no desenvolvimento da doença [24]. Estes dados corroboram estudos em que a administração de mavacamten, molécula que reduz a atividade da adenosina trifosfato, evitou o desenvolvimento de CMH apenas em modelos animais portadores de mutações no gene MYH7 sem doença expressa, mas falhou naqueles em estágio mais avançado da doença [26].

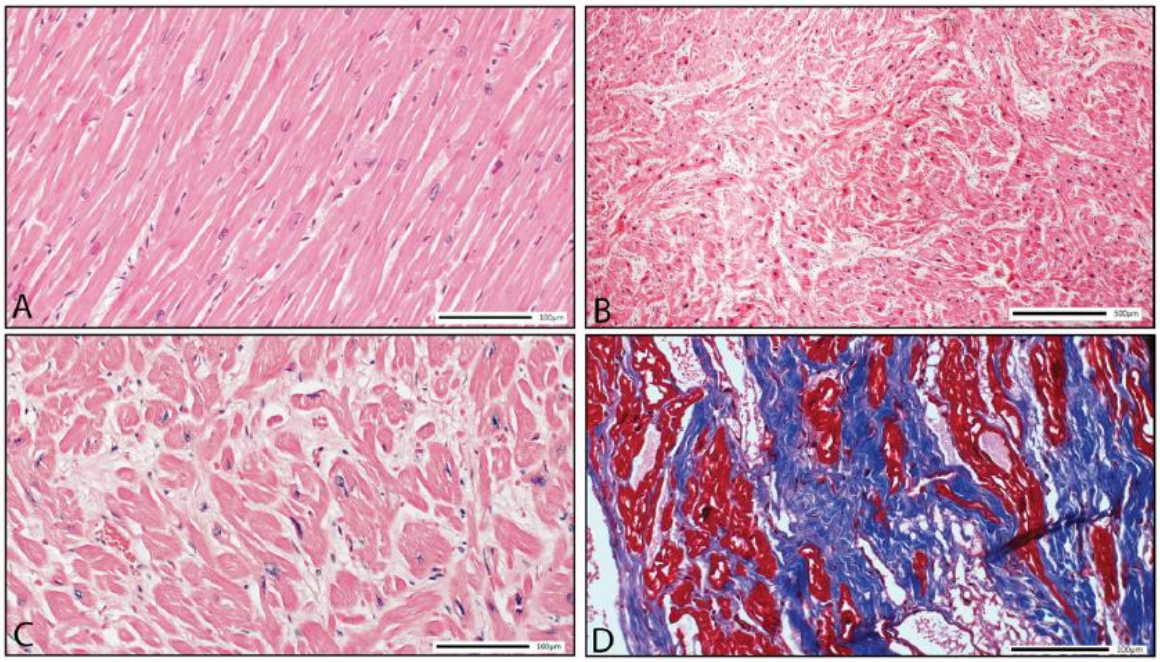

Figura 1. A. Tecido miocárdico normal corado com H&E. B. Miocárdio de paciente com cardiomiopatia hipertrófica demonstrando arquitetura miocárdica desorganizada (x4; H&E). C. Desorganização celular miocárdica (x20; H&E). D. Fibrose intersticial demonstrada por coloração de tricromo Masson em azul (x20) [17].

2.3) Diagnóstico

O diagnóstico da CMH é favorecido pela presença de sintomas cardiovasculares, detecção de sopro cardíaco ou por meio da avaliação de membros de famílias acometidas. A identificação das manifestações que caracterizam o fenótipo, ou seja, hipertrofia ventricular esquerda em grau, extensão e localização variáveis, associada à cavidade normal ou reduzida, é realizada através do ecocardiograma ou da RNM cardíaca [2,3,17,27]. Define-se pela identificação em qualquer método de imagem de espessura parietal máxima ≥ 15 mm ou ≥ 13 mm nos casos de mutação conhecida para CMH [2]. Predominam as formas septais assimétricas, em que a razão septo/parede posterior do VE é ≥ 1,3 [17]. O envolvimento isolado de outros segmentos ventriculares, como

septo posterior, parede ântero-lateral ou ápice do VE, é referido em menor número de casos e hipertrofia concêntrica em menos de 5% [17].

Obstrução dinâmica da via-de-saída do VE incide em 25% dos pacientes em repouso e em até 75% sob exercício ou manobras provocativas que reduzem a pré e a pós-carga ou aumentam a contratilidade miocárdica [28,29]. A obstrução decorre da geometria da via-de-saída anormal e da complexa interação entre o folheto anterior da válvula mitral e o septo interventricular promovida por vetores anômalos de fluxo gerados na cavidade ventricular durante a sístole [30]. Anomalias estruturais envolvem a válvula mitral e/ou músculos papilares com regurgitação em grau variável em 20% a 45% das formas obstrutivas [31,32]. O ecocardiograma é o método diagnóstico mais comumente empregado para avaliação da obstrução em via-de-saída do VE. A manobra de Valsalva, aplicada durante o exame em repouso, permite identificar apenas 50% dos pacientes com obstrução detectada ao exercício [33–35]. Neste contexto, o ecocardiograma com esforço aplica-se principalmente naqueles indivíduos sintomáticos com obstrução latente da via-de-saída do VE [34,35]. O ecocardiograma com exercício apresenta maior acurácia em relação à manobra de Valsalva ou à administração de nitrato, com resultados semelhantes quando é realizado em esteira rolante ou em cicloergômetro [36]. A presença de obstrução latente possui valor prognóstico e permite estender a indicação de redução septal em pacientes sintomáticos [34]. Outras anomalias envolvendo músculos papilares ou alongamento do folheto anterior da válvula mitral são descritas nas formas obstrutivas da doença e contribuiriam para desenvolvimento de gradiente intracavitário [37]. Há discordância na literatura se estas manifestações estão relacionadas ao substrato genético ou são decorrente de modificações na geometria da câmara ou do padrão de hipertrofia [31,38,39]. O comprometimento do ventrículo direito é excepcionalmente evidenciado [2,3].

A RNM cardíaca é fundamental no diagnóstico da CMH, pois permite a identificação de diversos padrões de hipertrofia além de definir formas confinadas a parede livre ântero-lateral, septo posterior e ápice [34,40–42]. A determinação das espessuras parietais pode apresentar discrepância entre o ecocardiograma e a RNM cardíaca, principalmente nos casos limítrofes ou com formas atípicas [40,43,44]. Além disso, este método diagnóstico permite a identificação de fibrose reparativa através da técnica de realce tardio, cuja presença está relacionada a pior prognóstico e ocorrência de morte súbita [45,46]. Apesar da fibrose não constituir critério independente para implante de cardiodesfibrilador implantável em prevenção primária de morte súbita, sua presença pode auxiliar na tomada de decisão [2,3,46]. O mapeamento T1 é uma nova técnica aplicada à RNM utilizada para quantificar a expansão do volume extracelular que se relaciona com a presença de fibrose intersticial [47]. Verifica-se que, em comparação com indivíduos saudáveis, indivíduos com CMH demonstraram T1 prolongado e volume extracelular elevado mesmo na ausência de realce tardio, além de forte correlação com índice de massa do VE [48]. Esta técnica foi estudada em carreadores de mutação sem expressão fenotípica. Identificou-se que estes indivíduos apresentaram expansão do volume extracelular, sugerindo seu emprego na detecção de formas pré-clínicas [49]. Por fim, a detecção da expansão do volume extracelular por mapeamento T1 apresenta valor prognóstico em relação a hospitalização por insuficiência cardíaca e mortalidade [50].

O diagnóstico diferencial da CMH com outras causas de hipertrofia ventricular pode ser desafiador. A RNM através de avaliação da morfologia septal, mapeamento T1 e *strain* longitudinal global pode auxiliar na diferenciação entre hipertrofia secundária a hipertensão arterial e a CMH [51,52]. Achados da CMH como espessura do septo interventricular médio e basal acima de 15 mm, presença de gradiente na via-de-saída do VE ≥ 30 mmHg, relação E/A ≥

2, aumento do átrio esquerdo e movimento sistólico anterior da válvula mitral sugerem o diagnóstico em indivíduos idosos [53]. A morfologia septal também auxilia no diagnóstico daqueles com CMH. Septo interventricular com curvatura reversa associa-se com 80 a 90% de identificação de mutação em genes sarcoméricos, além de incidir mais comumente em jovens, apresentar pior função diastólica e maior taxa de realce tardio [54,55]. No entanto, hipertrofia apical e septo neutro associam-se a mutações apenas em um terço dos casos e septo sigmoide em apenas 10-20% [53,54].

2.4) Evolução clínica e terapêutica

A evolução clínica é variável, enquanto alguns permanecem longo tempo assintomáticos ou com mínima restrição, outros apresentam morte prematura ou progridem tardiamente à fibrilação atrial e insuficiência cardíaca [17,56]. Dor torácica e síncope inexplicada são frequentes; contudo, sintomas de insuficiência cardíaca são predominantes na fase adulta e podem ocorrer em até 50% dos casos [4,17,18]. A expressão clínica pode divergir entre os sexos [57,58]. Apesar da discrepância na literatura quanto a diferenças no grau de obstrução da via-de-saída do VE entre gêneros, mulheres apresentam pior capacidade funcional, maior nível de biomarcadores e mortalidade mais elevada [57–60]. Além disso, sexo feminino apresentou-se como preditor independente para mortalidade em análise uni e multivariada [57,58]. Não está claro se estas diferenças se relacionam com fatores hormonais e/ou interação com processos genético-moleculares [61]. Fatores ambientais que possam modificar a evolução clínica da CMH ainda são pouco conhecidos. Evidências recentes sugerem que a presença de obesidade pode contribuir de forma independente para os sintomas relacionados a doença, bem como fisiologia obstrutiva e ocorrência de eventos cardiovasculares [62,63]. A restrição ao exercício imposta pela CMH e a maior limitação funcional pode ter relação com maior índice de massa encontrada nessa doença

[64]. Comorbidades como hipertensão arterial, diabete mellitus e tabagismo apresentam incidência semelhante à da população em geral [4,59].

Extrassístoles ventriculares são frequentes na CMH, compreendendo formas isoladas ou em salvas e se associam ao grau de hipertrofia ventricular esquerda [65]. A fibrilação atrial é a arritmia sustentada mais comum; acomete entre 20-30% dos pacientes e associa-se a piora da classe funcional [5,59]. Estudo com RNM cardíaca identificou que indivíduos com fibrilação atrial paroxística apresentam associação com fibrose do átrio esquerdo e configuram um fenótipo mais avançado da doença [66].

A morte súbita é mais frequente nos pacientes abaixo de 40 anos enquanto que óbito por insuficiência cardíaca ocorre nos pacientes em décadas de vida mais avançada [59]. A análise recente de uma ampla coorte de indivíduos adultos com CMH atendidos em centros de referência demonstra taxas de sobrevida em 5 e 10 anos de 98% e 94%, respectivamente, não distintas da população normal [67]. Contudo, estudo europeu demostrou maior mortalidade nos pacientes com CMH em relação à população geral quando estratificados por sexo, idade e anos de seguimento [59].

A morte súbita cardíaca é o desfecho mais temido em pacientes com CMH, podendo incidir em até 3 a 4% ao ano em indivíduos de alto risco [68]. Possui base arritmogênica e ocorre por desenvolvimento de taquicardia ventricular sustentada ou fibrilação ventricular [69]. Está indicado o implante de cardiodesfibrilador em prevenção secundária para aqueles pacientes que sobreviveram a uma parada cardiorrespiratória devido à taquicardia ventricular ou fibrilação ventricular, ou aqueles com taquicardia ventricular sustentada com comprometimento hemodinâmico [2,3]. Os principais fatores de risco para morte súbita de acordo com a recente

diretriz *American College of Cardiology/American Heart Association 2020* considerados como preditores independentes são história familiar de morte súbita em familiares de primeiro grau, síncope inexplicada, hipertrofia maciça do ventrículo esquerdo (VE), aneurismas apicais e fração de ejeção < 50% [3]. Taquicardia ventricular não-sustentada e realce tardio à RNM cardíaca são considerados fatores menores [3]. A presença destes indicadores ainda que isoladamente constituem critério para implante de cardiodesfibrilador em prevenção primária de morte súbita [3]. A Sociedade Europeia de Cardiologia, no entanto, propôs um modelo, denominado *ESC HCM-Risk SCD*, baseado em estimativa de risco que, além dos predisponentes conhecidos, inclui idade e diâmetro do átrio esquerdo [2]. Esta recomendação possuiria baixa sensibilidade para identificação de indivíduos que podem evoluir com morte súbita e poderia deixar desprotegidos aqueles em que o risco calculado situa-se abaixo de 6% ao ano [68]. A concordância entre a estratificação de risco para morte súbita entre essas diretrizes é baixa [70]. Verifica-se que a diretriz norte-americana é mais liberal em suas indicações e a diretriz europeia restringe o implante de CDI apenas em indivíduos de maior risco [70]. Desta forma, a estratificação de risco para morte súbita nesta doença é considerada ainda imperfeita visto que esta complicação pode incidir em indivíduos sem preditores conhecidos [69].

O tratamento clínico na CMH visa o controle de sintomas e tem base em estudos observacionais. Recomenda-se o uso de beta-bloqueadores sem efeito vasodilatador como fármacos de primeira escolha seguido dos bloqueadores de canais de cálcio não-diidropiridínicos [2,3]. Disopiramida pode ser utilizada em associação, mas não está disponível no Brasil. O uso de fármacos com efeito vasodilatador não está indicado pelo potencial efeito de piora da obstrução da via-de-saída. Contudo, ensaio clínico randomizado controlado por placebo avaliou o uso de losartana em 133 pacientes com CMH. Não houve modificação no padrão de hipertrofia

ventricular, mas mostrou-se seguro nessa população, sugerindo seu uso para outras indicações como controle anti-hipertensivo [71,72]. Diltiazem foi testado em ensaio clínico controlado por placebo em 38 indivíduos carreadores de mutação para CMH sem expressão fenotípica. No seguimento médio de 25 meses, o grupo tratamento demonstrou menor grau de remodelamento do VE [73]. Estudos adicionais, com maior população e tempo de seguimento deverão ser realizados para confirmar estes achados. Recentemente, ensaio clínico randomizado duplo cego demonstrou que o uso de mavacamten se associou a redução na obstrução da via-de-saída do VE, melhora do consumo de oxigênio no exercício e melhora em escore de sintomas [74]. Estes resultados promissores fornecem uma nova via de tratamento da doença. Esta droga atua através de inibição da ATPase miosina cardíaca, reduzindo a contratilidade e melhorando a performance energética [75,76].

A cirurgia de miectomia para redução septal foi um dos primeiros tratamentos propostos para a CMH e segue como terapêutica vigente para redução do gradiente na via-de-saída do VE para níveis inferiores a 10 mm Hg com marcado impacto na redução de sintomas e melhora da qualidade de vida [69]. A mortalidade perioperatória pode chegar a 10%; contudo, centros experientes relatam taxas de mortalidade menores de 1% [77–79]. A miectomia associa-se a distúrbios de condução, principalmente bloqueio de ramo esquerdo, e bloqueio átrio-ventricular em até 34%, sobretudo naqueles com bloqueio de ramo direito prévio ao procedimento [80]. Ablação septal alcoólica é uma alternativa segura, com redução >50% do gradiente na via-de-saída em torno de 90% dos casos, é menos invasiva e mais disponível [79,81]. A mortalidade do procedimento é baixa (<1%) e está relacionada a implante de marcapasso definitivo devido a bloqueio átrio-ventricular avançado em cerca de 10% dos casos principalmente naqueles com bloqueio de ramo esquerdo prévio ao procedimento [82,83].

2.5) Genética

A CMH é transmitida por herança autossômica dominante que leva ao desenvolvimento de formas familiares, ainda que casos esporádicos sejam também detectados. A ausência de caráter familiar é atribuída à penetrância incompleta, gene e idade dependentes e a ocorrência de mutações *de novo*. Mais de 2.500 mutações já foram descritas em 11 genes que codificam proteínas do sarcômero e, em menor número, dos discos Z [8,9,11,13]. Mais recentemente, foram descritas mutações em outros genes, relacionados a filamentos grossos, discos intercalados ou mediadores dos canais de cálcio, cuja patogenicidade ainda é interrogada [8,9]. Substituições em genes do metabolismo celular, *PRKAG2*, *LAMP2* e *GLA*, podem gerar doenças de armazenamento de glicogênio, consideradas fenocópias da CMH [8]. O comprometimento dos genes da cadeia pesada da beta-miosina cardíaca (*MYH7*) e da proteína C de ligação à miosina (*MYBPC3*) é considerado predominante, sendo ambos responsáveis por cerca de dois terços das mutações encontradas [8,9,27]. Reduzida prevalência individual é atribuída aos demais genes: troponina T (*TNNT2*), troponina I (*TNNI3*), cadeia leve da miosina reguladora (*MYL2*), cadeia leve da miosina essencial (*MYL3*), alfa-tropomiosina (*TPM1*), alfa-actina cardíaca (*ACTC1*), troponina C (*TNNC1*), alfa-actinina 2 (*ACTN2*) e miosenina 2 (*MYOZ2*). Os principais genes relacionados à CMH, as respectivas proteínas codificadas e sua função estão descritos na tabela 1 [17,84].

Tabela 1. Principais genes acometidos em pacientes com cardiomiopatia hipertrófica

Gene	Frequência	Proteína codificada	Função
MYH7	15-25%	Cadeia pesada da β-miosina	Atividade ATPase, interação com actina, contração miocárdica

MYBPC3	15-25%	Proteína C de ligação à miosina	Interação com os filamentos grossos, contração miocárdica
TNNT2	<5%	Troponina T cardíaca	Regulador da interação actinina-miosina
TNNI3	<5%	Troponina I cardíaca	Inibidor da interação actinina-miosina
TPM1	<5%	α-tropomiosina	Interação do complexo de troponina com actina cardíaca
MYL2	<2%	Miosina reguladora de cadeia leve	Proteína de ligação a cadeia pesada da miosina
MYL3	<1%	Miosina essencial de cadeia leve	Proteína de ligação a cadeia pesada da miosina
ACTC1	<1%	α-actina cardíaca	Interação actina-miosina
TNNC1	<1%	Troponina cardíaca C	Regulador cálcio sensível da função dos miofilamentos
ACTN2	<1%	α-2 actinina	Proteína do disco Z
MYOZ2	<1%	Miosenina 2 (calsarcina 1)	Proteína do disco Z

Até recentemente, a pesquisa de mutações no DNA era realizada pela técnica de *Sanger* na qual a ordem dos nucleotídeos nas sequências codificantes do gene é analisada em série, uma após a outra. Embora precisa e reprodutível, é considerada laboriosa e demorada [8]. O sequenciamento de nova geração (*Next Generation Sequencing* - NGS) possibilita a abordagem simultânea de um grande número de genes de forma mais rápida e com menor custo [85]. Esta técnica pode ser aplicada na modalidade de painel, o qual gera resultados semelhantes aos obtidos por *Sanger*, mas

apresenta maior rapidez e menor custo do que outras formas de NGS como WES *(Whole Exome Sequencing)* ou WGS *(Whole Genome Sequencing)* [10,86]. As anormalidades encontradas podem ser classificadas em cinco categorias: mutações patogênicas, provavelmente patogênicas, variantes de significado incerto (VUS), provavelmente benignas e benignas [8,10,87].

A correta interpretação destas variantes é fundamental para se estabelecer o diagnóstico em casos selecionados e para o aconselhamento familiar. A experiência acumulada em centros especializados com compartilhamento de banco de dados de mutações resulta em otimização da interpretação dos testes genéticos com reduzida discordância na classificação destas variantes [88]. Muitas das substituições de aminoácidos na sequência do DNA não causam doença e são interpretadas como polimorfismos de caráter benigno, com incidência média na população normal de 0,5 a 1% [8]. A introdução do NGS determina aumento da ocorrência de VUS e um grande número permanece com significado desconhecido mesmo após a aplicação de todos os instrumentos disponíveis para avaliação de patogenicidade [8,10]. Essas variações incidem em 5 a 50% dos casos na dependência do número de genes sequenciados e de classes utilizadas para definir a patogenicidade das mutações [8,89]. Para fins de diferenciação entre variações benignas e patogênicas, é preciso conhecer a frequência das substituições na população normal disponibilizada em bancos de dados, o registro prévio na literatura, o grau de cosegregação nas famílias, o possível impacto na estrutura da proteína, assim como proceder consulta aos sistemas de bioinformática na denominada análise *in silico* [10,86,89,90].

A aplicação de painéis com mais de 50 genes parece não adicionar sensibilidade ao diagnóstico, quando comparados àqueles contendo apenas os 11 genes considerados mais prevalentes [91]. A ampliação dos painéis genéticos a genes não-sarcoméricos produz um número elevado de variações raras, mas sem associação com a CMH. Desta forma, é recomendado apenas

a genotipagem dos genes não-sarcoméricos CSRP3, FHL1, FLNC, PLN, TNNC1, ACTN2 e MYOZ2 assim como aqueles relacionados a doenças metabólicas como LAMP2, GAA, GLA, PRKAG2 e TTR [92].

Mutações causadoras são atualmente identificadas em 60-70% dos pacientes com formas familiares e em 30-40% dos casos esporádicos [9,11,12]. Predominam aquelas denominadas *missense* ou de "sentido trocado" em que a substituição de uma única base no nucleotídeo leva à troca do aminoácido codificado. Entretanto, no gene *MYBPC3* são também encontradas deleções ou inserções que determinam mutações mais radicais na matriz do quadro de leitura denominadas de *frameshift* [8,11]. As mutações são frequentemente privativas de uma família e muitas são *de novo*, originando formas esporádicas [8]. Há marcada variabilidade da expressão fenotípica intra e interfamiliar em decorrência da ação de fatores ambientais, genes modificantes, dupla heterozigose e outros processos ainda desconhecidos [13].

As complexas características moleculares da CMH foram definidas com base na avaliação de populações europeias e norte-americanas [2,3]. Em nosso país, foram pesquisadas preliminarmente em coortes das regiões sudeste, norte e nordeste [93]. No sul do Brasil, cuja composição étnica é distinta, foi realizada a análise de uma amostra constituída por famílias com a doença [94]. A maior frequência de mutações de *MYH7* constatada neste estudo corrobora observações prévias em outras regiões do país, mas diverge do relatado em populações do hemisfério norte em que se verifica o predomínio de doença relacionada a *MYBPC3*. A aplicação em maior escala de testes genéticos em portadores de CMH poderá confirmar, futuramente, se tais achados representam ou não uma característica local.

Apesar do alto grau de recomendação da genotipagem nas diretrizes internacionais de indivíduos e familiares com CMH, ainda não está amplamente disponível, principalmente em países de média e baixa renda [2,87]. A probabilidade de um teste genético positivo aumenta na presença de algumas características clínicas como idade no diagnóstico, sexo feminino, caucasianos, padrão de hipertrofia e história familiar de CMH ou morte súbita [4,95–100]. Neste contexto, alguns escores foram propostos para auxiliar a identificação de pacientes com maior probabilidade de positivação do teste [96,97,100].

Com a aplicação em maior escala dos testes genéticos, os carreadores de mutação para doença sem o fenótipo, passaram a representar importante subgrupo dentro do extenso espectro de manifestações da CMH, a respeito dos quais persistem várias interrogações. Os indivíduos sem o fenótipo podem evidenciar alterações eletrocardiográficas que constituem critério para o seu reconhecimento [101]. Distúrbios da função sistólica e do enchimento diastólico do VE seriam detectados precocemente pelo ecocardiograma com Doppler tissular, assim como modificação do *strain* global longitudinal pode ser identificada por *speckle trecking* [102,103]. Anomalias intrínsecas da válvula mitral tais como alongamento excessivo do folheto anterior, deslocamento dos músculos papilares, bandas musculares anômalas e criptas identificadas à RNM cardíaca podem constituir expressão fenotípica precoce de mutações sarcoméricas [104–106]. O uso de RNM cardíaca pode auxiliar no reconhecimento daqueles com espessura parietal máxima do VE limítrofe [107]. A identificação de mutações patogênicas associa-se a maior mortalidade e maior incidência de complicações até mesmo após muitos anos do diagnóstico [108,109]. A presença de VUS relaciona-se a risco intermediário para o desenvolvimento de insuficiência cardíaca e fibrilação atrial [108].

Não se conhecem quantos e quais carreadores de mutação poderão desenvolver a doença e tampouco o risco ao qual estariam expostos na fase prévia ao surgimento de hipertrofia ventricular esquerda [1,9,13,27,94,110]. Recentemente, identificou-se que estes indivíduos apresentam aumento do diâmetro diastólico e da massa do VE quando expostos a exercício físico vigoroso; contudo, não houve maior incidência de arritmias [111]. Estes familiares carreadores de mutação possuem recomendação de avaliação por ecocardiograma a cada 5 anos [2]. Ainda não está claro quais desenvolverão a doença e o tempo necessário para tal. No seguimento de uma coorte de genótipo-positivos em fase pré-clínica por até 18 anos, foi identificado que apenas 4 de 14 indivíduos desenvolveram o fenótipo [112]. Outros estudos, com menor tempo de seguimento, observaram o desenvolvimento da CMH entre 6% e 14% dos indivíduos em um tempo médio de 5 a 12 anos dependendo da faixa etária inicial [106,109,113,114]. Recentemente, foi reportado estudo com 215 indivíduos de 186 famílias seguidos por até 15 anos. Identificou-se uma taxa de 50% de detecção de expressão clínica da CMH, a maior até o momento [115]. A intervenção farmacológica precoce em estágios evolutivos iniciais poderá favorecer, futuramente, a introdução de medicamentos que modulem a exteriorização fenotípica [73,116].

2.6) Avaliação familiar

A avaliação de familiares em primeiro grau de pacientes com CMH é preconizada pelas diretrizes vigentes através de avaliação clínica, eletrocardiográfica, ecocardiográfica e testagem genética [2,3]. Desta forma, pode-se realizar o rastreamento em cascata e identificar não apenas aqueles com o fenótipo, mas também aqueles que necessitarão seguimento a longo prazo (portadores de mutação) e excluir os negativos. Contudo, nos casos em que não se identifica mutação ou em que a testagem não foi realizada, recomenda-se o seguimento clínico com

eletrocardiograma e ecocardiograma a cada 2 a 5 anos ou em menor frequência se alterações subjacentes forem detectadas [2,3].

Embora o rastreamento apresente recomendação universal entre os familiares, algumas características associam-se com a detecção do fenótipo nesta população [117,118]. A análise de coortes contemporâneas demonstraram maior incidência do fenótipo naqueles com média > 40 anos de idade, sendo população de maior benefício para avaliação [117,119]. Embora seja recomendado rastreamento após 10-12 anos de idade, formas severas podem ocorrer em faixas etárias mais precoces [118,120]. Ainda que a incidência da CMH possa diferir conforme a idade, todos os familiares devem ser selecionados para avaliação [2]. A presença de sintomas pode ocorrer em 1% a 20% dos casos confirmados, sendo um indicativo da doença [117–119]. Contudo, há divergência entre estudos em relação a maior prevalência de sintomas entre aqueles com a doença clinicamente expressa em relação aos indivíduos normais [117,119]. Esta discrepância pode relacionar-se a baixa especificidade e a subjetividade da avaliação clínica baseada em sintomas.

Nas famílias com probandos genótipo-positivos, mutações podem ser detectadas em 30 a 80% dos familiares, modificando-se conforme os critérios de seleção aplicados [94,114,117,118,121]. Devido a maior acessibilidade dos testes genéticos, identificou-se um subgrupo de indivíduos carreadores de mutação sem o fenótipo, sobre os quais persistem interrogações quanto a penetrância das variantes e evolução clínica da doença [1]. Embora evidências sugiram pior prognóstico nos probandos com mutação patogênica identificada, estudo em familiares demostrou sobrevida semelhante entre aqueles com teste genético positivo em relação aos negativos e não testados [108,117]. Estas divergências podem estar relacionadas a

menor penetrância das mutações em familiares e a menor idade destes em relação aos pacientes índice, refletindo menor tempo de evolução da doença.

A avaliação eletrocardiográfica na CMH identifica anormalidades em > 90% dos casos, principalmente relacionadas a alterações de voltagem no QRS, ondas Q patológicas e distúrbios de repolarização [122]. Contudo, a frequência dos achados divergem entre os estudos, não demonstrando um padrão único em indivíduos acometidos [123–125]. Por outro lado, o número de anormalidades detectadas está diretamente relacionada à expressão fenotípica e há uma tendência de alteração de seu padrão no curso evolutivo da doença, refletindo sua relação com as alterações fisiopatológicas subjacentes [122,123,125–127]. Este dado é corroborado pela evidência de que indivíduos fenótipo-positivos sem alterações eletrocardiográficas apresentam melhor sobrevida [124]. Portanto, o eletrocardiograma torna-se ferramenta fundamental no rastreamento de familiares de indivíduos acometidos pela CMH devido a sua sensibilidade [128]. A descrição dos achados eletrocardiográficos na CMH restringe-se em sua maioria a indivíduos com a doença clinicamente expressa, ou seja, pacientes índice. Estes podem apresentar um fenótipo mais avançado em relação aos familiares em primeiro grau e, assim, ter um eletrocardiograma com maiores modificações [122]. Ainda não está estabelecido quais achados eletrocardiográficos estão mais associados a presença do fenótipo em familiares em primeiro grau. Contudo, entre os indivíduos carreadores de mutação sem o fenótipo, verificou-se maior presença de ondas Q e alterações de repolarização, mas com sensibilidade reduzida [129]. A avaliação eletrocardiográfica de indivíduos com CMH foi estudada recentemente através de algoritmo de inteligência artificial com altíssima performance, principalmente em indivíduos jovens < 40 anos [130]. Contudo, a alta taxa de falsos-positivos ainda é um desafio para o uso isolado do ECG como rastreamento da doença [123].

O ecocardiograma, como ferramenta fundamental no diagnóstico da CMH, é recomendado na avaliação dos familiares em primeiro grau [2,3]. Na presença de mutações causadoras, o diagnóstico nos familiares pode ser estabelecido com espessura parietal máxima ≥ 13 mm [2]. Na ausência de um fenótipo típico, alterações como elevação das pressões de enchimento, movimento anterior sistólico incompleto da válvula mitral ou alongamento dos seus folhetos, e anormalidade dos músculos papilares aumentam a probabilidade da doença, principalmente na presença de alterações eletrocardiográficas [31,131,132]. Recentemente, alterações pré-clínicas avaliadas por técnica de *strain*, tanto em ecocardiograma como em RNM cardíaca, foram capazes de identificar indivíduos carreadores de mutação em fase pré-clínica da doença, podendo ser ferramentas úteis na identificação daqueles sob maior risco [133,134].

2.7) MicroRNA

Os microRNAs são moléculas que exercem função através da regulação da expressão gênica a nível pós-transcripcional. Atuam por meio de interações com vários sítios de ligação no RNA mensageiro (mRNA), modulando cerca de 30% do código proteico do nosso genoma [14,135]. Os microRNAs podem regular diversos sítios de um mRNA assim como outros microRNAs [14]. Desta forma, possuem papel na modulação da proliferação, diferenciação, desenvolvimento e apoptose celular, com ação fundamental na resposta fisiopatológica ao estresse [136–138]. As publicações relacionadas aos microRNAs sofreram incremento na última década devido ao fato de serem potenciais biomarcador e alvos terapêuticos [139]. Dentre os mais de 2000 identificados, cerca de 200 apresentam associação com doenças cardiovasculares [139,140]. Os microRNAs podem ser detectados tanto em tecidos como em sangue periférico, no qual encontram-se ligados a complexos lipoproteicos, em vesículas extracelulares ou livres no plasma [14]. Assim, atuam na modulação da transcrição gênica em sítios distantes, demonstrando sua

função parácrina. Diversos métodos foram descritos para sua quantificação, mas a técnica de *polymerase chain reaction* (PCR) em tempo real é mais utilizada [139,141]. Técnica de microarranjo também pode ser empregada na qual diversos microRNAs podem ser quantificados em uma única amostra, embora resulte em menor acurácia e maior custo [139,142]. Esta técnica limita-se por utilizar *primers* pré-definidos, ou seja, não são capazes de identificar novos microRNAs. Os resultados são analisados de forma relativa e não com unidade quantitativa, utilizando-se uma amostra pareada com a dosagem de microRNA não-humano [139].

Em estudo recente, a combinação de 8 microRNAs associada ao biomarcador NT-pro-BNP possibilitou a identificação de pacientes com insuficiência cardíaca com fração de ejeção preservada (ICFEP) quando comparados àqueles com fração de ejeção reduzida [143]. A nível molecular, estes resultados corroboram diferenças na patogênese destas duas formas de insuficiência cardíaca. Além disso, a introdução de novos biomarcadores, além dos peptídeos natriuréticos, poderiam auxiliar na definição diagnóstica mais precisa da ICFEP, tão discutida atualmente [144]. Na insuficiência cardíaca aguda, identificou-se que níveis reduzidos de mir-423-5p se associaram a pior prognóstico, mas esta relação não foi verificada em pacientes com insuficiência cardíaca compensada [145]. Este achado nos permite a hipótese de que os microRNAs possuem diversos padrões de expressão, ou seja, um conjunto de microRNAs podem estar expressos apenas na fase aguda e outros cronicamente expressos.

Alguns microRNAs tem sido amplamente estudados no contexto das doenças cardiovasculares. O mir-133 apresenta maior densidade nos cardiomiócitos, mas também está presente no músculo esquelético. Este microRNA atua na proliferação de mioblastos com ação anti-apoptótica [146,147]. Sua expressão encontra-se reduzida na presença de hipertrofia ventricular em modelo animal, em indivíduos com CMH e no infarto agudo do miocárdio[148].

Todavia, em níveis elevados, relaciona-se à melhora da função cardíaca [146–148]. Por sua vez, o mir-1, transcrito no mesmo loci que o mrR-133, apresenta papel pró-apoptótico e atua na diferenciação miofibroblástica, opondo-se ao mir-133 [148]. Igualmente abundante, o mir-1 encontra-se reduzido em pacientes com insuficiência cardíaca. Está relacionado a vias de ativação dos fatores de crescimento relacionados à insulina de forma a contribuir na gênese da hipertrofia dos miócitos e desenvolvimento de arritmias [139,149].

2.8) MicroRNAs na CMH

A CMH tem sido tema de estudos que objetivam caracterizar o perfil de expressão dos microRNAs envolvidos em sua fisiopatologia [15,16,150]. Nos casos associados à fibrose miocárdica difusa, foi verificada elevação sérica de 14 microRNAS circulantes, atribuída à super-regulação consequente ao estresse miocárdico [16]. No entanto, somente a expressão conjunta de oito destas moléculas evidenciou alto valor preditivo para identificação e quantificação de fibrose difusa avaliada por RNM cardíaca através de realce tardio, com melhor performance do que outros biomarcadores como os pró-colágenos tipo I e III [16]. Em análise recente, foi constatada elevação dos níveis séricos de 12 subtipos nesta doença: mir-27a, mir-199a-5p, mir-26a, mir-143, mir-199a-3p, mir-29a, mir-145, mir-133a, mir-126-3p, mir-155, mir-30a e mir-21. Apenas os mir-27a, mir-29a e mir-199a-5p correlacionaram-se com hipertrofia, determinada ao ecocardiograma transtorácico e RNM cardíaca, em quatro segmentos distintos da parede ventricular esquerda. No entanto, apenas o mir-29a e mir-21 evidenciaram associação específica tanto com fibrose quanto com hipertrofia, o que os tornam potenciais biomarcadores para avaliação do remodelamento miocárdico nesta doença [15]. Tais resultados corroboram dados obtidos em análise subsequente que evidenciou correlação da expressão de mir-29a com áreas de fibrose difusa [16]. Ainda, identificou-se que carreadores de mutações em *MYH7* e *MYBPC3* apresentam distintos padrões de

expressão destas moléculas. O mir-29a circulante mostrou-se elevado naqueles com mutação de *MYH7*, enquanto que o mir-155 estava reduzido nos casos com acometimento de *MYBPC3* [150]. No entanto, estes resultados ainda não são conclusivos para determinar um padrão específico gene-microRNA.

O mir-29a, situado no cromossomo 7, posição 7q32.3, tem sua expressão ligada à regulação da síntese de proteínas da matriz extracelular e ao processo de apoptose [151,152]. A expressão de mir-29a não é exclusiva ao miócito, mas possui íntima relação com expressão de fibrose em diversos tecidos, como demonstrado em indivíduos com esclerose sistêmica [153]. No miocárdio, foi demonstrado ser suprimido através de expressão de TGF-β. Está relacionado a uma série de genes, como o DNMT3A (*DNA methyltransferase enzymes A*), envolvidos na expressão de substâncias como colágeno, fibrilinas e elastina e resultam em ativação de fibroblastos e desenvolvimento de fibrose [142,152,154]. Uma coorte de quase 6.000 indivíduos com doença arterial coronariana identificou associação da expressão de mir-29a com a ocorrência de morte súbita. Embora estes indivíduos não tenham se submetido à RNM para detecção de fibrose, especula-se que estes resultados se devam à relação do mir-29a com ativação de fibroblastos [155]. Em pacientes com CMH, identifica-se supra-regulação do mir-29a e correlação com aumento da espessura parietal máxima do VE avaliada tanto ao ecocardiograma quanto à RNM [15,16,150,156]. Associa-se igualmente com a fibrose detectada através de técnica de realce tardio à RNM [15,16,156]. Em comparação a outros biomarcadores como a troponina C ultrassensível e o peptídeo natriurético tipo B estudados previamente na CMH, o mir-29a apresenta maior valor preditivo para detecção de fibrose miocárdica [15,16,157].

O mir-21 é músculo específico (estriado e esquelético), considerado o mais abundante no miocárdio [158]. Sua função está relacionada a ação anti-apoptótica e ao crescimento celular [158].

Assim como o mir-29a, o mir-21 apresenta papel fundamental na gênese da fibrose miocárdica através de ativação de fibroblastos cardíacos via TGF-β1 [159]. Foi identificado mecanismo de *feedback* entre TGF-β1 e mir-21, havendo ativação recíproca entre ambos [160,161]. Em modelo de isquemia miocárdica, o mir-21 apresentou expressão reduzida em áreas infartadas, supra-regulação em regiões limítrofes e associação com a presença de fibrose pós-infarto intermediada por TGF-β1 [162,163]. Além disso, apresentou relação com vias relacionadas à hipertrofia ventricular esquerda em modelo animal [161]. Em estudos clínicos, o mir-21 demonstrou supra-regulação em indivíduos com cardiomiopatia dilatada, CMH e fibrilação atrial [15,143,164]. Sua expressão apresenta correlação com grau de hipertrofia no ecocardiograma transtorácico e presença de fibrose à RNM na CMH [15,16].

A elucidação das bases moleculares da CMH, através do estudo dos microRNAs, possibilitaria a introdução de novas estratégias diagnósticas e terapêuticas desenvolvidas com intuito de modificar a história natural da doença e favorecer a detecção precoce. Os miR-29a e miR-21 destacam-se como biomarcadores com funções reguladoras relacionadas simultaneamente aos processos de hipertrofia e fibrose [15,16]. Deste modo, poderão ser futuramente utilizados para rastreamento da doença, ou ainda como marcadores de prognósticos ou alvos terapêuticos.

3. JUSTIFICATIVA E OBJETIVOS

A CMH é a doença cardiovascular de origem genética mais prevalente e o conhecimento de suas bases moleculares avançou sensivelmente nas últimas décadas. O rastreamento da CMH entre familiares de primeiro grau encontra-se recomendado em diretrizes internacionais com base em estudos genéticos. Contudo, estes não se encontram disponíveis em algumas regiões. Além disso, em cerca de metade das famílias não há identificação de mutação causadora. Assim, faz-se necessário o seguimento clínico destes familiares por longos períodos. Ainda não está estabelecida qual a melhor estratégia de identificação e acompanhamento destes familiares nestes cenários.

Os processos moleculares responsáveis pela modulação da expressão clínica nesses indivíduos ainda necessitam elucidação. Nos últimos anos, alguns fatores ambientais foram reconhecidos como prováveis moduladores da expressão fenotípica da CMH, mas não a explicam integralmente. Os microRNAs, como moléculas reguladoras da expressão gênica, estão relacionados com a presença de hipertrofia do VE e fibrose detectada à RNM cardíaca e podem representar fatores moduladores das distintas expressões fenotípicas.

Na CMH, os miR-29a e miR-21 apresentam relação tanto com hipertrofia miocárdica quanto com fibrose em pacientes com doença expressa. Seu desempenho em formas pré-clínicas ainda é desconhecido. A ampliação do entendimento dos microRNAs nas bases moleculares da doença poderá ter um efeito decisivo no desenvolvimento de novos alvos terapêuticos, assim como na identificação precoce de indivíduos com CMH.

Em decorrência do exposto acima, este projeto tem como objetivos principais:

a. Realizar análise genético-molecular através de painel de mutações em pacientes com CMH.

b. Avaliar estratégia de rastreamento de familiares em primeiro grau através de avaliação clínica, eletrocardiográfica e ecocardiográfica para identificação da CMH.

c. Por meio de uma revisão sistemática, avaliar a literatura existente sobre o padrão de expressão dos microRNAs em pacientes com CMH, sua correlação fenotípica e seu potencial como biomarcadores da doença.

4. REFERÊNCIAS DA REVISÃO DA LITERATURA

[1] Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol 2015;65:1249–54. https://doi.org/10.1016/j.jacc.2015.01.019.

[2] Authors/Task Force members, Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J 2014;35:2733–79. https://doi.org/10.1093/eurheartj/ehu284.

[3] Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, et al. 2020 AHA/ACC guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy. Circulation 2020:Epub ahead of print. https://doi.org/10.1161/CIR.0000000000000937.

[4] Neubauer S, Kolm P, Ho CY, Kwong RY, Desai MY, Dolman SF, et al. Distinct subgroups in hypertrophic cardiomyopathy in the NHLBI HCM registry. J Am Coll Cardiol 2019;74:2333–45. https://doi.org/10.1016/j.jacc.2019.08.1057.

[5] Finocchiaro G, Magavern E, Sinagra G, Ashley E, Papadakis M, Tome-Esteban M, et al. Impact of demographic features, lifestyle, and comorbidities on the clinical expression of hypertrophic cardiomyopathy. J Am Heart Assoc 2017;6:1–11. https://doi.org/10.1161/JAHA.117.007161.

[6] Grace A. Sudden death in hypertrophic cardiomyopathy is rare. Circulation 2019;140:1717–9. https://doi.org/10.1161/CIRCULATIONAHA.119.043525.

[7] Maron MS, Maron BJ, Harrigan C, Buros J, Gibson CM, Olivotto I, et al. Hypertrophic cardiomyopathy phenotype revisited after 50 years with cardiovascular magnetic resonance. J Am Coll Cardiol 2009;54:220–8. https://doi.org/10.1016/j.jacc.2009.05.006.

[8] Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: Clinical perspectives. J Am Coll Cardiol 2012;60:705–15. https://doi.org/10.1016/j.jacc.2012.02.068.

[9] Ho CY, Charron P, Richard P, Girolami F, Van Spaendonck-Zwarts KY, Pinto Y. Genetic advances in sarcomeric cardiomyopathies: state of the art. Cardiovasc Res 2015;105:397–408. https://doi.org/10.1093/cvr/cvv025.

[10] Mogensen J, Van Tintelen JP, Fokstuen S, Elliott P, Van Langen IM, Meder B, et al. The current role of next-generation DNA sequencing in routine care of patients with hereditary cardiovascular conditions: A viewpoint paper of the European Society of Cardiology working group on myocardial and pericardial diseases and members of the Europ. Eur Heart J 2015;36:1367–70. https://doi.org/10.1093/eurheartj/ehv122.

[11] Sen-chowdhry S, Jacoby D, Moon JC, Mckenna WJ. Update on hypertrophic. Nat Publ Gr 2016;13:651–75. https://doi.org/10.1038/nrcardio.2016.140.

[12] Gersh B, BJ Maron, RO Bonow, JA Dearani, MA Fifer, MS Link, et al. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy. J Thorac Cardiovasc Surg 2011;142:e153–203. https://doi.org/10.1016/j.jtcvs.2011.10.020.

[13] Olivotto I, D'Amati G, Basso C, Van Rossum A, Patten M, Emdin M, et al. Defining phenotypes and disease progression in sarcomeric cardiomyopathies: contemporary role of clinical investigations. Cardiovasc Res 2015;105:409–23.

https://doi.org/10.1093/cvr/cvv024.

[14] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008;9:102–14. https://doi.org/10.1038/nrg2290.

[15] Roncarati R, Anselmi V, Losi A, Papa L, Cavarretta E, Costa D, et al. Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2014;63:920–7. https://doi.org/10.1016/j.jacc.2013.09.041.

[16] Fang L, Ellims AH, Moore X, White DA, Taylor AJ, Chin-Dusting J, et al. Circulating microRNAs as biomarkers for diffuse myocardial fibrosis in patients with hypertrophic cardiomyopathy. J Transl Med 2015;13:314. https://doi.org/10.1186/s12967-015-0672-0.

[17] Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: Genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ Res 2017;121:749–70. https://doi.org/10.1161/CIRCRESAHA.117.311059.

[18] Eberly LA, Day SM, Ashley EA, Jacoby DL, Jefferies JL, Colan SD, et al. Association of race with disease expression and clinical outcomes among patients with hypertrophic cardiomyopathy. JAMA Cardiol 2020;5:83–91. https://doi.org/10.1001/jamacardio.2019.4638.

[19] Elliott PM, Anastasakis, Aris, Michael A Borger MB, Cecchi F, Charron P, Alain A, Hagege, Lafont AHM, et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy. Eur Heart J 2014;35:2733–79. https://doi.org/10.1093/eurheartj/ehu284.

[20] Varnava AM, Elliott PM, Sharma S, McKenna WJ, Davies MJ. Hypertrophic cardiomyopathy: The interrelation of disarray, fibrosis and small vessel disease. Heart 2000;84:476–82. https://doi.org/10.1136/heart.84.5.476.

[21] Maron BJ, Ferrans VJ, Henry WL, Clark CE, Redwood DR, Roberts WC, et al. Differences in distribution of myocardial abnormalities in patients with obstructive and nonobstructive asymmetric septal hypertrophy (ASH). Light and electron microscopic findings. Circulation 1974;50:436–46. https://doi.org/10.1161/01.cir.50.3.436.

[22] Williams L, Rakowski H. Predicting the future in hypertrophic cardiomyopathy: From histopathology to flow to function. J Am Soc Echocardiogr 2012;25:190–3. https://doi.org/10.1016/j.echo.2011.12.017.

[23] Petersen SE, Jerosch-Herold M, Hudsmith LE, Robson MD, Francis JM, Doll HA, et al. Evidence for microvascular dysfunction in hypertrophic cardiomyopathy: new insights from multiparametric magnetic resonance imaging. Circulation 2007;115:2418–25. https://doi.org/10.1161/CIRCULATIONAHA.106.657023.

[24] Sewanan LR, Schwan J, Kluger J, Park J, Jacoby DL, Qyang Y, et al. Extracellular matrix from hypertrophic myocardium provokes impaired twitch dynamics in healthy cardiomyocytes. JACC Basic to Transl Sci 2019;4:495–505. https://doi.org/10.1016/j.jacbts.2019.03.004.

[25] Marian AJ. Role of the extracellular matrix in the pathogenesis of hypertrophic cardiomyopathy. JACC Basic to Transl Sci 2019;4:506–8. https://doi.org/10.1016/j.jacbts.2019.03.011.

[26] Green EM, Wakimoto H, Anderson RL, Evanchik MJ, Gorham JM, Harrison BC, et al. A

small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. Science 2016;351:617–21. https://doi.org/10.1126/science.aad3456.

[27] Maron BJ, Ommen SR, Semsarian C, Spirito P, Olivotto I, Maron MS. Hypertrophic cardiomyopathy: Present and future, with translation into contemporary cardiovascular medicine. J Am Coll Cardiol 2014;64:83–99. https://doi.org/10.1016/j.jacc.2014.05.003.

[28] Maron MS, Olivotto I, Zenovich AG, Link MS, Pandian NG, Kuvin JT, et al. Hypertrophic cardiomyopathy is predominantly a disease of left ventricular outflow tract obstruction. Circulation 2006;114:2232–9. https://doi.org/10.1161/CIRCULATIONAHA.106.644682.

[29] Shah JS, Esteban MTT, Thaman R, Sharma R, Mist B, Pantazis a, et al. Prevalence of exercise-induced left ventricular outflow tract obstruction in symptomatic patients with non-obstructive hypertrophic cardiomyopathy. Heart 2008;94:1288–94. https://doi.org/10.1136/hrt.2007.126003.

[30] Sherrid M V., Wever-Pinzon O, Shah A, Chaudhry FA. Reflections of inflections in hypertrophic cardiomyopathy. J Am Coll Cardiol 2009;54:212–9. https://doi.org/10.1016/j.jacc.2009.03.052.

[31] Maron MS, Olivotto I, Harrigan C, Appelbaum E, Gibson CM, Lesser JR, et al. Mitral valve abnormalities identified by cardiovascular magnetic resonance represent a primary phenotypic expression of hypertrophic cardiomyopathy. Circulation 2011;124:40–7. https://doi.org/10.1161/CIRCULATIONAHA.110.985812.

[32] Sherrid M V., Balaram S, Kim B, Axel L, Swistel DG. The mitral valve in obstructive

hypertrophic cardiomyopathy: A test in context. J Am Coll Cardiol 2016;67:1846–58. https://doi.org/10.1016/j.jacc.2016.01.071.

[33] Tower-Rader A, Betancor J, Lever HM, Desai MY. A comprehensive review of stress testing in hypertrophic cardiomyopathy: Assessment of functional capacity, identification of prognostic indicators, and detection of coronary artery disease. J Am Soc Echocardiogr 2017;30:829–44. https://doi.org/10.1016/j.echo.2017.05.017.

[34] Rowin EJ, Maron BJ, Maron MS. The hypertrophic cardiomyopathy phenotype viewed through the prism of multimodality imaging: Clinical and etiologic implications. JACC Cardiovasc Imaging 2020;13:2002–16. https://doi.org/10.1016/j.jcmg.2019.09.020.

[35] Mattos BP, Torres MAR, Rebelatto TF, de Loreto MS, Scolari FL. The diagnosis of left ventricular outflow tract obstruction in hypertrophic cardiomyopathy. Arq Bras Cardiol 2012;99:665–75. https://doi.org/10.1590/S0066-782X2012000700013.

[36] Ayoub C, Geske JB, Larsen CM, Scott CG, Klarich KW, Pellikka PA. Comparison of Valsalva maneuver, amyl nitrite, and exercise echocardiography to demonstrate latent left ventricular outflow obstruction in hypertrophic cardiomyopathy. Am J Cardiol 2017;120:2265–71. https://doi.org/10.1016/j.amjcard.2017.08.047.

[37] Klues HG, Proschan MA, Dollar AL, Spirito P, Roberts WC, Maron BJ. Echocardiographic assessment of mitral valve size in obstructive hypertrophic cardiomyopathy: Anatomic validation from mitral valve specimen. Circulation 1993;88:548–55. https://doi.org/10.1161/01.CIR.88.2.548.

[38] Tarkiainen M, Sipola P, Jalanko M, Heliö T, Laine M, Järvinen V, et al. Cardiovascular magnetic resonance of mitral valve length in hypertrophic cardiomyopathy. J Cardiovasc

Magn Reson 2016;18:33. https://doi.org/10.1186/s12968-016-0250-5.

[39] Chung H, Kim Y, Park CH, Kim JY, Min PK, Yoon YW, et al. Genetic relevance and determinants of mitral leaflet size in hypertrophic cardiomyopathy. Cardiovasc Ultrasound 2019;17:21. https://doi.org/10.1186/s12947-019-0171-1.

[40] Hindieh W, Weissler-Snir A, Hammer H, Adler A, Rakowski H, Chan RH. Discrepant measurements of maximal left ventricular wall thickness between cardiac magnetic resonance imaging and echocardiography in patients with hypertrophic cardiomyopathy. Circ Cardiovasc Imaging 2017;10:1–9. https://doi.org/10.1161/CIRCIMAGING.117.006309.

[41] Moon JCC, Fisher NG, McKenna WJ, Pennell DJ. Detection of apical hypertrophic cardiomyopathy by cardiovascular magnetic resonance in patients with non-diagnostic echocardiography. Heart 2004;90:645–9. https://doi.org/10.1136/hrt.2003.014969.

[42] Corona-Villalobos CP, Sorensen LL, Pozios I, Chu L, Eng J, Abraham MR, et al. Left ventricular wall thickness in patients with hypertrophic cardiomyopathy: a comparison between cardiac magnetic resonance imaging and echocardiography. Int J Cardiovasc Imaging 2016;32:945–54. https://doi.org/10.1007/s10554-016-0858-4.

[43] Scolari FL, Mattos BP, Garbin HI, Bellagamba CC, Ecco G, Barcellos PA MD. Discordância entre as medidas das espessuras parietais do ventrículo esquerdo avaliadas através do ecocardiograma e ressonância magnética na cardiomiopatia hipertrófica - Resumo das Comunicações SOCERGS 2019 Congresso da Sociedade de Cardiologia do Estado. Arq Bras Cardiol 2019;113:1.

[44] Bois JP, Geske JB, Foley TA, Ommen SR, Pellikka PA. Comparison of maximal wall

thickness in hypertrophic cardiomyopathy differs between magnetic resonance imaging and transthoracic echocardiography. Am J Cardiol 2017;119:643–50. https://doi.org/10.1016/j.amjcard.2016.11.010.

[45] Weng Z, Yao J, Chan RH, He J, Yang X, Zhou Y, et al. Prognostic value of LGE-CMR in HCM: A meta-analysis. JACC Cardiovasc Imaging 2016;9:1392–402. https://doi.org/10.1016/j.jcmg.2016.02.031.

[46] Bittencourt MI, Cader SA, Araújo DV, Salles ALF, de Albuquerque FN, Spineti PP de M, et al. Role of myocardial fibrosis in hypertrophic cardiomyopathy: A systematic review and updated meta-analysis of risk markers for sudden death. Arq Bras Cardiol 2019;112:281–9. https://doi.org/10.5935/abc.20190045.

[47] Minegishi S, Kato S, Takase-Minegishi K, Horita N, Azushima K, Wakui H, et al. Native T1 time and extracellular volume fraction in differentiation of normal myocardium from non-ischemic dilated and hypertrophic cardiomyopathy myocardium: A systematic review and meta-analysis. IJC Heart Vasc 2019;25:100422. https://doi.org/10.1016/j.ijcha.2019.100422.

[48] Xu J, Zhuang B, Sirajuddin A, Li S, Huang J, Yin G, et al. MRI T1 mapping in hypertrophic cardiomyopathy: evaluation in patients without late gadolinium enhancement and hemodynamic obstruction. Radiology 2019;294:275–86. https://doi.org/10.1148/radiol.2019190651.

[49] Ho CY, Abbasi SA, Neilan TG, Shah R V, Chen Y, Heydari B, et al. T1 measurements identify extracellular volume expansion in hypertrophic cardiomyopathy sarcomere mutation carriers with and without left ventricular hypertrophy. Circ Cardiovasc Imaging

2013;6:415–22. https://doi.org/10.1161/CIRCIMAGING.112.000333.

[50] Treibel TA, Fridman Y, Bering P, Sayeed A, Maanja M, Frojdh F, et al. Extracellular volume associates with outcomes more strongly than native or post-contrast myocardial T1. JACC Cardiovasc Imaging 2020;13:44–54. https://doi.org/10.1016/j.jcmg.2019.03.017.

[51] Hinojar R, Varma N, Child N, Goodman B, Jabbour A, Yu CY, et al. T1 mapping in discrimination of hypertrophic phenotypes: hypertensive heart disease and hypertrophic cardiomyopathy: Findings from the international T1 multicenter cardiovascular magnetic resonance study. Circ Cardiovasc Imaging 2015;8:1–9. https://doi.org/10.1161/CIRCIMAGING.115.003285.

[52] Neisius U, Myerson L, Fahmy AS, Nakamori S, El-Rewaidy H, Joshi G, et al. Cardiovascular magnetic resonance feature tracking strain analysis for discrimination between hypertensive heart disease and hypertrophic cardiomyopathy. PLoS One 2019;14:1–18. https://doi.org/10.1371/journal.pone.0221061.

[53] Canepa M, Pozios I, Vianello PF, Ameri P, Brunelli C, Ferrucci L, et al. Distinguishing ventricular septal bulge versus hypertrophic cardiomyopathy in the elderly. Heart 2016;102:1087–94. https://doi.org/10.1136/heartjnl-2015-308764.

[54] Binder J, Ommen SR, Gersh BJ, Van Driest SL, Tajik AJ, Nishimura RA, et al. Echocardiography-guided genetic testing in hypertrophic cardiomyopathy: Septal morphological features predict the presence of myofilament mutations. Mayo Clin Proc 2006;81:459–67. https://doi.org/10.4065/81.4.459.

[55] Turer AT, Samad Z, Valente AM, Parker MA, Hayes B, Kim RJ, et al. Anatomic and

clinical correlates of septal morphology in hypertrophic cardiomyopathy. Eur J Echocardiogr 2011;12:131–9. https://doi.org/10.1093/ejechocard/jeq163.

[56] Maron BJ, Rowin EJ, Casey SA, Link MS, Lesser JR, Chan RHM, et al. Hypertrophic cardiomyopathy in adulthood associated With low cardiovascular mortality with contemporary management strategies. J Am Coll Cardiol 2015;65:1915–28. https://doi.org/10.1016/j.jacc.2015.02.061.

[57] Geske JB, Ong KC, Siontis KC, Hebl VB, Ackerman MJ, Hodge DO, et al. Women with hypertrophic cardiomyopathy have worse survival. Eur Heart J 2017;38:3434–40. https://doi.org/10.1093/eurheartj/ehx527.

[58] Ghiselli L, Marchi A, Fumagalli C, Maurizi N, Oddo A, Pieri F, et al. Sex-related differences in exercise performance and outcome of patients with hypertrophic cardiomyopathy. Eur J Prev Cardiol 2020;27:1821–31. https://doi.org/10.1177/2047487319886961.

[59] Lorenzini M, Anastasiou Z, O'Mahony C, Guttman OP, Gimeno JR, Monserrat L, et al. Mortality among referral patients with hypertrophic cardiomyopathy vs the general european population. JAMA Cardiol 2020;5:73–80. https://doi.org/10.1001/jamacardio.2019.4534.

[60] Lu DY, Ventoulis I, Liu H, Kudchadkar SM, Greenland G V., Yalcin H, et al. Sex-specific cardiac phenotype and clinical outcomes in patients with hypertrophic cardiomyopathy. Am Heart J 2020;219:58–69. https://doi.org/10.1016/j.ahj.2019.10.004.

[61] Constantine A, Dimopoulos K, Rafiq I, Vazir A. Sex differences in hypertrophic cardiomyopathy: Time to tailor risk stratification and therapy? Eur J Prev Cardiol

2020;27:1816–8. https://doi.org/10.1177/2047487319890996.

[62] Fumagalli C, Maurizi N, Day SM, Ashley EA, Michels M, Colan SD, et al. Association of obesity with adverse long-term outcomes in hypertrophic cardiomyopathy. JAMA Cardiol 2020;5:65–72. https://doi.org/10.1001/jamacardio.2019.4268.

[63] Park J-B, Kim DH, Lee H, Hwang I-C, Yoon YE, Park HE, et al. Obesity and metabolic health status are determinants for the clinical expression of hypertrophic cardiomyopathy. Eur J Prev Cardiol 2020;27:1849–57. https://doi.org/10.1177/2047487319889714.

[64] Reineck E, Rolston B, Bragg-Gresham JL, Salberg L, Baty L, Kumar S, et al. Physical activity and other health behaviors in adults with hypertrophic cardiomyopathy. Am J Cardiol 2013;111:1034–9. https://doi.org/10.1016/j.amjcard.2012.12.018.

[65] Mattos BP, Torres MAR, Freitas VC, Scolari FL, Loreto MS. Ventricular arrhythmias and left ventricular hypertrophy in hypertrophic cardiomyopathy. Arq Bras Cardiol 2013;100:452–9. https://doi.org/10.5935/abc.20130078.

[66] Sivalokanathan S, Zghaib T, Greenland G V., Vasquez N, Kudchadkar SM, Kontari E, et al. Hypertrophic cardiomyopathy patients with paroxysmal atrial fibrillation have a high burden of left atrial fibrosis by cardiac magnetic resonance imaging. JACC Clin Electrophysiol 2019;5:364–75. https://doi.org/10.1016/j.jacep.2018.10.016.

[67] Maron BJ, Rowin EJ, Casey SA, Link MS, Lesser JR, Chan RHM, et al. Hypertrophic cardiomyopathy in adulthood associated with low cardiovascular mortality with contemporary management strategies. J Am Coll Cardiol 2015;65:1915–28. https://doi.org/10.1016/j.jacc.2015.02.061.

[68] Maron BJ, Rowin EJ, Maron MS. Paradigm of sudden death prevention in hypertrophic cardiomyopathy. Circ Res 2019;125:370–8. https://doi.org/10.1161/CIRCRESAHA.119.315159.

[69] Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. N Engl J Med 2018;379:655–68. https://doi.org/10.1056/nejmra1710575.

[70] Mattos BP e, Scolari FL, Garbin HI. Discordância entre diretrizes internacionais sobre critérios de prevenção primária de morte súbita na cardiomiopatia hipertrófica. Arq Bras Cardiol 2020;115:197–204. https://doi.org/10.36660/abc.20190161.

[71] Axelsson A, Iversen K, Vejlstrup N, Ho C, Norsk J, Langhoff L, et al. Efficacy and safety of the angiotensin II receptor blocker losartan for hypertrophic cardiomyopathy: The INHERIT randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol 2015;3:123–31. https://doi.org/10.1016/S2213-8587(14)70241-4.

[72] Axelsson A, Iversen K, Vejlstrup N, Ho CY, Havndrup O, Kofoed KF, et al. Functional effects of losartan in hypertrophic cardiomyopathy-A randomised clinical trial. Heart 2016;102:285–91. https://doi.org/10.1136/heartjnl-2015-308343.

[73] Ho CY, Lakdawala NK, Cirino AL, Lipshultz SE, Sparks E, Abbasi SA, et al. Diltiazem treatment for pre-clinical hypertrophic cardiomyopathy sarcomereMutation carriers: A pilot randomized trial to modify disease expression. JACC Heart Fail 2015;3:180–8. https://doi.org/10.1016/j.jchf.2014.08.003.

[74] Olivotto I, Oreziak A, Barriales-Villa R, Abraham TP, Masri A, Garcia-Pavia P, et al. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial.

Lancet 2020;396:759–69. https://doi.org/10.1016/S0140-6736(20)31792-X.

[75] Kawas RF, Anderson RL, Bartholomew Ingle SR, Song Y, Sran AS, Rodriguez HM. A small-molecule modulator of cardiac myosin acts on multiple stages of the myosin chemomechanical cycle. J Biol Chem 2017;292:16571–7. https://doi.org/10.1074/jbc.M117.776815.

[76] Anderson RL, Trivedi D V., Sarkar SS, Henze M, Ma W, Gong H, et al. Deciphering the super relaxed state of human β-cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers. Proc Natl Acad Sci U S A 2018;115:E8143–52. https://doi.org/10.1073/pnas.1809540115.

[77] Maron BJ, Dearani JA, Ommen SR, Maron MS, Schaff H V, Nishimura RA, et al. Low operative mortality achieved with surgical septal myectomy: role of dedicated hypertrophic cardiomyopathy centers in the management of dynamic subaortic obstruction. J Am Coll Cardiol 2015;66:1307–8. https://doi.org/10.1016/j.jacc.2015.06.1333.

[78] Shimada YJ, Goto T, Takayama H, Brown DFM, Homma S, Maurer MS, et al. Comparison of effectiveness of alcohol septal ablation versus ventricular septal myectomy on acute care use for cardiovascular disease in patients with hypertrophic cardiomyopathy. Am J Cardiol 2019;124:1272–8. https://doi.org/10.1016/j.amjcard.2019.07.031.

[79] Nishimura RA, Seggewiss H, Schaff H V. Hypertrophic obstructive cardiomyopathy: Surgical myectomy and septal ablation. Circ Res 2017;121:771–83. https://doi.org/10.1161/CIRCRESAHA.116.309348.

[80] Cui H, Schaff H V., Nishimura RA, Geske JB, Dearani JA, Lahr BD, et al. Conduction

abnormalities and long-term mortality following septal myectomy in patients with obstructive hypertrophic cardiomyopathy. J Am Coll Cardiol 2019;74:645–55. https://doi.org/10.1016/j.jacc.2019.05.053.

[81] Batzner A, Pfeiffer B, Neugebauer A, Aicha D, Blank C, Seggewiss H. Survival after alcohol septal ablation in patients with hypertrophic obstructive cardiomyopathy. J Am Coll Cardiol 2018;72:3087–94. https://doi.org/10.1016/j.jacc.2018.09.064.

[82] Faber L, Welge D, Fassbender D, Schmidt HK, Horstkotte D, Seggewiss H. Percutaneous septal ablation for symptomatic hypertrophic obstructive cardiomyopathy: managing the risk of procedure-related AV conduction disturbances. Int J Cardiol 2007;119:163–7. https://doi.org/10.1016/j.ijcard.2006.07.179.

[83] Kashtanov MG, Rzhannikova AD, Chernyshev SD, Kardapoltsev L V., Idov EM, Berdnikov S V., et al. Outcomes of hypertrophic obstructive cardiomyopathy patients undergoing alcohol septal ablation with a standard 3 mL dose of ethanol: Focus on long-term safety. Catheter Cardiovasc Interv 2020;95:1212–8. https://doi.org/10.1002/ccd.28499.

[84] Geske JB, Ommen SR, Gersh BJ. Hypertrophic cardiomyopathy: clinical update. JACC Heart Fail 2018;6:364–75. https://doi.org/10.1016/j.jchf.2018.02.010.

[85] Sikkema-Raddatz B, Johansson LF, de Boer EN, Almomani R, Boven LG, van den Berg MP, et al. Targeted next-generation sequencing can replace Sanger sequencing in clinical diagnostics. Hum Mutat 2013;34:1035–42. https://doi.org/10.1002/humu.22332.

[86] Cirino AL, Lakdawala NK, McDonough B, Conner L, Adler D, Weinfeld M, et al. A Comparison of whole genome sequencing to multigene panel testing in hypertrophic

cardiomyopathy patients. Circ Cardiovasc Genet 2017;10:e001768. https://doi.org/10.1161/CIRCGENETICS.117.001768.

[87] Cirino AL, Seidman CE, Ho CY. Genetic testing and counseling for hypertrophic cardiomyopathy. Cardiol Clin 2019;37:35–43. https://doi.org/10.1016/j.ccl.2018.08.003.

[88] Furqan A, Arscott P, Girolami F, Cirino AL, Michels M, Day SM, et al. Care in specialized centers and data sharing increase agreement in hypertrophic cardiomyopathy genetic test interpretation. Circ Cardiovasc Genet 2017;10:e001700. https://doi.org/10.1161/CIRCGENETICS.116.001700.

[89] Ingles J, Burns C, Barratt A, Semsarian C. Application of genetic testing in hypertrophic cardiomyopathy for preclinical disease detection. Circ Cardiovasc Genet 2015;8:852–9. https://doi.org/10.1161/CIRCGENETICS.115.001093.

[90] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24. https://doi.org/10.1038/gim.2015.30.

[91] Alfares AA, Kelly MA, McDermott G, Funke BH, Lebo MS, Baxter SB, et al. Results of clinical genetic testing of 2,912 probands with hypertrophic cardiomyopathy: expanded panels offer limited additional sensitivity. Genet Med 2015;17:880–8. https://doi.org/10.1038/gim.2014.205.

[92] Walsh R, Buchan R, Wilk A, John S, Felkin LE, Thomson KL, et al. Defining the genetic architecture of hypertrophic cardiomyopathy: re-evaluating the role of non-sarcomeric genes. Eur Heart J 2017;38:3461–8. https://doi.org/10.1093/eurheartj/ehw603.

[93] Marsiglia JDC, Credidio FL, De Oliveira TGM, Reis RF, Antunes MDO, De Araujo AQ, et al. Screening of MYH7, MYBPC3, and TNNT2 genes in Brazilian patients with hypertrophic cardiomyopathy. Am Heart J 2013;166:775–82. https://doi.org/10.1016/j.ahj.2013.07.029.

[94] Mattos BP, Scolari FL, Torres MAR, Simon L, Freitas VC, Giugliani R, et al. Prevalence and phenotypic expression of mutations in the MYH7, MYBPC3 and TNNT2 genes in families with hypertrophic cardiomyopathy in the south of Brazil: a cross-sectional study. Arq Bras Cardiol 2016;107:257–65. https://doi.org/10.5935/abc.20160133.

[95] Marsiglia JDC, Credidio FL, de Oliveira TGM, Reis RF, Antunes M de O, de Araujo AQ, et al. Clinical predictors of a positive genetic test in hypertrophic cardiomyopathy in the Brazilian population. BMC Cardiovasc Disord 2014;14:1–7. https://doi.org/10.1186/1471-2261-14-36.

[96] Murphy SL, Anderson JH, Kapplinger JD, Kruisselbrink TM, Gersh BJ, Ommen SR, et al. Evaluation of the Mayo clinic phenotype-based genotype predictor score in patients with clinically diagnosed hypertrophic cardiomyopathy. J Cardiovasc Transl Res 2016;9:153–61. https://doi.org/10.1007/s12265-016-9681-5.

[97] Newman R, Jefferies JL, Chin C, He H, Shikany A, Miller EM, et al. Hypertrophic cardiomyopathy genotype prediction models in a pediatric population. Pediatr Cardiol 2018;39:709–17. https://doi.org/10.1007/s00246-018-1810-2.

[98] Lopes LR, Brito D, Belo A, Cardim N. Genetic characterization and genotype-phenotype associations in a large cohort of patients with hypertrophic cardiomyopathy – An ancillary study of the Portuguese registry of hypertrophic cardiomyopathy. Int J Cardiol

2019;278:173–9. https://doi.org/10.1016/j.ijcard.2018.12.012.

[99] Walsh R, Mazzarotto F, Whiffin N, Buchan R, Midwinter W, Wilk A, et al. Quantitative approaches to variant classification increase the yield and precision of genetic testing in Mendelian diseases: The case of hypertrophic cardiomyopathy. Genome Med 2019;11:5. https://doi.org/10.1186/s13073-019-0616-z.

[100] Gruner C, Ivanov J, Care M, Williams L, Moravsky G, Yang H, et al. Toronto hypertrophic cardiomyopathy genotype score for prediction of a positive genotype in hypertrophic cardiomyopathy. Circ Cardiovasc Genet 2013;6:19–26. https://doi.org/10.1161/CIRCGENETICS.112.963363.

[101] McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. Heart 1997;77:130–2. https://doi.org/10.1136/hrt.77.2.130.

[102] Tower-Rader A, Mohananey D, To A, Lever HM, Popovic ZB, Desai MY. Prognostic value of global longitudinal strain in hypertrophic cardiomyopathy: A systematic review of existing literature. JACC Cardiovasc Imaging 2019;12:1930–42. https://doi.org/10.1016/j.jcmg.2018.07.016.

[103] Yiu KH, Atsma DE, Delgado V, Ng ACT, Witkowski TG, Ewe SH, et al. Myocardial structural alteration and systolic dysfunction in preclinical hypertrophic cardiomyopathy mutation carriers. PLoS One 2012;7:e36115. https://doi.org/10.1371/journal.pone.0036115.

[104] Groarke JD, Galazka PZ, Cirino AL, Lakdawala NK, Thune JJ, Bundgaard H, et al. Intrinsic mitral valve alterations in hypertrophic cardiomyopathy sarcomere mutation

carriers. Eur Heart J Cardiovasc Imaging 2018;19:1109–16. https://doi.org/10.1093/ehjci/jey095.

[105] Maron MS, Maron BJ. Clinical impact of contemporary cardiovascular magnetic resonance imaging in hypertrophic cardiomyopathy. Circulation 2015;132:292–8. https://doi.org/10.1161/CIRCULATIONAHA.114.014283.

[106] Ho CY, Cirino AL, Lakdawala NK, Groarke J, Valente AM, Semsarian C, et al. Evolution of hypertrophic cardiomyopathy in sarcomere mutation carriers. Heart 2016;102:1805–12. https://doi.org/10.1136/heartjnl-2016-310015.

[107] Valente AM, Lakdawala NK, Powell AJ, Evans SP, Cirino AL, Orav EJ, et al. Comparison of echocardiographic and cardiac magnetic resonance imaging in hypertrophic cardiomyopathy sarcomere mutation carriers without left ventricular hypertrophy. Circ Cardiovasc Genet 2013;6:230–7. https://doi.org/10.1161/CIRCGENETICS.113.000037.

[108] Ho CY, Day SM, Ashley EA, Michels M, Pereira AC, Jacoby D, et al. Genotype and lifetime burden of disease in hypertrophic cardiomyopathy insights from the sarcomeric human cardiomyopathy registry (SHaRe). Circulation 2018;138:1387–98. https://doi.org/10.1161/CIRCULATIONAHA.117.033200.

[109] van Velzen HG, Schinkel AFL, Oldenburg RA, Van Slegtenhorst MA, Frohn-Mulder IME, Van Der Velden J, et al. Clinical characteristic and long-term outcome of hypertrophic cardiomyopathy in individuals with a MYBPC3 (myosin-binding protein C) founder mutation. Circ Cardiovasc Genet 2017;10:1–8. https://doi.org/10.1161/CIRCGENETICS.116.001660.

[110] Mattos BP, Torres MAR, Freitas VC de. Diagnostic evaluation of hypertrophic cardiomyopathy in its clinical and preclinical phases. Arq Bras Cardiol 2008;91:51–62. https://doi.org/10.1590/s0066-782x2008001300009.

[111] Dejgaard LA, Haland TF, Lie OH, Ribe M, Bjune T, Leren IS, et al. Vigorous exercise in patients with hypertrophic cardiomyopathy. Int J Cardiol 2018;250:157–63. https://doi.org/10.1016/j.ijcard.2017.07.015.

[112] McTaggart DR, Ogden KJ, Marathe JA. A long term follow-up study of carriers of hypertrophic cardiomyopathy mutations. Heart Lung Circ 2017;26:18–24. https://doi.org/10.1016/j.hlc.2016.04.019.

[113] van Velzen HG, Schinkel AFL, Menting ME, van den Bosch AE, Michels M. Prognostic significance of anterior mitral valve leaflet length in individuals with a hypertrophic cardiomyopathy gene mutation without hypertrophic changes. J Ultrasound 2018;21:217–24. https://doi.org/10.1007/s40477-018-0302-9.

[114] Jensen MK, Havndrup O, Christiansen M, Andersen PS, Diness B, Axelsson A, et al. Penetrance of hypertrophic cardiomyopathy in children and adolescents: A 12-year follow-up study of clinical screening and predictive genetic testing. Circulation 2013;127:48–54. https://doi.org/10.1161/CIRCULATIONAHA.111.090514.

[115] Lorenzini M, Norrish G, Field E, Ochoa JP, Cicerchia M, Akhtar MM, et al. Penetrance of hypertrophic cardiomyopathy in sarcomere protein mutation carriers. J Am Coll Cardiol 2020;76:550–9. https://doi.org/10.1016/j.jacc.2020.06.011.

[116] Marian AJ. Hypertrophic cardiomyopathy: From genetics to treatment. Eur J Clin Invest 2010;40:360–9. https://doi.org/10.1111/j.1365-2362.2010.02268.x.

[117] van Velzen HG, Schinkel AFLL, Baart SJ, Oldenburg RA, Frohn-Mulder IMEE, van Slegtenhorst MA, et al. Outcomes of contemporary family screening in hypertrophic cardiomyopathy. Circ Genomic Precis Med 2018;11:e001896. https://doi.org/10.1161/CIRCGEN.117.001896.

[118] Lafreniere-Roula M, Bolkier Y, Zahavich L, Mathew J, George K, Wilson J, et al. Family screening for hypertrophic cardiomyopathy: is it time to change practice guidelines? Eur Heart J 2019;40:3672–81. https://doi.org/10.1093/eurheartj/ehz396.

[119] Christiaans I, Birnie E, Bonsel GJ, Mannens MMAM, Michels M, Majoor-Krakauer D, et al. Manifest disease, risk factors for sudden cardiac death, and cardiac events in a large nationwide cohort of predictively tested hypertrophic cardiomyopathy mutation carriers: Determining the best cardiological screening strategy. Eur Heart J 2011;32:1161–70. https://doi.org/10.1093/eurheartj/ehr092.

[120] Norrish G, Jager J, Field E, Quinn E, Fell H, Lord E, et al. Yield of clinical screening for hypertrophic cardiomyopathy in child first-degree relatives. Circulation 2019;140:184–92. https://doi.org/10.1161/CIRCULATIONAHA.118.038846.

[121] Ko C, Arscott P, Concannon M, Saberi S, Day SM, Yashar BM, et al. Genetic testing impacts the utility of prospective familial screening in hypertrophic cardiomyopathy through identification of a nonfamilial subgroup. Genet Med 2018;20:69–75. https://doi.org/10.1038/gim.2017.79.

[122] Finocchiaro G, Sheikh N, Biagini E, Papadakis M, Maurizi N, Sinagra G, et al. The electrocardiogram in the diagnosis and management of patients with hypertrophic cardiomyopathy. Heart Rhythm 2019;17:142–51.

https://doi.org/10.1016/j.hrthm.2019.07.019.

[123] Delcrè SDL, Di Donna P, Leuzzi S, Miceli S, Bisi M, Scaglione M, et al. Relationship of ECG findings to phenotypic expression in patients with hypertrophic cardiomyopathy: A cardiac magnetic resonance study. Int J Cardiol 2013;167:1038–45. https://doi.org/10.1016/j.ijcard.2012.03.074.

[124] McLeod CJ, Ackerman MJ, Nishimura RA, Tajik AJ, Gersh BJ, Ommen SR. Outcome of patients with hypertrophic cardiomyopathy and a normal electrocardiogram. J Am Coll Cardiol 2009;54:229–33. https://doi.org/10.1016/j.jacc.2009.02.071.

[125] Montgomery J V, Harris KM, Casey SA, Zenovich AG, Maron BJ. Relation of electrocardiographic patterns to phenotypic expression and clinical outcome in hypertrophic cardiomyopathy. Am J Cardiol 2005;96:270–5. https://doi.org/10.1016/j.amjcard.2005.03.058.

[126] Biagini E, Pazzi C, Olivotto I, Musumeci B, Limongelli G, Boriani G, et al. Usefulness of electrocardiographic patterns at presentation to predict long-term risk of cardiac death in patients with hypertrophic cardiomyopathy. Am J Cardiol 2016;118:432–9. https://doi.org/10.1016/j.amjcard.2016.05.023.

[127] Ledieu N, Larnier L, Auffret V, Marie C, Fargeau D, Donal E, et al. Prognostic value of the 12-lead surface electrocardiogram in sarcomeric hypertrophic cardiomyopathy: data from the REMY French register. Europace 2020;22:139–48. https://doi.org/10.1093/europace/euz272.

[128] Ryan MP, Cleland JGF, French JA, Joshi J, Choudhury L, Chojnowska L, et al. The standard electrocardiogram as a screening test for hypertrophic cardiomyopathy. Am J

Cardiol 1995;76:689–94. https://doi.org/10.1016/S0002-9149(99)80198-2.

[129] Lakdawala NK, Thune JJ, Maron BJ, Cirino AL, Havndrup O, Bundgaard H, et al. Electrocardiographic features of sarcomere mutation carriers with and without clinically overt hypertrophic cardiomyopathy. Am J Cardiol 2011;108:1606–13. https://doi.org/10.1016/j.amjcard.2011.07.019.

[130] Ko WY, Siontis KC, Attia ZI, Carter RE, Kapa S, Ommen SR, et al. Detection of hypertrophic cardiomyopathy using a convolutional neural network-enabled electrocardiogram. J Am Coll Cardiol 2020;75:722–33. https://doi.org/10.1016/j.jacc.2019.12.030.

[131] Nagueh SF, Bachinski LL, Meyer D, Hill R, Zoghbi WA, Tam JW, et al. Tissue Doppler imaging consistently detects myocardial abnormalities in patients with hypertrophic cardiomyopathy and provides a novel means for an early diagnosis before and independently of hypertrophy. Circulation 2001;104:128–30.

[132] Cardim N, Perrot A, Ferreira T, Pereira A, Osterziel KJ, Palma Reis R, et al. Usefulness of Doppler myocardial imaging for identification of mutation carriers of familial hypertrophic cardiomyopathy. Am J Cardiol 2002;90:128–32. https://doi.org/10.1016/S0002-9149(02)02434-7.

[133] van Velzen HG, Schinkel AFL, van Grootel RWJ, van Slegtenhorst MA, van der Velden J, Strachinaru M, et al. Five-year prognostic significance of global longitudinal strain in individuals with a hypertrophic cardiomyopathy gene mutation without hypertrophic changes. Netherlands Heart J 2019;27:117–26. https://doi.org/10.1007/s12471-019-1226-5.

[134] Vigneault DM, Yang E, Jensen PJ, Tee MW, Farhad H, Chu L, et al. Left ventricular strain is abnormal in preclinical and overt hypertrophic cardiomyopathy: cardiac MR feature tracking. Radiology 2019;290:640–8. https://doi.org/10.1148/radiol.2018180339.

[135] Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J 2016;37:2315–81. https://doi.org/10.1093/eurheartj/ehw106.

[136] Liu N, Olson EN. MicroRNA regulatory networks in cardiovascular development. Dev Cell 2010;18:510–25. https://doi.org/10.1016/j.devcel.2010.03.010.

[137] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97. https://doi.org/10.1016/S0092-8674(04)00045-5.

[138] Leung AKL, Sharp PA. MicroRNA functions in stress responses. Mol Cell 2010;40:205–15. https://doi.org/10.1016/j.molcel.2010.09.027.

[139] Barwari T, Joshi A, Mayr M. MicroRNAs in cardiovascular disease. J Am Coll Cardiol 2016;68:2577–84. https://doi.org/10.1016/j.jacc.2016.09.945.

[140] Condorelli G, Latronico MVG, Dorn GW. microRNAs in heart disease: putative novel therapeutic targets? Eur Heart J 2010;31:649–58. https://doi.org/10.1093/eurheartj/ehp573.

[141] Rooij E Van. The art of microRNA research. Circ Res 2011;108:219–34. https://doi.org/10.1161/CIRCRESAHA.110.227496.

[142] Thum T, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. Circ Res 2015;116:751–62.

https://doi.org/10.1161/CIRCRESAHA.116.303549.

[143] Li M, Chen X, Chen L, Chen K, Zhou J, Song J. MiR - 1 - 3p that correlates with left ventricular function of HCM can serve as a potential target and differentiate HCM from DCM. J Transl Med 2018;16:161. https://doi.org/10.1186/s12967-018-1534-3.

[144] Shah SJ. The urgent need for biomarkers beyond B-type natriuretic peptide for the diagnosis and management of heart failure with preserved ejection fraction. JAMA Cardiol 2018;3:1211. https://doi.org/10.1001/jamacardio.2018.3702.

[145] Seronde M-F, Vausort M, Gayat E, Goretti E, Ng LL, Squire IB, et al. Circulating microRNAs and outcome in patients with acute heart failure. PLoS One 2015;10:e0142237. https://doi.org/10.1371/journal.pone.0142237.

[146] Carè A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, et al. MicroRNA-133 controls cardiac hypertrophy. Nat Med 2007;13:613–8. https://doi.org/10.1038/nm1582.

[147] Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 2006;38:228–33. https://doi.org/10.1038/ng1725.

[148] Boštjančič E, Zidar N, Štajer D, Glavač D. MicroRNAs miR-1, miR-133a, miR-133b and miR-208 are dysregulated in human myocardial infarction. Cardiology 2010;115:163–9. https://doi.org/10.1159/000268088.

[149] Karakikes I, Chaanine AH, Kang S, Mukete BN, Jeong D, Zhang S, et al. Therapeutic cardiac-targeted delivery of miR-1 reverses pressure overload-induced cardiac hypertrophy and attenuates pathological remodeling. J Am Heart Assoc 2013;2:e000078.

https://doi.org/10.1161/JAHA.113.000078.

[150] Derda AA, Thum S, Lorenzen JM, Bavendiek U, Heineke J, Keyser B, et al. Blood-based microRNA signatures differentiate various forms of cardiac hypertrophy. Int J Cardiol 2015;196:115–22. https://doi.org/10.1016/j.ijcard.2015.05.185.

[151] Bargaje R, Gupta S, Sarkeshik A, Park R, Xu T, Sarkar M, et al. Identification of novel targets for miR-29a using miRNA proteomics. PLoS One 2012;7:e43243. https://doi.org/10.1371/journal.pone.0043243.

[152] van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci 2008;105:13027–32. https://doi.org/10.1073/pnas.0805038105.

[153] Jafarinejad-Farsangi S, Gharibdoost F, Farazmand A, Kavosi H, Jamshidi A, Karimizadeh E, et al. MicroRNA-21 and microRNA-29a modulate the expression of collagen in dermal fibroblasts of patients with systemic sclerosis. Autoimmunity 2019;52:108–16. https://doi.org/10.1080/08916934.2019.1621856.

[154] Qin RH, Tao H, Ni SH, Shi P, Dai C, Shi KH. microRNA-29a inhibits cardiac fibrosis in Sprague-Dawley rats by downregulating the expression of DNMT3A. Anatol J Cardiol 2018;20:198–205. https://doi.org/10.14744/AnatolJCardiol.2018.98511.

[155] Silverman MG, Yeri A, Moorthy MV, Camacho Garcia F, Chatterjee NA, Glinge CSA, et al. Circulating miRNAs and risk of sudden death in patients with coronary heart disease. JACC Clin Electrophysiol 2020;6:70–9. https://doi.org/10.1016/j.jacep.2019.08.011.

[156] Zhou J, Zhou Y, Wang C xia. LncRNA-MIAT regulates fibrosis in hypertrophic cardiomyopathy (HCM) by mediating the expression of miR-29a-3p. J Cell Biochem 2019;120:7265–75. https://doi.org/10.1002/jcb.28001.

[157] Kawasaki T, Sakai C, Harimoto K, Yamano M, Miki S, Kamitani T. Usefulness of high-sensitivity cardiac troponin T and brain natriuretic peptide as biomarkers of myocardial fibrosis in patients with hypertrophic cardiomyopathy. Am J Cardiol 2013;112:867–72. https://doi.org/10.1016/j.amjcard.2013.04.060.

[158] Kura B, Kalocayova B, Devaux Y, Bartekova M. Potential clinical implications of mir-1 and mir-21 in heart disease and cardioprotection. Int J Mol Sci 2020;21:700. https://doi.org/10.3390/ijms21030700.

[159] Zhou X liang, Xu H, Liu Z bo, Wu Q cai, Zhu R rong, Liu J chun. miR-21 promotes cardiac fibroblast-to-myofibroblast transformation and myocardial fibrosis by targeting Jagged1. J Cell Mol Med 2018;22:3816–24. https://doi.org/10.1111/jcmm.13654.

[160] Liu R-H, Ning B, Ma X-E, Gong W-M, Jia T-H. Regulatory roles of microRNA-21 in fibrosis through interaction with diverse pathways (Review). Mol Med Rep 2016;13:2359–66. https://doi.org/10.3892/mmr.2016.4834.

[161] Watanabe K, Narumi T, Watanabe T, Otaki Y, Takahashi T, Aono T, et al. The association between microRNA-21 and hypertension-induced cardiac remodeling. PLoS One 2020;15:e0226053. https://doi.org/10.1371/journal.pone.0226053.

[162] Dong S, Cheng Y, Yang J, Li J, Liu X, Wang X, et al. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. J Biol Chem 2009;284:29514–25. https://doi.org/10.1074/jbc.M109.027896.

[163] Yuan J, Chen H, Ge D, Xu Y, Xu H, Yang Y, et al. Mir-21 promotes cardiac fibrosis after myocardial infarction via targeting Smad7. Cell Physiol Biochem 2017;42:2207–19. https://doi.org/10.1159/000479995.

[164] Barana A, Matamoros M, Dolz-Gaitón P, Pérez-Hernández M, Amorós I, Núñez M, et al. Chronic atrial fibrillation increases microRNA-21 in human atrial myocytes decreasing L-type calcium current. Circ Arrhythm Electrophysiol 2014;7:861–8. https://doi.org/10.1161/CIRCEP.114.001709.

5. ARTIGO ORIGINAL 1

Family screening of first-degree relatives of patients with hypertrophic cardiomyopathy in a middle-income country: the role of electrocardiogram

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Abstract

Background: The family screening of hypertrophic cardiomyopathy (HCM) based on the clinical and genetic evaluation has not been systematically studied in middle-income countries. Our aim is to evaluate a family screening strategy in first-degree relatives of patients with HCM.

Methods: Patients with HCM from a tertiary hospital in Brazil and their first-degree relatives were submitted to clinical evaluation, electrocardiogram (ECG), echocardiogram and genetic testing.

Results: A total of 81 individuals of 23 unrelated families were recruited, 23 probands and 58 first-degree relatives. Among probands, 11 (47%) were found to harbor sarcomere mutations, mainly in MYH7 and MYBPC3 genes. HCM phenotype was identified in ten (17%) first-degree relatives at the clinical screening. The occurrence of 2 out of 4 clinical markers (HCM-related symptoms, family history of sudden cardiac death, systolic murmur and an modified abnormal ECG model) or an isolated abnormal ECG showed 100% sensitivity and 100% negative predictive value to detect the phenotype among the first-degree relatives. A modified abnormal ECG criterion was applied with a higher accuracy among all other markers considered for evaluation.

Conclusions: The screening of first-degree relatives based on clinical evaluation and ECG is feasible in a middle-income country. The ECG has a central role on the identification of the clinically affected relatives with a high accuracy.

Keywords:

hypertrophic cardiomyopathy, screening, first-degree relatives, genetic testing

Introduction

Hypertrophic cardiomyopathy (HCM) is considered the most common genetic cardiovascular disease affecting 1 in 200-500 individuals [1]. The disease is characterized by left ventricular (LV) hypertrophy in the absence of other causes [2,3]. An autosomal dominant genetic pattern is described with over 2,500 mutations identified mainly in the β-myosin heavy chain (MYH7) and in the cardiac myosin-binding protein C (MYBPC3) genes [4–6].

The HCM-related mutations are recognized in 30-60% of probands leading to a cascade family evaluation due to the 50% risk of first-degree relatives inherit the same genetic mutation [2,6,7]. Current guidelines recommend family screening based on genetic testing and/or clinical evaluation every 3-5 years [2,3]. The use of genetic testing in this setting promotes the recognition of the genotype-positive relatives at risk of developing HCM and the discharge of genotype-negative from long term follow-up [2,3]. Probands with a known sarcomere mutation have a worse prognosis, but recent data show that relatives harbouring a sarcomere mutation had similar survival than negative or untested subjects [8,9]. Whether or not relatives may have a different mutation penetrance, these results put in question if a family genetic-based screening is capable to modify long-term outcomes in HCM.

The electrocardiogram (ECG) is a highly available method with great sensitivity in detecting sudden cardiac death (SCD) related disorders and has been advocated as a reliable screening tool of HCM for a long time [10–12]. The most common ECG patterns in HCM are related to increased QRS voltages, pathological Q waves and repolarization abnormalities, occurring in more than 90% of cases [12]. The ECG changes along the clinical course of HCM, reflecting increased late morbidity [12,13]. Moreover, a normal ECG at presentation is associated

with better survival [14]. These findings reflect the intrinsic relation of the ECG abnormalities with the underlying myocardial pathological processes [12–14].

The HCM family screening strategies in middle-income countries must consider the local resources and economic boundaries. The use of clinical and electrocardiographic easily assessed criteria with this purpose may have an impact on the assessment of HCM families. In this study, we sought to evaluate the feasibility of a clinical and electrocardiographic-based screening strategy to detect HCM in first-degree relatives in a middle-income country.

Methods

Patient selection

This is a single-center cohort study that recruited 23 unrelated consecutive probands and their first-degree relatives at an HCM clinic from a tertiary hospital in Brazil between March 2017 and November 2019. HCM diagnosis was based on echocardiographic identification of unexplained LV hypertrophy with a maximal wall thickness ≥ 15 mm at any segment in the proband or ≥ 13 mm in first-degree relatives in the absence of other causes according to current guidelines [2,3]. Probands were excluded if there were no first-degree relatives able to be submitted to evaluation. The study was approved by the local ethics committees (nº 2015-0607, 2017-0042, 2018-0152, 2019-0213) and complied with the principles of the Helsinki Declaration (2008 revision). Written informed consent was obtained from all individuals.

Clinical evaluation

All subjects were submitted to clinical evaluation, standard 12-lead electrocardiogram (ECG), two-dimensional transthoracic Doppler echocardiogram, and blood sample collection. Cardiac magnetic resonance imaging (MRI) was performed in the probands in cases with no

restrictions. Symptoms such as shortness of breath, chest pain, palpitations, syncope or any other related to the cardiovascular system were considered when relevant. The family history of HCM was defined as the presence of a first-degree relative with a well-documented HCM diagnosis. A family history of SCD was considered when it occurred in one or more relatives under 40 years of age or at any age with a previous diagnosis of the disease.

Electrocardiogram

A standard 12-lead ECG was performed in all individuals. LV hypertrophy was considered according to the following criteria: Sokolow-Lyon, SV1 + RV5/6 > 35 mV; Cornel, RaVL+SV3 > 28 mV in men and > 20 mV in women; Lewis, RI-RIII + SIII-SI > 16 mV; and Romhilt-Estes, ≥ 5 points. It was also evaluated HCM typical ECG characteristics such as the presence of T wave inversion in leads I and aVL >3mm (with QRS-T wave axis difference > 30º), V3-V6 (>3mm) or II and III and aVF (>5mm); abnormal Q wave (>40 ms or >25% R wave) in at least 2 leads from II, III, aVF (in absence of left anterior hemiblock), V1-V4; or I, aVL, V5-V6, and deep S wave in V2 > 25 mm [15]. In probands on pacemaker rhythm, a non-paced ECG was analyzed.

Echocardiogram

All patients were evaluated with two-dimensional transthoracic Doppler echocardiography according to the American Society of Echocardiography on commercially available ultrasound equipment [16]. The following measurements were considered for analysis: left atrial diameter, interventricular septal and posterior wall diastolic thickness, ejection fraction, end-diastolic and end-systolic LV diameters, as well as the presence of mitral valve anterior motion. LV outflow tract (LVOT) gradient at rest and under Valsalva maneuver was assessed by the Bernoulli equation. LVOT obstruction was defined as a gradient ≥ 30 mmHg and severe obstruction of ≥ 50 mmHg at

rest or during provocation. LV diastolic function was evaluated based on Doppler mitral inflow filling and tissue Doppler velocities.

The evaluation of the LV myocardial deformation usin global longitudinal strain (GLS) was performed by a single investigator (W.R.M.) with a B-mod speckle-tracking software (2D CPA TTA2.20.01, TomTec). At end-systole, three landmarks were established at the endocardial edge (two basal and one apical), with automatic detection of speckles along the LV endocardial edge. Peak GLS for each 2D apical view (two-, three-, and four-chamber) was obtained from the mean of the 6 traced segments, while the mean peak GLS was calculated by averaging the peak GLS of apical available views. It was excluded images from patients with non-sinus rhythm, poor quality precluded speckle analysis in two or more consecutive segments, images covering less than one complete cardiac cycle, or excessively tangential views.

Blood samples

All samples were collected from peripheral blood in EDTA containing tubes on the same day of clinical evaluation. Samples were processed within 1 hour after collection, centrifuged at 1000xg for 10 minutes on 4^{o}C. DNA was extracted according to the current technic [17]. RNA was extracted with the mirVana Paris (Ambion) kit. Sample quantification was performed with NanoDrop 1000 (ThermoScientific). All samples were stored at -80^{o}C.

Genetic testing

The genetic-molecular analysis was performed in the proband with next-generation sequencing (NGS) panel containing the following genes: ACTC1, ACTN2, ANKRD1, CALR3, CSRP3, FHL1, FXN, GLA, JPH2, LAMP2, LDB3/ZASP, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPN, NEXN, PLN, PRKAG2, PTPN11, RAF1, TCAP, TNNC1,

TNNI3, TNNT2, TPM1, TTN, TTR, and VCL. All fragments were purified with Exo-SAP according to the manufacture instructions (USB Corporations, USA), following by direct sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and capillary electrophoresis analyzed with ABI3500 Genetic Analyzer (Applied Biosystems, USA). All sequences obtained were compared with those considered references for each gene. *In silico* analysis was applied to evaluate the substitution effect of the amino acid in the protein based on the conservation affected regions through the following programs: PolyPhen [18], SIFT [19], PROVEAN [20], MutationTaster [21], and MutPred [22]. MutPred system was applied as a hypothesis generator over the mutation structural and functional properties. Synonymous mutations and intronic substitutions in codifying exons, not reported as polymorphisms (SNPs) or found at the 1000 Exome Variant Server (EVS) database, were also submitted to *in silico* analysis for identification of a possible splice-site alteration. NetGene [23] and Human Splicing Finder [24] were adopted for the calculation of the consensus values of the potential splice-site. All variants found were classified according to the American College of Medical Genetics and Genomics criteria [25].

Statistical analysis

Continuous variables were submitted to normality test with histogram analysis and a Shapiro-Wilk test. Data were presented as mean ± standard deviation or median (interquartile range) when appropriate. Categorical data are shown as counts and percentages. The Student T or Mann-Whitney tests were applied to detect differences between continuous variables. Pearson chi-square test and Fisher exact test were used to evaluate differences between categorical variables. A logistic regression analysis was performed to assess whether clinical, demographical or electrocardiographic characteristics were associated with a greater likelihood of an HCM

phenotype in first-degree relatives. Clinically relevant variables were included in the model as well as those with a P-value < 0.1. ROC curves were applied to evaluate the discriminatory power of clinical and electrocardiographic data to recognize first-degree relatives with the HCM phenotype. Areas under the ROC curve (AUC) were compared by the DeLong test. All variables were tested for multicollinearity in the multivariable models by the variance inflation test. Significance was accepted at $P < 0.05$ for all tests. Data were analyzed in SPSS, Version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 81 individuals of 23 unrelated families with HCM were recruited, 23 probands and 58 first-degree relatives. Probands and relatives demographic and clinical characteristics are shown in table 1. Probands were older [62 (23) vs. 36 (28) years, P=0.022), more likely to be male [15 (65%) vs. 3 (30%), P=0.068)], presented with more HCM-related symptoms [18 (78%) vs. 6 (60%), P=0.022], had a greater body mass index [29.7 (4.4) vs. 25.5 (4.3) kg/m^2, P=0.051], and a higher burden of comorbidities such as hypertension [14 (61%) vs. 2 (20%), P=0.031] and diabetes [8 (35%) vs. none], than first-degree relatives.

Genetics

Genetic testing was positive for a pathogenic/likely pathogenic mutation in 11 (48%) probands, and for a variant of uncertain significance (VUS) in 4 (17%). The most affected genes were MYH7 in 5 (46%) individuals and MYBPC3 in 3 (27%). KCNH2 and RYR2 were affected in one (9%) case respectively, and MYH7 and MYBPC3 cosegregation in other one (9%) case. VUS were present in KCNH2, LAMP2, MYH6, and RYR2 genes in one (25%) proband each.

Table 2 summarizes the pathogenic/likely pathogenic and VUS found in probands and their associated clinical characteristics.

Family evaluation

The initial screening was able to detect 10 (17%) first-degree relatives with HCM. Table 1 summarizes the demographic and clinical characteristics of phenotype-positive and phenotype-negative subjects. Dyspnea [5 (50%)] and palpitations [4 (40%)] were the most common symptoms among them, independently of the HCM phenotype presence, but the frequency of overall HCM-related symptoms was similar in phenotype-positive and phenotype-negative first-degree relatives [7 (70%) vs. 24 (50%), P=0.311]. A family history of SCD was more common in phenotype-positive first-degree relatives than in phenotype-negative [7 (70%) vs. 15 (31%), P=0.032]. Also, phenotype-positive first-degree relatives were older [36 (28) vs. 31 (16) years, P=0.083] and more likely to have a systolic murmur [6 (60%) vs. 13 (27%), P=0.065) than phenotype-negative, although with borderline statistics results.

All probands [23 (100%)] and clinically affected first-degree relatives [10 (100%)] had an abnormal ECG. The summary of the ECG patterns is shown in table 3. LV hypertrophy was the most common finding followed by abnormal Q waves; both were more prevalent in phenotype-positive individuals, [9 (90%) vs. 13 (27%), P=0.001) and [7 (70%) vs. 11 (23%), P=0.008], than in phenotype-negative. In relation to LV hypertrophy criteria, Cornell [4 (40%) vs none], Lewis [6 (60%) vs. 4 (8%), P=0.001] and Romhilt-Estes $\geq$ 5 points [6 (60%) vs. 9 (19%), P=0.007] differed between these groups. Sokolow-Lyon LV criteria [2 (22%) vs. 3 (6%), P=0.178] and S waves in V2 > 25 mm [2 (22%) vs. 3 (6%), P=0.178] were also more frequent in the phenotype-positive, but did not reached a statistical difference. T wave inversion was found in only one

relative phenotype-positive. Besides these differences, phenotype-negative relatives had an abnormal ECG in 20 (42%) of cases.

Echocardiographic evaluation of the phenotype-positive relatives in comparison to probands showed a lower left atrial (left atrial volume 36.3±5.4 vs. 48.2±11.7 ml/m^2, P=0.017), LV end-diastolic diameter (42.3±2.8 vs. 46.1±5.8 mm, P=0.018), LV mass index [143 (47) vs. 178 (89) g/m^2, P=0.042] and LVOT gradient [0 (0) vs. 16 (61) mmHg, P=0.034], as shown in Table 4. In addition to the greater LV maximal wall thickness [18 (7) vs. 8 (2) mm, P=0.001], the phenotype-positive first-degree relatives in comparison to phenotype-negative also showed a higher left atrial diameter [40.1±5.5 vs. 34.9±4.1 mm, P=0.002], lower end-diastolic (42.3±2.8 vs. 46.7±4.1 mm, P=0.002) and end-systolic LV diameters (26.7±2.6 vs. 29.0±2.8, P=0.022), LV mass index [143 (47) vs. 72 (19) g/m2, P=0.001], LV obstruction gradient [0 (2) vs. 0 (0) mmHg, P=0.002], E/e' [10.6±4.2 vs. 6.8±1.7, P=0.001], and GLS (-17±4 vs. -21.8±2, P=0.001].

Phenotype screening strategy

Unadjusted and adjusted analyses were performed to assess the association of demographic, clinical and electrocardiographic characteristics with a greater likelihood to detect an HCM phenotype among first-degree relatives. As shown in Table 5, age [OR 1.04 (1.00-1.09), P=0.037], family history of SCD [OR 3.81 (1.1-13.2), P=0.035] and abnormal ECG [OR 11.88 (1.60-0.015), P=0.015] were associated with HCM among relatives in the unadjusted analysis. A known mutation in the proband, systolic murmur or HCM-related symptoms showed borderline results. We considered HCM-related symptoms, systolic murmur, family history of SCD and an abnormal ECG as clinical markers to recognize the phenotype-positive first-degree relatives at initial screening, based on the unadjusted analysis. Although an older age showed a borderline result for an independent association with the HCM phenotype, this variable was not added to the

model considering that all family members should be evaluated independently of age. The adjusted analysis for these clinical markers showed that only abnormal ECG [OR 7.6 (1.07-53.71), P=0.042] was independently associated with the occurrence of the HCM phenotype in the first-degree relatives. A family history of SCD showed borderline results.

The accuracy of all clinical markers and ECG findings to identify first-degree relatives with a HCM phenotype is shown in table 6. HCM-related symptoms [AUC 0.60 (0.41-0.78), P=0.323), family history of SCD [AUC 0.68 (0.51-0.87), P=0.056], and heart murmur [AUC 0.66 (0.47-0.85), P=0.104) alone were not able to discriminate those with the phenotype. Among ECG findings, LV hypertrophy by any criteria showed the highest AUC (0.81, 0.67-0.95, P=0.002). Lewis [AUC 0.75 (0.56-0.95), P=0.011] and Cornell [AUC 0.70 (0.48-0.91), P=0.048] demonstrated the greatest accuracy, 86% and 89% respectively. Abnormal Q waves had an accuracy of 75% and an AUC of 0.73 (0.55-0.91, P=0.022). Although T wave abnormalities had an accuracy of 84%, the AUC [0.55 (0.34-0.75), P=0.620] did not reach statistical significance. The array of ECG abnormalities reached 65% accuracy for detecting phenotype-positive first-degree relatives with an AUC of 0.79 (0.67-0.90, P=0.004). A modified abnormal ECG model was created to increase accuracy, in which were included the variables with a higher AUC: two LV hypertrophy criteria (Cornell and Lewis) and abnormal Q waves. The modified abnormal ECG model showed an accuracy of 85% with an AUC of 0.85 (0.75-0.95, P=0.001). A model based on all clinical markers (HCM-related symptoms, family history of HCM, heart murmur and modified abnormal ECG) showed an AUC of 0.89 (0.80-0.99, P=0.001). As shown in figure 1, there is an increase in the rate of detection of phenotype-positive first-degree relatives according to the number of clinical markers and the presence of electrocardiographic findings in the modified model. It was applied a cut-off ≥2 clinical markers and ≥1 ECG abnormalities in both employed

criteria (abnormal ECG and modified abnormal ECG), with all of them showing 100% sensitivity and 100% negative predictive value. However, the modified abnormal ECG criteria presented with a higher AUC 0.85 (0.75-0.95), P=0.001 in comparison with the cut-off ≥ 2 clinical markers [0.78 (0.66-0.90), P=0.005], (P=0.02) and ≥1 abnormal ECG findings [0.79 (0.67-0.90), P=0.004], (P=0.009).

Phenotype-negative first-degree relatives

It was observed that 48 (83%) of first-degree relatives were phenotype-negative. HCM-related symptoms and heart murmur were present in 13 (27%) of these subjects, respectively, and family history of SCD in 15 (31%) (Table 1). ECG abnormalities were found in 20 (42%) individuals, especially LV hypertrophy by any criteria in 13 (27%), and abnormal Q waves in 11 (23%). A modified abnormal ECG model was present in 14 (29%). The absence of clinical markers was not able to discriminate those members without the phenotype [AUC 0.57 (0.39-76), P=0.471]. However, a normal ECG showed an AUC of 0.79 (0.67-0.90) P=0.004, and a modified ECG model an AUC of 0.85 (0.759-0.950), P=0.0001 to detect normal individuals. The comparison between ROC curves showed a greater discriminatory power of the modified abnormal ECG to identify normal family members (P=0.009). A normal modified ECG showed sensitivity of 71%, specificity of 100%, positive predictive value of 100%, negative predictive value of 42% and accuracy of 76%.

Discussion

This study evaluated 23 unrelated families from an HCM clinic of a tertiary public hospital in Brazil. Approximately a fifth of the first-degree relatives showed the HCM phenotype presenting with younger age, fewer symptoms, and a lower degree of atrial and ventricular

remodeling. It was identified four easily assessed clinical markers that predicted with high accuracy those individuals with a positive clinical screening for HCM, especially in cases with a cut-off of ≥ 2 clinical markers and/or ECG abnormalities. The ECG played a central role in the identification of the affected first-degree relatives.

A positive genetic screening may show different results according to the selected population [9,26,27]. In our study, 47% of probands were found to harbor a sarcomere mutation, mainly in MYH7 and MYBPC3 genes. The genetic panel comprised the sequencing of over 30 genes including those related to HCM phenocopies such as LAMP2, GLA and TTR. This genetic profile is similar to previous findings in the Brazilian population [28,29].

Up to our knowledge, this is the largest HCM family screening reported in Brazil. Previous studies showed a large range of HCM recognition in relatives from 2.5% to 46% [7,29,30]. Our study showed an intermediate (17%) prevalence of HCM phenotype among first-degree relatives, perhaps justified by the fact that the disease penetrance is incomplete [6]. These members presented with several differences in comparison to probands, such as a lower burden of symptoms and comorbidities, as well as lower body mass index and lower left atrial and LV remodeling. These findings may be explained by the younger age of the first-degree relatives, which implies a shorter disease course. Besides, it has been shown that the incidence of HCM-related symptoms and the phenotype expression increases according to the age of the individuals under screening [9,30,31].

An abnormal ECG may be the first phenotypic manifestation of HCM [32]. These electrocardiographic changes have been studied previously showing abnormalities in >90% of cases [10,12,33]. However, its prognostic role is still uncertain [13,34]. In our study, both probands and phenotype-positive first-degree relatives showed frequent ECG abnormalities. However,

probands showed a higher rate of LV hypertrophy (by any criteria) and T wave inversion, although not statistically significant. It is known that ECG changes are detected in the long-term follow-up of HCM patients and may have a lower burden in younger-age relatives [12]. Nevertheless, the lack of power due to the small sample size may explain our results. ECG abnormalities were more common in the phenotype-positive first-degree relatives in comparison to phenotype-negative. LV hypertrophy criteria were the major finding in 90% of them, but abnormal Q waves also showed a high prevalence (70%). Phenotype-negative first-degree relatives showed an abnormal ECG in 42% of cases, mainly due to the presence of LV hypertrophy and abnormal Q waves. Moreover, a normal ECG had a higher accuracy to detect phenotype-negative relatives when compared to other clinical markers. The genotype-positive with no overt HCM may present with ECG abnormalities, such as increased precordial voltages and deep Q waves, although no specific pattern has been described yet [12,35]. HCM phenocopies related to storage diseases are also associated with very high amplitude QRS, but may present with very low voltages when extracellular infiltration of the myocardium occurs, such as in amyloidosis [12]. It has been proposed that genotype-positive/phenotype-negative relatives with abnormal ECG should be followed closely in contrast to those with a normal test, in which no restrictions are applied [32]. A QRS complex ECG criteria have been used as a strategy tool for screening HCM and other SCD-related disorders with reasonable sensitivity and negative predictive value [11]. Recently, an automated HCM screening using ECG showed similar performance when compared to a trained electrophysiologist [36]. Therefore, due to its availability and low cost, the ECG should be applied as a reliable HCM screening tool.

The low rate of application of genetic testing in middle-income countries may be related not only to limit budget but also to ethical and cultural issues [37]. In these settings, the clinical

screening of families with HCM should use locally available resources. Based on the adjusted analysis, a screening strategy was built integrating easily and accessible data from clinical and electrocardiographic evaluation to detect phenotype among first-degree relatives. Despite the association with a positive phenotype in relatives, age was not added to the screening strategy. All relatives should be included independent of age in these cases [31]. In relation to the set of 4 clinical markers (HCM-related symptoms, family history of SCD, systolic murmur, and a modified abnormal ECG model), it was observed an increase in accuracy according to the number of identified variables. It reached an AUC of 0.89, the greatest among all studied criteria, with sensitivity and negative predictive value of 100%. The fact that ECG has added diagnostic power to an early screening of HCM families, points out that electrical remodeling occurs simultaneously or even precedes the development of morphological and functional abnormalities in this disease [12]. In this sense, a normal ECG showed good discrimination ability in detecting family members without the phenotype.

The ECG abnormalities described in our study showed a high sensitivity to recognize phenotype-positive first-degree relatives. Among those, LV hypertrophy criteria had higher accuracy in comparison to abnormal Q waves and T waves abnormalities. Despite the good performance of an abnormal ECG to detect HCM phenotype among relatives, it was proposed a modified abnormal ECG model, which was able to detect HCM phenotype with higher accuracy (76%) and sensitivity (100%). Previous studies show that LV hypertrophy and abnormal Q waves are the most common findings in HCM [10,12]. However, few reports have included first-degree relatives and it may impact the results. The probands usually have a longer disease course and a more severe ECG pattern in comparison to these younger phenotype-positive relatives [12,31]. In

our study, LV hypertrophy criteria and abnormal Q waves differed between probands and first-degree relatives, but it failed to reach statistical difference probably due to the small sample size.

The strategy to detect phenotype-positive first-degree relatives must be highly sensitive, but with the lowest possible false-positive rate. HCM guidelines recommend the use of genetic testing for family screening, but there is not a formal strategy in cases of none availability [2,3]. The 4 clinical markers and an abnormal ECG showed a higher AUC, but when cut-offs were applied, the ECG became more accurate. It is still unclear if a family screening strategy without genetic testing should include a broader clinical evaluation or if a single ECG is accurate enough to identify those with the phenotype. Also, should the absence of clinical markers or a normal ECG avoid routine echocardiography evaluation? Further studies with larger samples are needed to clarify this hypothesis.

Future perspectives

Genetic testing in middle-income countries is not widely available. Indeed, it is still to be performed in the relatives included in this study. The phenotype-negative relatives will be re-assessed in 3 and 5 years of follow-up and results will be analyzed to evaluate the cost-effectiveness of genetic screening in comparison to clinical follow-up.

Strengths and study limitations

This study proposes an easy and widely available tool to screen first-degree relatives of patients with HCM in a middle-income environment. However, this is a single-center study and results should be taken carefully. The small sample size may lack the power to show other associations of clinical and electrocardiographic characteristics to detect phenotype-positive first-degree relatives. These results are not definitive and a larger sample should be studied to better

assess the accuracy of the proposed clinical strategy. The absence of pediatric patients also limits the results to an early adulthood screening. Furthermore, a follow-up of the phenotype-negative subjects must be performed to establish the best time for reassessment.

Conclusions

The best strategy for the family screening of first-degree relatives of patients with HCM is yet to be established in settings without genetic testing. Clinical and ECG evaluation should be applied to this aim due to its great availability. We showed that the presence of 4 clinical markers (HCM-related symptoms, systolic murmur, family history of SCD and modified abnormal ECG model) or an isolated abnormal ECG may be useful to detect phenotype-positive first-degree relatives. The ECG has shown to play a central role in family screening, signaling the presence of an electrical remodeling even in subjects with early disease course. A normal ECG showed good reliability in detecting phenotype-negative family members.

References

[1] Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol 2015;65:1249–54. https://doi.org/10.1016/j.jacc.2015.01.019.

[2] Authors/Task Force members, Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J 2014;35:2733–79. https://doi.org/10.1093/eurheartj/ehu284.

[3] Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, et al. 2020 AHA/ACC

guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy. Circulation 2020:Epub ahead of print. https://doi.org/10.1161/CIR.0000000000000937.

[4] Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: Clinical perspectives. J Am Coll Cardiol 2012;60:705–15. https://doi.org/10.1016/j.jacc.2012.02.068.

[5] Ho CY, Charron P, Richard P, Girolami F, Van Spaendonck-Zwarts KY, Pinto Y. Genetic advances in sarcomeric cardiomyopathies: state of the art. Cardiovasc Res 2015;105:397–408. https://doi.org/10.1093/cvr/cvv025.

[6] Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. N Engl J Med 2018;379:655–68. https://doi.org/10.1056/nejmra1710575.

[7] Lorenzini M, Norrish G, Field E, Ochoa JP, Cicerchia M, Akhtar MM, et al. Penetrance of hypertrophic cardiomyopathy in sarcomere protein mutation carriers. J Am Coll Cardiol 2020;76:550–9. https://doi.org/10.1016/j.jacc.2020.06.011.

[8] Ho CY, Day SM, Ashley EA, Michels M, Pereira AC, Jacoby D, et al. Genotype and lifetime burden of disease in hypertrophic cardiomyopathy insights from the sarcomeric human cardiomyopathy registry (SHaRe). Circulation 2018;138:1387–98. https://doi.org/10.1161/CIRCULATIONAHA.117.033200.

[9] van Velzen HG, Schinkel AFLL, Baart SJ, Oldenburg RA, Frohn-Mulder IMEE, van Slegtenhorst MA, et al. Outcomes of contemporary family screening in hypertrophic cardiomyopathy. Circ Genomic Precis Med 2018;11:e001896. https://doi.org/10.1161/CIRCGEN.117.001896.

[10] Ryan MP, Cleland JGF, French JA, Joshi J, Choudhury L, Chojnowska L, et al. The standard electrocardiogram as a screening test for hypertrophic cardiomyopathy. Am J Cardiol 1995;76:689–94. https://doi.org/10.1016/S0002-9149(99)80198-2.

[11] Rodday AM, Triedman JK, Alexander ME, Cohen JT, Ip S, Newburger JW, et al. Electrocardiogram screening for disorders that cause sudden cardiac death in asymptomatic children: A meta-analysis. Pediatrics 2012;129:999–1010. https://doi.org/10.1542/peds.2011-0643.

[12] Finocchiaro G, Sheikh N, Biagini E, Papadakis M, Maurizi N, Sinagra G, et al. The electrocardiogram in the diagnosis and management of patients with hypertrophic cardiomyopathy. Heart Rhythm 2019;17:142–51. https://doi.org/10.1016/j.hrthm.2019.07.019.

[13] Biagini E, Pazzi C, Olivotto I, Musumeci B, Limongelli G, Boriani G, et al. Usefulness of electrocardiographic patterns at presentation to predict long-term risk of cardiac death in patients with hypertrophic cardiomyopathy. Am J Cardiol 2016;118:432–9. https://doi.org/10.1016/j.amjcard.2016.05.023.

[14] McLeod CJ, Ackerman MJ, Nishimura RA, Tajik AJ, Gersh BJ, Ommen SR. Outcome of patients with hypertrophic cardiomyopathy and a normal electrocardiogram. J Am Coll Cardiol 2009;54:229–33. https://doi.org/10.1016/j.jacc.2009.02.071.

[15] McKenna WJ, Spirito P, Desnos M, Dubourg O, Komajda M. Experience from clinical genetics in hypertrophic cardiomyopathy: proposal for new diagnostic criteria in adult members of affected families. Heart 1997;77:130–2. https://doi.org/10.1136/hrt.77.2.130.

[16] Doherty JU, Kort S, Mehran R, Schoenhagen P, Soman P, Dehmer GJ, et al.

ACC/AATS/AHA/ASE/ASNC/HRS/SCAI/SCCT/SCMR/STS 2019 appropriate use criteria for multimodality imaging in the assessment of cardiac structure and function in nonvalvular heart disease. J Am Soc Echocardiogr 2019;32:553–79. https://doi.org/10.1016/j.echo.2019.01.008.

[17] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215. https://doi.org/10.1093/nar/16.3.1215.

[18] Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248–9. https://doi.org/10.1038/nmeth0410-248.

[19] Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009;4:1073–82. https://doi.org/10.1038/nprot.2009.86.

[20] Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. PLoS One 2012;7:e46688. https://doi.org/10.1371/journal.pone.0046688.

[21] Schwarz JM, Cooper DN, Schuelke M, Seelow D. Mutationtaster2: Mutation prediction for the deep-sequencing age. Nat Methods 2014;11:361–2. https://doi.org/10.1038/nmeth.2890.

[22] Li B, Krishnan VG, Mort ME, Xin F, Kamati KK, Cooper DN, et al. Automated inference of molecular mechanisms of disease from amino acid substitutions. Bioinformatics 2009;25:2744–50. https://doi.org/10.1093/bioinformatics/btp528.

[23] Hebsgaard S. Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. Nucleic Acids Res 1996;24:3439–52. https://doi.org/10.1093/nar/24.17.3439.

[24] Desmet FO, Hamroun D, Lalande M, Collod-Bëroud G, Claustres M, Béroud C. Human splicing finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 2009;37. https://doi.org/10.1093/nar/gkp215.

[25] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24. https://doi.org/10.1038/gim.2015.30.

[26] Geske JB, Ommen SR, Gersh BJ. Hypertrophic cardiomyopathy: clinical update. JACC Heart Fail 2018;6:364–75. https://doi.org/10.1016/j.jchf.2018.02.010.

[27] Murphy SL, Anderson JH, Kapplinger JD, Kruisselbrink TM, Gersh BJ, Ommen SR, et al. Evaluation of the Mayo clinic phenotype-based genotype predictor score in patients with clinically diagnosed hypertrophic cardiomyopathy. J Cardiovasc Transl Res 2016;9:153–61. https://doi.org/10.1007/s12265-016-9681-5.

[28] Marsiglia JDC, Credidio FL, De Oliveira TGM, Reis RF, Antunes MDO, De Araujo AQ, et al. Screening of MYH7, MYBPC3, and TNNT2 genes in Brazilian patients with hypertrophic cardiomyopathy. Am Heart J 2013;166:775–82. https://doi.org/10.1016/j.ahj.2013.07.029.

[29] Mattos BP, Scolari FL, Torres MAR, Simon L, Freitas VC, Giugliani R, et al. Prevalence and phenotypic expression of mutations in the MYH7, MYBPC3 and TNNT2 genes in

families with hypertrophic cardiomyopathy in the south of Brazil: a cross-sectional study. Arq Bras Cardiol 2016;107:257–65. https://doi.org/10.5935/abc.20160133.

[30] Norrish G, Jager J, Field E, Quinn E, Fell H, Lord E, et al. Yield of clinical screening for hypertrophic cardiomyopathy in child first-degree relatives. Circulation 2019;140:184–92. https://doi.org/10.1161/CIRCULATIONAHA.118.038846.

[31] Lafreniere-Roula M, Bolkier Y, Zahavich L, Mathew J, George K, Wilson J, et al. Family screening for hypertrophic cardiomyopathy: is it time to change practice guidelines? Eur Heart J 2019;40:3672–81. https://doi.org/10.1093/eurheartj/ehz396.

[32] Caselli S, Pelliccia A. The electrocardiogram and the phenotypic expression of hypertrophic cardiomyopathy. Eur Heart J 2019;40:982–5. https://doi.org/10.1093/eurheartj/ehy403.

[33] Delcrè SDL, Di Donna P, Leuzzi S, Miceli S, Bisi M, Scaglione M, et al. Relationship of ECG findings to phenotypic expression in patients with hypertrophic cardiomyopathy: A cardiac magnetic resonance study. Int J Cardiol 2013;167:1038–45. https://doi.org/10.1016/j.ijcard.2012.03.074.

[34] Ledieu N, Larnier L, Auffret V, Marie C, Fargeau D, Donal E, et al. Prognostic value of the 12-lead surface electrocardiogram in sarcomeric hypertrophic cardiomyopathy: data from the REMY French register. Europace 2020;22:139–48. https://doi.org/10.1093/europace/euz272.

[35] Lakdawala NK, Thune JJ, Maron BJ, Cirino AL, Havndrup O, Bundgaard H, et al. Electrocardiographic features of sarcomere mutation carriers with and without clinically overt hypertrophic cardiomyopathy. Am J Cardiol 2011;108:1606–13.

https://doi.org/10.1016/j.amjcard.2011.07.019.

[36] Campbell MJ, Zhou X, Han C, Abrishami H, Webster G, Miyake CY, et al. Pilot study analyzing automated ECG screening of hypertrophic cardiomyopathy. Heart Rhythm 2017;14:848–52. https://doi.org/10.1016/j.hrthm.2017.02.011.

[37] Zhong A, Darren B, Loiseau B, He LQB, Chang T, Hill J, et al. Ethical, social, and cultural issues related to clinical genetic testing and counseling in low- and middle-income countries: a systematic review. Genet Med 2018h. https://doi.org/10.1038/s41436-018-0090-9.

TABLES

Table 1. Demographic and clinical characteristics of probands with hypertrophic cardiomyopathy and their first-degree relatives

	Probands	First-degree relatives				
	All (N=23)	All (N=58)	Phenotype + (N=10)	Phenotype - (N=48)	P*	P**
Age (years)	62 (13)	31 (16)	36 (28)	31 (16)	0.022	0.083
Male sex, (%)	15 (65%)	26 (45%)	3 (30%)	23 (48%)	0.062	0.487
BMI, (kg/m²)	29.7 (4.4)	25.5 (4.3)	25.9 (5.6)	25.5 (4.1)	0.051	0.853
HCM-related symptoms, (%)	18 (78%)	19 (33%)	6 (60%)	13 (27%)	0.252	0.065
Dyspnea, (%)	19 (83%)	18 (31%)	5 (50%)	13 (27%)	0.053	0.258
Functional class NYHA, (%)						
I/II, (%)	17 (74%)	18 (31%)	5 (50%)	13 (27%)	0.032	0.135
III/IV, (%)	3 (13%)	-	-	-		
Chest pain, (%)	7 (30%)	8 (14%)	3 (30%)	5 (10%)	0.980	0.131
Syncope, (%)	5 (22%)	4 (7%)	-	4 (8%)	0.142	0.592
Palpitations, (%)	5 (22%)	19 (33%)	4 (40%)	15 (31%)	0.279	0.714
FH of HCM, (%)	8 (35%)	-	-	-	-	-
FH of SCD, (%)	15 (65%)	22 (38%)	7 (70%)	15 (31%)	0.789	0.032
Hypertension, (%)	14 (61%)	5 (9%)	2 (20%)	3 (6%)	0.031	0.202
Diabetes, (%)	8 (35%)	-	-	-	0.032	
AF, (%)	6 (26%)	1 (2%)	1 (10%)	-	0.299	0.172
Heart rate, (%)	64 (28)	80 (13)	80 (15)	78 (18)	0.305	0.726
Systolic BP, (%)	130 (20)	123 (19)	120 (19)	124 (18)	0.105	0.337
Diastolic BP, (%)	80 (10)	80 (12)	75 (11)	80 (14)	0.954	0.238
Systolic murmur, (%)	18 (78%)	19 (33%)	6 (60%)	13 (27%)	0.252	0.065
Betablocker, (%)	17 (74%)	5 (9%)	4 (40%)	1 (2%)	0.063	0.002
CC blocker, (%)	7 (30%)	1 (2%)	1 (10%)	-	0.208	0.172

Diuretic, (%)	12 (52%)	3 (5%)	2 (20%)	1 (2%)	0.086	0.074
ACEi/ARB, (%)	6 (26%)	4 (7%)	1 (10%)	3 (6%)	0.299	0.541
Alcohol septal ablation, (%)	2 (9%)	-	-	-	-	-
Myectomy, (%)	2 (9%)	-	-	-	-	-
ICD, (%)	6 (26%)	-	-	-	-	-

ACEi, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; BP, blood pressure; ARB, angiotensin II receptor blocker; BMI, body mass index; CC, calcium channel; FH, family history; G+, genotype-positive; G-, genotype-negative; ICD, implantable cardioverter-defibrillator; Phenotype+, phenotype-positive, Phenotype-, phenotype-negative; SCD, sudden cardiac death.

P* test between probands and phenotype-positive relatives

P** test between phenotype-positive and phenotype-negative first-degree relatives

Table 2. Results of the next-generation genetic sequencing and clinical characteristics of probands with hypertrophic cardiomyopathy.

Family	Gene	cDNa change	Protein	Interpretation	MWT (mm)	FH SCD
AD	MYH7	c.4508G>A	p.E1468K	P/LP	18	+
AK	KCNH2	c.526C>T	p.R176W	VUS	24	-
AC	MYBPC3	c.1484G>A	p.R495Q	P/LP	19	+
AH	MYH7	c.4508G>A	p.E1468K	P/LP	13	-
	MYBPC3	c.2686G>A	p.V896M	P/LP		
AD	MYH7	c.2389G>A	p.A797T	P/LP	20	-
AM	MYBPC3	c.1505G>A	p.R502Q	P/LP	17	-
AN	LAMP2	c.1234T>C	p.T412Q	VUS	21	+
AQ	MYH7	c.2750C>T	p.A917V	P/LP	18	-
AS	MYH7	c.1063G>A	p.A355T	P/LP	23	+
AT	MYH6	c.3010G>T	p.A1004S	VUS	16	+
AU	MYBPC3	c.1484G>A	p.R495Q	P/LP	17	+
AW	KCNH2	c.2863C>G	p.L955V	P/LP	22	+
AX	RYR2	c.593A>T	p.N198I	VUS	15	-
AY	MYH7	c.788T>C	p.I263T	P/LP	23	+
BB	RYR2	c.147G>C	p.L49F	P/LP	15	+

FH SCD, family history of sudden cardiac death; MWT, maximal wall thickness; P/LP, pathogenic/likely pathogenic; VUS, variant of unknown significance

Table 3. Electrocardiographic characteristics of hypertrophic cardiomyopathy probands and their first-degree relatives.

	Probands	First-degree relatives				
	All (N=23)	All (N=58)	Phenotype+ (N=10)	Phenotype- (N=48)	P*	P**
Any ECG abnormalities	23 (100%)	30 (52%)	10 (100%)	20 (42%)	1.00	0.001
LV hypertrophy by any criteria	22 (96%)	13 (22%)	9 (90%)	13 (27%)	0.532	0.001
Sokolow-Lyon	11 (48%)	5 (9%)	2 (22%)	3 (6%)	0.245	0.178
Cornell	16 (70%)	4 (7%)	4 (40%)	-	0.139	0.001
Lewis	15 (65%)	10 (17%)	6 (60%)	4 (8%)	0.775	0.001
Romhilt-Estes ≥ 5 points	20 (87%)	15 (26%)	6 (60%)	9 (19%)	0.161	0.007
Abnormal Q waves	8 (35%)	18 (31%)	7 (70%)	11 (23%)	0.126	0.008
S waves in V2 > 25 mm	5 (22%)	5 (9%)	2 (22%)	3 (6%)	0.911	0.178
T wave inversions						
DI and aVL (>3mm)	9 (39%)	1 (2%)	1 (10%)	-	0.123	0.175
DII and DIII and aVF (>5mm)	1 (4%)	-	-	-	-	-
V3-V6 (>3mm)	9 (39%)	-	-	-	-	-

LV, left ventricle; Phenotype+, phenotype-positive; Phenotype-, phenotype-negative

P* test between probands and phenotype-positive relatives

P** test between phenotype-positive and phenotype-negative first-degree relatives

Table 4. Phenotypic characteristics of probands and relatives evaluated by echocardiogram and cardiac magnetic resonance imaging.

	Probands	First-degree relatives				
	All (N=23)	All (N=58)	Phenotype+ (N=10)	Phenotype- (N=48)	P*	P**
Echocardiogram						
LA, (mm)	45.7±5.7	35.8±4.8	40.1±5.5	34.9±4.1	0.013	0.002
LA volume, (ml/m^2)	48.2±11.7	31.0±8.6	36.3±5.4	29.8±8.7	0.017	0.071
LVDD, (mm)	46.1±5.8	45.9±4.2	42.3±2.8	46.7±4.1	0.018	0.002
LVSD, (mm)	29.1±4.5	28.6±0.9	26.7±2.6	29.0±2.8	0.06	0.022
MWT, (mm)	19 (5)	8 (3)	18 (7)	8 (2)	0.343	0.001
PW, (mm)	11 (2)	8 (1)	8 (2)	8 (1)	0.001	0.169
EF, (%)	66.3±6.2	67.7±4.4	67±4.7	67.8±4.4	0.772	0.589
LV mass index, (g/m²)	178.4 (89)	73 (28)	143 (47)	72 (19)	0.042	0.001
PASP, (mmHg)	30 (79)	19.5 (26)	24 (28)	18 (25)	0.09	0.158
LVOT gradient (rest), mmHg	16 (61)	0 (0)	0 (2)	0 (0)	0.034	0.002
LVOT gradient (Valsalva), mmHg	18 (80)	0 (0)	0 (2)	0 (0)	0.028	0.002
LVOT obstruction	11 (48%)	1 (2%)	1 (10%)	-	0.038	0.175
LVOT severe obstruction	10 (44%)	1 (2%)	1 (10%)	-	0.061	0.175
SAM	8 (35%)	2 (4%)	1 (10%)	1 (2%)	0.142	0.339
E/e'	14.9±6.3	7.47±2.6	10.6±4.2	6.8±1.7	0.09	0.001
GLS, (%)	-16.5±3	-21±3	-17±4	-21.8±2	0.741	0.001

Cardiac MRI (n=16/23)	
LGE, (%)	9 (56%)
MWT, (mm)	21 (3.7)

ECG, electrocardiogram; EF, ejection fraction; Gn+, genotype-positive; Gn-, genotype-negative; GLS, global longitudinal strain; LA, left atrium; LGE, late gadolinium enhancement; LVDD, left ventricular diastolic diameter; LVSD, left ventricular systolic diameter; LVOT, left ventricular outflow tract; MRI, magnetic resonance imaging, MWT, maximal wall thickness; Ph+, phenotype-positive, Ph-, phenotype-negative; PASP, pulmonary artery systolic pressure; PW, posterior wall; SCD, sudden cardiac death.

P* test between probands and phenotype-positive relatives

P** test between phenotype-positive and phenotype-negative first-degree relatives

Table 5. Unadjusted and adjusted analysis to evaluate demographic, clinical, and electrocardiographic characteristics associated with HCM diagnosis in first-degree relatives.

	Unadjusted			Adjusted		
	OR	CI	P	OR	CI	P
Age (years)	1.04	1.00-1.09	0.037			
Known mutation in family	2.62	0.61-11.2	0.194			
HCM-related symptoms	2.33	0.53-10.1	0.257	2.10	0.79-5.56	0.136
FH of SCD	3.81	1.1-13.2	0.035	2.62	0.94-7.29	0.064
Systolic murmur	3.07	0.98-9.62	0.053	1.59	0.59-4.25	0.353
Abnormal ECG	11.88	1.60-87.7	0.015	7.60	1.07-53.71	0.042

CI, confidence interval; ECG, electrocardiogram; FH, family history; OR, odds ratio; SCD, sudden cardiac death;

Table 6. Accuracy of clinical markers and abnormal ECG to detect phenotype-positive first-degree relatives.

	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	CI	P
HCM-related symptoms	70%	50%	23%	89%	53%	0.60	0.41-0.78	0.323
Family history of SCD	70%	69%	32%	92%	69%	0.68	0.51-0.87	0.056
Heart murmur	60%	73%	32%	90%	71%	0.66	0.47-0.85	0.104
LV hypertrophy by any criteria	90%	73%	41%	97%	76%	0.81	0.67-0.95	0.002
Sokolow-Lyon	20%	94%	40%	85%	81%	0.56	0.36-0.77	0.497
Lewis	60%	91%	60%	91%	86%	0.75	0.56-0.95	0.011
Cornell	40%	100%	100%	89%	89%	0.70	0.48-0.91	0.048
Romhilt-Estes ≥ 5 points	60%	81%	40%	91%	78%	0.70	0.51-0.89	0.042
Abnormal Q waves*	70%	77%	39%	92%	75%	0.73	0.55-0.91	0.022
T wave abnormalities**	10%	100%	100%	84%	84%	0.55	0.34-0.75	0.620
S wave > 25mm in V2	20%	94%	40%	85%	81%	0.56	0.36-0.77	0.490
Abnormal ECG	100%	58%	33%	100%	65%	0.79	0.67-0.90	0.004
Modified abnormal ECG ***	100%	71%	42%	100%	76%	0.85	0.75-0.95	0.001
Clinical markers****	-	-	-	-	-	0.89	0.80-0.99	0.001
≥1	100%	17%	20%	100%	31%	0.58	0.40-0.76	0.410
≥2	100%	56%	32%	100%	64%	0.78	0.66-0.90	0.005
≥3	70%	90%	58%	94%	86%	0.79	0.62-0.97	0.003
≥4	30%	100%	75%	87%	88%	0.65	0.43-0.86	0.138

AUC, area under the receiver operating characteristics curve; CI, confidence interval; ECG, electrocardiogram; NPV, negative predictive value; PPV, positive predictive value;

*Abnormal Q: >40ms or >25% R wave) at least 2 leads from DII, DIII, aVF; V1-V4; I, aVL, orV5-V6;

**T wave inversion in leads DI and aVL (>3mm) or T wave inversion at DII and DIII and aVF (>5mm) or T wave Inversion at V3-V6 (>3mm)

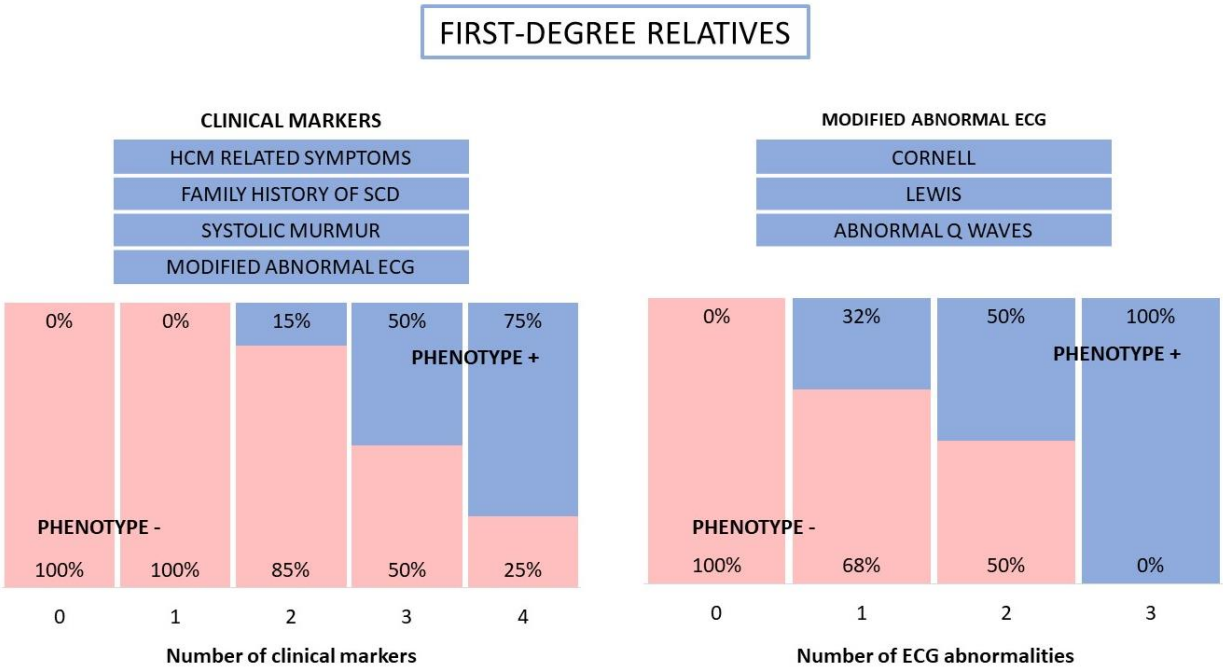
***LV hypertrohy criteria (Lewis and Cornell) and abnormal Q wave

**** HCM-related symptoms, family history of SCD, systolic murmur, modified abnormal ECG model

FIGURE LEGENDS

Figure 1. Screening strategy in first-degree relatives of probands with hypertrophic cardiomyopathy

FIGURE 1



HCM related symptoms:
- Dyspnea
- Palpitations
- Chest pain
- Syncope

Systolic murmur
- Murmur between S1 and S2 ≥ 2+

Family history of SCD
- SCD in in one or more relatives under 40 years of age or at any age with a previous diagnosis of the disease

Abnormal ECG
- LV hypertrophy
  - Sokolow-Lyon (SV1 + RV5/6 > 35 mV)
  - Lewis (RI-RIII + SIII-SI > 16 mV)
  - Cornell (RaVL+SV3 > 28 mV in men and > 20 mV in women)
- T wave inversion in leads I and aVL (>3mm) (with QRS-T wave axis difference > 30°), V3-V6 (>3mm) or II and III and aVF (>5mm)
- Abnormal Q (>40 ms or >25% R wave) in at least 2 leads from II, III, aVF (in absence of left anterior hemiblock), V1-V4; or I, aVL, V5-V6
- Deep S wave in V2 > 25 mm

Modified abnormal ECG model
- Cornell and Lewis LV hypertrophy criteria, and abnormal Q waves

6. ARTIGO ORIGINAL 2

A systematic review of microRNAs in patients with hypertrophic cardiomyopathy

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Dr. Fernando Luís Scolari and Lucas Simnonetto Faganello selected the studies, extracted and analyzed data from all studies included and drafted the manuscript. Dr. Henrique Iahnke Garbin independently evaluated the quality of selected reports. Dr. Beatriz Piva e Mattos and Dr. Andreia Biolo critically reviewed the data collected and the manuscript. All authors approved the final version of the manuscript.

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The authors report no conflicts.

Keywords: hypertrophic cardiomyopathy, microRNAs, left ventricular hypertrophy, myocardium fibrosis, cardiomyopathy, epigenetic

Highlights

- Several miRNAs have been associated with hypertrophic cardiomyopathy
- Main phenotype correlation has been found for mir-1-3p, mir-19b, mir-21, mir-29a, mir-155, and mir-221
- Mir-29a showed the greatest consistency of findings regarding hypertrophy and fibrosis association
- miRNAs are biomarkers candidates in hypertrophic cardiomyopathy

Abstract

Background: Several microRNAs (miRNA) have been associated with hypertrophic cardiomyopathy (HCM), but studies differ regarding methods employed. In an attempt to understand their role in the disease, we performed a systematic review of studies assessing miRNAs and their association with HCM.

Methods: The literature search was based on The Medical Subject Headings (MeSH) terms “Hypertrophic Cardiomyopathy” and “MicroRNA" combined with other synonyms on Embase, Medline and LILACS databases in April 2020. The selected studies and data extraction were independently evaluated. Only human reports with a clear definition of HCM diagnosis were included.

Results: The search found 68 studies, 13 fulfilled the selection criteria, with a total of 329 patients. Eighty-seven miRNA were differentially expressed in HCM patients, being mir-21, mir-29a and mir-133 the most reported. The miRNA were mainly up-regulated, where mir-29a was up-regulated in 6 studies, followed by mir-133 in 4 and mir-21 in 3. Blood samples were evaluated in the majority of patients (86%), but a greater number of miRNAs (79%) were assessed in myocardium. Six studies evaluating the phenotype correlation demonstrated that several miRNAs, mainly mir-1-3p, mir-19b, mir-21, mir-29a, mir-155, and mir-221, were related to either hypertrophy or fibrosis. Mir-29a showed a more consistent phenotypic correlation.

Conclusion: Eighty-seven miRNAs were differentially expressed in HCM patients, the majority in up-regulation. Mir-21, mir-29a and mir-133 were the most reported. Correlation with left ventricular hypertrophy and fibrosis was evaluated in six studies for several miRNAs, nevertheless, mir-29a showed more consistent findings and seems to be a promising biomarker.

Introduction

Hypertrophic cardiomyopathy (HCM) is a cardiovascular genetic disorder with an autosomal dominant pattern with more than 1,500 mutations described mainly on sarcomeric genes and Z disks in different populations worldwide[1,2]. Its prevalence ranges from 1:200 to 1:500, being the major cause of sudden cardiac death in young and athletes[3]. It is characterized by left ventricular (LV) hypertrophy in the absence of other causes[4]. The clinical expression may vary from asymptomatic carriers to severe ventricular hypertrophy even between first-degree relatives[1–3,5]. This phenotype expression variability may be explained by incomplete mutation penetrance, but the underlying process is still unknown[1,5].

MicroRNAs (miRNA) are small noncoding ribonucleic acid molecules that act at a post-transcriptional level on gene expression regulation[6]. The tissue evaluation of miRNA with microarray technic set the basis of the understanding of their role on several key processes related to myocardial hypertrophy, fibrosis, and apoptosis[7,8]. Therefore, they are implicated in the pathogenesis of several cardiovascular diseases such as heart failure, myocardial ischemia, and atherosclerosis[9–11]. The stability of blood plasma and the resistance to pH and temperature variance makes these molecules good candidates to be used as clinical biomarkers[9]. However, there are some challenges in the measurement of extracellular miRNA due to its lower concentration, high sensibility to pre and post-processing factors, and high concentration of miRNA in blood cells that can confound the results of the study[12]. While their use as biomarkers in peripheral samples seems promising and could add useful information in the clinical setting, careful evaluation of current studies is necessary in order to understand the performance of miRNAs in HCM.

The potential role of miRNAs in HCM and their association with the expression of myocardial hypertrophy and/or fibrosis has been suggested in some studies[13–15]. However, most

reports are small, and there is variability in clinical settings, methods, sample tissue, miRNAs selection and results. This systematic review sought to evaluate available data regarding miRNAs and their association with HCM phenotypic expression, in an attempt to identify those more consistently related to hypertrophy and fibrosis.

Methods

Search strategy

A systematic literature search was performed on the Embase, Medline and LILACS databases in April 2020. The Medical Subject Headings (MeSH) terms “Hypertrophic Cardiomyopathy” and “MicroRNA" were combined with other synonyms to detect papers that assessed miRNA expression in HCM. The full search strategy is found in the supplementary material. This systematic review is reported according to PRISMA guidelines.

Article selection

We selected studies that evaluated the miRNA profile in human HCM. The articles were select by two independent authors (F.L.S and L.S.F) after initial screening by title and abstract and, after initial eligibility, by full-text evaluation. For articles that were discordant on first judgment, a third author (A.B.) was consulted. References from selected studies were revised to detect additional papers, especially in reviews. Data selected for extraction were the number of patients, the miRNA submitted to an evaluation, the microarray assessment validated with real-time polymerase chain reaction PCR (RT-PCR), the system biology approach and the phenotype correlation. All pre-selected studies were submitted to the Downs and Black checklist for quality assessment[16].

Exclusion criteria

Exclusion criteria included non-HCM studies, animal models, reviews, letters, editorials, duplicates and articles not available in English. In studies that evaluated the miRNA profile in HCM and non-HCM, the authors extracted only the information on HCM. Reports that used data from unpublished studies or those that did not evaluated a profile of miRNA in HCM patients, such as bioinformatics analysis, were not included.

Data collection

Quality among studies was evaluated through miRNA extraction technic regarding the tissue sample, such as blood serum or myocardium, the employment of microarray and the validation with RT-PCR. We searched for the main report and the supplementary material to assess the miRNAs evaluated in each study. Some studies evaluated many miRNAs but reported only those up- or down-regulated. In these cases, we extracted the available information. Data was also classified using the number of studies checking the same miRNA, the number of subjects and the phenotype correlation. Up, down or mixed regulations were evaluated only in those miRNAs mentioned in more than one report. Up-regulation was defined as the absence of down-regulation for a single miRNA in all studies and down-regulation as the absence of up-regulation. Up- or down-regulation were classified independently of the number of studies with neutral effect. Mixed-regulation was defined as the presence of at least one report with up-regulation and one with down-regulation for a single miRNA. Exclusively up- or down-regulation were described when all articles showed the same result without a neutral effect. In cases in which the correlation between miRNA expression and HCM phenotype was described, data was extracted if studies presented a clear definition of the diagnosis, imaging methods and exclusion criteria. Left ventricular hypertrophy detected on transthoracic echocardiogram (TTE) or cardiac magnetic resonance

(CMR) and fibrosis evaluated by late gadolinium enhancement (LGE) on CMR were considered as the main phenotype expressions.

Results

The search strategy is presented in Figure 1. Sixty-eight articles were identified; 19 (28%) were excluded for not corresponding to HCM and other 17 (25%) for not including microRNA profile. From the remaining 32 (47%) articles, 5 (7%) were reviews, 5 (7%) were letters or editorials, 4 (6%) were not written in English, 4 (6%) were experimental animal models, and one (1%) did not provide results for microRNA expression. Thirteen (19%) articles fulfilled the selection criteria and were included[13,14,24–26,15,17–23]. Quality evaluation through Down and Blacks checklist is shown in Table 1.

Characteristics of selected studies

The search identified 87 microRNAs expressed in a total of 329 HCM subjects. Most of the studies have included controls (10 out of 13)[13–15,17–21,24,26]. Five (38%) evaluated microRNAs on blood serum[13–15,20,25], 7 (54%) on myocardial samples[17–19,21–24] and one (8%) study employed both blood serum and myocardial tissue[26]. All reports applied RT-PCR assays, but two used a preliminary microarray technic[15,22] to identify miRNAs and further validation was determined with RT-PCR. Table 2 shows the characteristics of selected studies: the inclusion of controls, sample, methods, and miRNAs evaluated in each report.

miRNA levels in HCM

Thirty-four (39%) microRNAs were assessed in more than one study. The mir-21, mir-29a and mir-133a were evaluated in 7 studies, followed by mir-1, mir-155 and mir-199a-3p in 5, mir-27a, mir-143-3p, mir-145-3p, mir-199a-5p, mir-208a, mir-208b, mir-214, mir-451, mir-499-3p in

3, and mir-10b, mir-10b*, mir-19b, mir-26a, mir-30a, mir-30b, mir-30d-5p, mir-92a, mir-93, mir-96-5p, mir-126-3p, mir-126-5p, mir-133b, mir-142, mir-195, mir-221, mir-222, mir-483-5p and mir-497 in 2. The remaining microRNAs were assessed in only one report. Figure 2 summarizes in a heat map all evaluated microRNAs.

Blood/serum samples

Among the studies, 6 (46%) evaluated miRNAs on peripheral blood comprising 282 (86%) of studied patients and 42 (48%) miRNAs[13–15,20,25,26]. According to prespecified criteria, among the 20 (47%) miRNAs evaluated in more than one study, 8 (40%) were found to be exclusively up-regulated in HCM (29a, mir-27a, 143-3p, 145-3p, 199a-5p, 30a and 125-3p) in all of them, although 7 (88%) of those were assessed in only 2 reports. Another group of 7 (35%) miRNAs were also up-regulated (mir-133a, mir-21, mir-199a-3p, mir-214, mir-499-3p, mir-126-5p, and mir-195). Results were mixed in 2 (10%) miRNAs (mir-155 and mir-19b) showing both up- and down-regulation and 3 (15%) were neutral (mir-1, mir-208a, and mir-208b). There was no exclusive down-regulation. Mir-133a was the most reported (5 studies), followed by mir-29a and 21 (4 studies). Of the 22 miRNAs evaluated in only one study, 14 (64%) were up-regulated, and 8 (36%) had a neutral effect.

Myocardium tissue

Myocardium tissue was evaluated in 8 reports comprising 89 (27%) patients and 69 (79%) miRNA[17–19,21–24,26]. Twelve (17%) miRNA were evaluated in more than one report. Exclusive up-regulation was found in 3 (25%) miRNA (mir-221, mir-451, and mir-222) and exclusively down-regulation in 1 (8%) (mir-1). Another 5 (42%) were up-regulated (mir-29a, mir-133a, mir-199-3p, mir-93 and mir-497), 1 (8%) down-regulated (mir-21), and 2 (17%) were mixed (mir-155 and mir-

10b*). The most reported miRNA was mir-29a (3 studies). The remaining 56 (81%) miRNA were evaluated in only one study, up-regulated in 35 (63%), down-regulated in 13 (23%) and neutral in 8 (14%).

Phenotype correlation

Six studies evaluated whether miRNAs correlated with the HCM phenotype, either hypertrophy or fibrosis (Table 3)[13–15,24–26]. Most of them (4 out of 6) studied miRNAs in blood samples[13–15,25], one evaluated both tissues[26] and one only myocardium[24]. In Roncarati 2014 et al., 21 miRNAs were assessed in peripheral blood samples of 41 patients and sex- and age-matched controls. Mir-29a was the only miRNA found to be correlated with both LV hypertrophy and fibrosis determined by TTE and/or CMR[13]. Fang 2015 et al. selected 14 miRNAs after microarray validation and assessed diffuse fibrosis by T1 mapping on CMR. Mir-29a and mir-133 showed a moderate area under the receiver operating characteristics curve (AUC) (0.663 and 0.742, respectively) for the presence of diffuse fibrosis, while the combination of all 14 miRNAs showed a more increased AUC (0.870)[15].

In the study by Derda 2015 et al., patients with obstructive and non-obstructive HCM, severe aortic stenosis, senile cardiac amyloidosis, and healthy controls were compared. Mir-29a levels correlated with interventricular septum thickness and were higher on obstructive HCM patients with a MYH7 gene mutation. In contrast, mir-155 was found to be down-regulated in patients with a MYBPC3 gene mutation. Mir-29a and mir-155 were able to discriminate non-obstructive HCM from senile amyloidosis[14].

Li 2018 et al. employed tissue samples from explanted hearts of respectively 10 HCM and dilated cardiomyopathy (DCM) patients submitted to heart transplantation, which were compared

to 10 controls. Only two miRNAs showed different expressions in both cardiomyopathy samples. Mir-27a and mir-1-3p were found down-regulated respectively in DCM and HCM. Mir-1-3p showed to be directly correlated with LV ejection fraction and inversely with LV end-diastolic diameter[24].

Zhou 2019 et al. evaluated 69 HCM patients and found that only mir-29a was increased in those with fibrosis[25]. Huang 2020 et al. employed both tissue samples from septal myectomy and peripheral blood. In the myocardium, only mir-221 showed correlation with collagen volume fraction, but both mir-221 and mir-19b were associated with LGE on CMR. On peripheral blood, mir-221 showed a relation with LGE and collagen volume fraction, as well as with maximal LV wall thickness and LV mass index on TTE. However, mir-19b was correlated only with LGE on CMR and LV mass index on TTE. AUC to detect fibrosis was higher for mir-221 than mir-19b (0.764 vs. 0.201)[26].

Discussion

This systematic review sought to evaluate available studies regarding the expression of miRNAs in patients with HCM. In the 13 included reports, a total of 87 miRNAs were found to be related to the disease with either up or down-regulation[13,14,24–26,15,17–23]. However, most miRNAs were assessed in only one study. Mir-29a was evaluated in 7 studies, showing up-regulation in 6[13–15,23,25,26]. Only six studies correlated miRNAs with phenotype findings such as left ventricular hypertrophy or fibrosis[13–15,24–26]. Among these, mir-29a appeared as the most reported and was correlated both with left ventricular hypertrophy and myocardial fibrosis[13–15,25].

miRNA in HCM and cardiovascular diseases

The study of miRNAs in cardiovascular diseases has grown in the last decades due to their ability to be measured on blood serum which makes them valuable biomarkers candidates[11,13,27]. In this systematic review, only 6 articles evaluated miRNAs on blood samples, but they accounted for the majority of the study patients[13–15,20,25,26]. As shown in Table 2, these articles assessed mainly symptomatic not-end stage HCM patients, representing those seen in daily clinical practice. A miRNA must have a consistent pattern in a given condition, preferentially replicated in several studies, to be a reliable disease biomarker[6]. Mir-1-3p and mir-27a showed an AUC of 0.850 and 0.860 respectively that allowed the differentiation between HCM and DCM[24]. Mir-29a expresses differently among patients with HCM, aortic stenosis, and senile amyloidosis[14]. These findings corroborate the hypothesis that miRNAs may be used as biomarkers to differentiate left ventricular hypertrophy produced by several similar conditions with borderline genotype or phenotype. As it was shown that patients with heart failure with preserved ejection fraction had a different miRNA profile than those with reduced ejection fraction[10]. However, the intracellular miRNA assessment on the target tissue shows a higher performance than the peripheral blood, but it requires a tissue sample[12]. The blood serum, however, is widely available and less invasive. New techniques have been developed to minimize the errors related to serum evaluation and increase the reliability of the results, essential for a biomarker candidate[28].

miRNA profile in HCM and tissue relationship

We attempted to identify patterns of miRNAs that could be associated with HCM and to evaluate the consistency of these findings in different studies. The majority of miRNAs were found to be up-regulated in HCM, whereas only a few were exclusively up-regulated. Although blood samples were assessed in the majority of studies[13–15,20,26,29], a greater number of miRNAs were evaluated in the myocardium[17–19,21–24,26]. According to our systematic review, among the three

most reported miRNAs, only mir-29a showed a consistent pattern of up-regulation in 6 out of 7 studies based on whether in serum blood or myocardium tissue[13–15,24–26,30]. The mir-133a, mir-199a-3p, mir-221, 143-3p, 145-3p, 30d-5p and 96-5p were also up-regulated in both tissues. In particular, mir-221 showed to have a moderate correlation between its circulating levels and myocardium expression level[26], which may explain its up-regulation in studies[18,26]. A down-regulation pattern was found for mir-1, mir-208b and mir-30b in myocardium tissue, but blood samples showed no expression[13,14,17,20,24]. Mir-1 is considered a muscle-specific miRNA and its down-regulation is associated with myocyte injury[31]. However, the mir-208b, also considered muscle-specific, was detected in the plasma of patients with myocardial infarction[32,33].Mir-30b is also related to the myocardial injury process, but its tissue specificity is unknown[34]. Mir-21 is known as the most common miRNA in cardiac muscle and it is related to myocardial fibrosis mediated by fibroblast activation via TGF-β1 signaling[29,31]. It was mainly up-regulated in blood[13,15,25], but in myocardium, it was not expressed in two studies[24,26] and down-regulated in one[22]. Another four miRNAs showed a similar results, with either up or down-regulation in different tissues (mir-155, mir-19b, and mir-10b)[13,14,21,24–26]. These differences in miRNA expression may be related to the sample characteristics. The dynamic expression of miRNAs has been described previously in other clinical scenarios and may explain this mixed pattern of some miRNAs in HCM[35].

Association with ventricular hypertrophy

Left ventricular hypertrophy is the main component of the HCM phenotype and it is related to important outcomes, such as heart failure and sudden cardiac death[4]. There is sufficient evidence to state that miRNAs have a role in ventricular hypertrophy expression. This systematic review identified that mir-21, mir-29a, mir-155, and mir-221 were related to maximal LV wall thickness

or hypertrophy index on TTE while mir-27a, mir-29a, mir-155, and mir-221 were associated with maximal LV wall thickness on CMR in HCM. A study of patients with hypertension and left ventricular hypertrophy showed that mir-21 was up-regulated and correlated moderately with left ventricular mass index[36]. In an animal model-based study, it was also correlated with left ventricular hypertrophy mediated by aldosterone[37]. Mir-29a seems to have a central role in pathological cardiac hypertrophy as shown in a system biology approach[38]. In a clinical study, it was able to differentiate various forms of cardiac hypertrophy, including HCM[14]. However, patients with hypertension also showed greater levels of mir-29a and higher correlation with LV hypertrophy[39]. The inhibition of mir-29a with an antagomir in a pressure hypertrophy induced animal model improved the remodeling of LV, signalling its role on the pathogenesis of cardiac hypertrophy[40,41]. The mir-221 showed to be up-regulated in an animal model and related to cardiac hypertrophy suppressor p27[18]. Other miRNAs were related to distinct forms of hypertrophy such as mir-27a and mir-155, respectively decreased in exercise-induced ventricular hypertrophy and pressure overload model[42,43]. Moreover, it is still unknown whether a single or a group of miRNAs have a role in specific forms of hypertrophy[13,14,26].

Association with myocardial fibrosis

Myocardial fibrosis is an important component of LV remodeling in HCM present as a replacement or interstitial process[44,45]. A pro-fibrotic state may be found even in the early disease when chamber morphology is still normal[44]. The occurrence of myocardial fibrosis in HCM patients has several implications, including the risk of sudden cardiac death and progression to end-stage forms[45]. The sixteen miRNAs were able to identify HCM patients with fibrosis on CMR: mir-10b-5p, mir-15a-5p, mir-17-5p, mir-18a-5p, mir-19b-3p, mir-21, mir-27a, mir-29a, mir-30d-5p, mir-133a, mir-146a-5p, mir-192-5p, mir-193-5p, mir-200a-3p and mir-296-5p. In a myocardial

infarction model, the mir-29 family, mainly 29b, was expressed preferentially in fibroblasts, suggesting the influence of mir-29a in fibrosis expression[46]. Whether it plays a role in HCM related fibrosis or if it is associated with any specific pattern is still unknown, but there is some evidence showing that it may be expressed in other conditions, such as systemic sclerosis, liver and diabetic renal diseases[47–49]. Up to our knowledge, there is no study comparing different causes of myocardial fibrosis and the expression of mir-29a. Mir-133a was recognized as an antifibrotic targeting the connective growth factor in only one study[50]. However, it has been related previously to other cardiovascular diseases[15,50]. Among the other miRNAs related to fibrosis, discrepant results were also found. The mir-19b showed a moderate AUC to detect fibrosis on CMR in one report, but a negative correlation with LGE was found in another[15,26]. The mir-221 showed a relation to myocardial fibrosis in only one study, but it was negatively correlated with the extent of myocardial fibrosis in DCM and aortic stenosis patients[26,51].

Mir-29a in HCM

The mir-29a is related to extracellular matrix proteins synthesis and apoptosis processes[46,52]. In a large trial, mir-29a was associated with sudden cardiac death in patients with coronary artery disease, probably due to its relation with fibroblast activation[53]. However, the association of mir-29a with fibrosis is not exclusive to the myocardium and was found in several tissues[47–49]. Interestingly, mir-29 family was associated with myocardial infarction-related fibrosis[46], but mir-29b and mir-29c were not expressed in the myocardium[54]. In this systematic review, it was shown to be the most reported miRNA in HCM, predominantly expressed in up-regulation, and correlated with LV hypertrophy assessed on TTE or CMR as well as with fibrosis[13–15,23,25,26]. The association of mir-29a with hypertrophy and fibrosis in HCM may suggest that different processes share a common molecular basis and pathogenic pathways[5]. It remains to be

demonstrated if mir-29a is associated with prognosis and increased risk for sudden cardiac death in HCM. It is unknown whether mir-29a is up-regulated only in clinically expressed HCM or also in pre-clinical disease. Further studies are needed to demonstrate the role of mir-29a as a biomarker in order to assure its current use in clinical practice.

In summary, it seems that although several miRNAs are related to HCM, only a few were evaluated in a clinical context and submitted to phenotype correlation. Mir-1-3p, mir-19b, mir-21, mir-29a, mir-155, and mir-221 showed association with either myocardial hypertrophy or fibrosis, but only mir-21, mir-29a and mir-221 were related to both phenotypes. Mir-133 showed a correlation with fibrosis in only one study. Mir-29a was the most reported and consistent regarding the phenotypical correlation.

Studies limitations

The study of miRNA expression in HCM patients is still limited. Reports differed on samples employed such as blood serum or myocardial tissue. Only a few studies included blood serum allowing its current use as a non-invasive clinical biomarker[13–15,20,23,25]. There was also a lack of uniformity regarding miRNAs selection approach. One articles employed a microarray strategy to identify those highly expressed before evaluating a larger sample, others performed RT-PCR and some did not provide the miRNAs selection approach. Another limitation is that some reports described only miRNAs found to be up- or down-regulated and did not inform about those without a specific pattern of expression. Finally, this systematic review did not included the pattern of miRNA expression found in animal models.

Conclusion

In this systematic review, we identified data on eighty-seven miRNAs which are differentially expressed in HCM patients, mostly in an up-regulation pattern. Only a few studies have analyzed phenotype correlation with left ventricular hypertrophy and fibrosis. The major phenotypic correlation has been demonstrated with mir-29a, which appears to be up-regulated in HCM patients and related both to hypertrophy and fibrosis. Further studies should evaluate its potential diagnostic, prognostic and even therapeutic role in the disease.

References

1. Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. *N Engl J Med*. 2018;379:655–668. doi: 10.1056/nejmra1710575

2. Mattos BP, Scolari FL, Torres MAR, Simon L, Freitas VC, Giugliani R, Matte Ú. Prevalence and phenotypic expression of mutations in the MYH7, MYBPC3 and TNNT2 genes in families with hypertrophic cardiomyopathy in the south of Brazil: a cross-sectional study. *Arq Bras Cardiol*. 2016;107:257–265. doi: 10.5935/abc.20160133

3. Semsarian C, Ingles J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2015;65:1249–1254. doi: 10.1016/j.jacc.2015.01.019

4. Elliott PM, Anastasakis, Aris, Michael A Borger MB, Cecchi F, Charron P, Alain A, Hagege, Lafont AHM, Mckenna WJJM, Nihoyannopoulos P, Tillmanns C, Watkins H. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy. *Eur Heart J*. 2014;35:2733–2779. doi: 10.1093/eurheartj/ehu284

5. Olivotto I, D'Amati G, Basso C, Van Rossum A, Patten M, Emdin M, Pinto Y, Tomberli B, Camici PG, Michels M. Defining phenotypes and disease progression in sarcomeric

cardiomyopathies: contemporary role of clinical investigations. Cardiovasc. Res. 2015;105:409–423. doi: 10.1093/cvr/cvv024

6. Thum T, Condorelli G. Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. *Circ Res*. 2015;116:751–762. doi: 10.1161/CIRCRESAHA.116.303549

7. Kumarswamy R, Thum T. Non-coding RNAs in cardiac remodeling and heart failure. *Circ Res*. 2013;113:676–689. doi: 10.1161/CIRCRESAHA.113.300226

8. Piccoli MT, Bär C, Thum T. Non-coding RNAs as modulators of the cardiac fibroblast phenotype. *J Mol Cell Cardiol*. 2016;92:75–81. doi: 10.1016/j.yjmcc.2015.12.023

9. Kumarswamy R, Anker SD, Thum T. MicroRNAs as circulating biomarkers for heart failure: questions about MiR-423-5p. *Circ Res*. 2010;106:e8; author reply e9. doi: 10.1161/CIRCRESAHA.110.220616

10. Wong LL, Zou R, Zhou L, Lim JY, Phua DCY, Liu C, Chong JPC, Ng JYX, Liew OW, Chan SP, Chen Y-TT, Chan MMY, Yeo PSD, Ng TP, Ling LH, Sim D, Leong KTG, Ong HY, Jaufeerally F, Wong R, Chai P, Low AF, Lund M, Devlin G, Troughton R, Cameron VA, Doughty RN, Lam CSP, Too HP, Richards AM. Combining circulating microRNA and NT-proBNP to detect and categorize heart failure subtypes. *J Am Coll Cardiol*. 2019;73:1300–1313. doi: 10.1016/j.jacc.2018.11.060

11. Condorelli G, Latronico MVG, Cavarretta E. MicroRNAs in cardiovascular diseases: current knowledge and the road ahead. *J Am Coll Cardiol*. 2014;63:2177–2187. doi: 10.1016/j.jacc.2014.01.050

12. Parikh VN, Chan SY. Analysis of microRNA niches: Techniques to measure extracellular

microRNA and intracellular microRNA in situ. *Methods Mol Biol*. 2013;1024:157–172. doi: 10.1007/978-1-62703-453-1_12

13. Roncarati R, Anselmi V, Losi A, Papa L, Cavarretta E, Costa D, Contaldi C, Jotti S, Franzone A, Galastri L, Latronico MVGG, Imbriaco M, Esposito G, Windt L De, Betocchi S, Condorelli G. Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2014;63:920–927. doi: 10.1016/j.jacc.2013.09.041

14. Derda AA, Thum S, Lorenzen JM, Bavendiek U, Heineke J, Keyser B, Stuhrmann M, Givens RC, Kennel PJ, Christian Schulze P, Widder JD, Bauersachs J, Thum T. Blood-based microRNA signatures differentiate various forms of cardiac hypertrophy. *Int J Cardiol*. 2015;196:115–122. doi: 10.1016/j.ijcard.2015.05.185

15. Fang L, Ellims AH, Moore X, White DA, Taylor AJ, Chin-Dusting J, Dart AM. Circulating microRNAs as biomarkers for diffuse myocardial fibrosis in patients with hypertrophic cardiomyopathy. *J Transl Med*. 2015;13:314. doi: 10.1186/s12967-015-0672-0

16. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *J Epidemiol Community Health*. 1998;52:377–384. doi: 10.1136/jech.52.6.377

17. Palacin M, Reguero JR, Martin M, Diaz Molina B, Moris C, Alvarez V, Coto E. Profile of microRNAs differentially produced in hearts from patients with hypertrophic cardiomyopathy and sarcomeric mutations. *Clin Chem*. 2011;57:1614–1616. doi:

10.1373/clinchem.2011.168005

18. Wang C, Wang S, Zhao P, Wang X, Wang J, Wang Y, Song L, Zou Y, Hui R. MiR-221 promotes cardiac hypertrophy in vitro through the modulation of p27 expression. *J Cell Biochem*. 2012;113:2040–2046. doi: 10.1002/jcb.24075

19. Leptidis S, el Azzouzi H, Lok SI, de Weger R, Olieslagers S, Kisters N, Silva GJ, Heymans S, Cuppen E, Berezikov E, de Windt LJ, da Costa Martins P. A deep sequencing approach to uncover the miRNOME in the human heart. *PLoS One*. 2013;8:1–9. doi: 10.1371/journal.pone.0057800

20. Palacín M, Coto E, Reguero JR, Morís C, Alvarez V. Profile of microRNAs in the plasma of hypertrophic cardiomyopathy patients compared to healthy controls. *Int J Cardiol*. 2013;167:3075–3076. doi: 10.1016/j.ijcard.2012.11.069

21. Kuster DWD, Mulders J, ten Cate FJ, Michels MM, dos Remedios CG, da Costa Martins PA, van der Velden J, Oudejans CBM, Cate FJ, Michels MM, Remedios CG, Paula A, Martins C, Velden J Van Der, Oudejans CBM. MicroRNA transcriptome profiling in cardiac tissue of hypertrophic cardiomyopathy patients with MYBPC3 mutations. *J Mol Cell Cardiol*. 2013;65:59–66. doi: 10.1016/j.yjmcc.2013.09.012

22. Song L, Su M, Wang S, Zou Y, Wang X, Wang Y, Cui H, Zhao P, Hui R, Wang J. MiR-451 is decreased in hypertrophic cardiomyopathy and regulates autophagy by targeting TSC1. *J Cell Mol Med*. 2014;18:2266–2274. doi: 10.1111/jcmm.12380

23. Ntelios D, Meditskou S, Efthimiadis G, Pitsis A, Nikolakaki E, Girtovitis F, Parcharidou D, Zegkos T, Kouidou S, Karvounis H, Tzimagiorgis G. Elevated plasma levels of miR-29a are associated with hemolysis in patients with hypertrophic cardiomyopathy. *Clin*

Chim Acta. 2017;471:321–326. doi: 10.1016/j.cca.2017.07.004

24. Li M, Chen X, Chen L, Chen K, Zhou J, Song J. MiR - 1 - 3p that correlates with left ventricular function of HCM can serve as a potential target and differentiate HCM from DCM. *J Transl Med*. 2018;1–10. doi: 10.1186/s12967-018-1534-3

25. Zhou J, Zhou Y, Wang C xia. LncRNA-MIAT regulates fibrosis in hypertrophic cardiomyopathy (HCM) by mediating the expression of miR-29a-3p. *J Cell Biochem*. 2019;120:7265–7275. doi: 10.1002/jcb.28001

26. Huang D, Chen Z, Wang J, Chen Y, Liu D, Lin K. MicroRNA-221 is a potential biomarker of myocardial hypertrophy and fibrosis in hypertrophic obstructive cardiomyopathy. *Biosci Rep*. 2020;40:1–13. doi: 10.1042/BSR20191234

27. Barwari T, Joshi A, Mayr M. MicroRNAs in cardiovascular disease. *J Am Coll Cardiol*. 2016;68:2577–2584. doi: 10.1016/j.jacc.2016.09.945

28. Cheng Y, Dong L, Zhang J, Zhao Y, Li Z. Recent advances in microRNA detection [Internet]. Analyst. 2018;143:1758–1774. doi: 10.1039/c7an02001e

29. Zhou X liang, Xu H, Liu Z bo, Wu Q cai, Zhu R rong, Liu J chun. miR-21 promotes cardiac fibroblast-to-myofibroblast transformation and myocardial fibrosis by targeting Jagged1. *J Cell Mol Med*. 2018;22:3816–3824. doi: 10.1111/jcmm.13654

30. Michels M, Soliman OII, Kofflard MJ, Hoedemaekers YM, Dooijes D, Majoor-Krakauer D, ten Cate FJ. Diastolic abnormalities as the first feature of hypertrophic cardiomyopathy in Dutch myosin-binding protein C founder mutations. *JACC Cardiovasc Imaging*. 2009;2:58–64. doi: 10.1016/j.jcmg.2008.08.003

31. Kura B, Kalocayova B, Devaux Y, Bartekova M. Potential clinical implications of mir-1 and mir-21 in heart disease and cardioprotection. *Int J Mol Sci*. 2020;21:700. doi: 10.3390/ijms21030700

32. Liu X, Yuan L, Chen F, Zhang L, Chen X, Yang C, Han Z. Circulating miR-208b: A potentially sensitive and reliable biomarker for the diagnosis and prognosis of acute myocardial infarction. *Clin Lab*. 2017;63:101–109. doi: 10.7754/Clin.Lab.2016.160632

33. Lu F, Li SZ, Gao X, Gong YN, Shi PX, Zhang C. Diagnostic value of circulating miR-208b and miR-499 in peripheral blood of patients with acute myocardial infarction. *J Biol Regul Homeost Agents*. 2020;34. doi: 10.23812/20-171-L-2

34. Hu X, Liu B, Wu P, Lang Y, Li T. LncRNA Oprm1 overexpression attenuates myocardial ischemia/reperfusion injury by increasing endogenous hydrogen sulfide via Oprm1/miR-30b-5p/CSE axis. *Life Sci*. 2020;254. doi: 10.1016/j.lfs.2020.117699

35. Seronde M-F, Vausort M, Gayat E, Goretti E, Ng LL, Squire IB, Vodovar N, Sadoune M, Samuel J-L, Thum T, Solal AC, Laribi S, Plaisance P, Wagner DR, Mebazaa A, Devaux Y. Circulating microRNAs and outcome in patients with acute heart failure. *PLoS One*. 2015;10:e0142237. doi: 10.1371/journal.pone.0142237

36. Kontaraki JE, Marketou ME, Parthenakis FI, Maragkoudakis S, Zacharis EA, Petousis S, Kochiadakis GE, Vardas PE. Hypertrophic and antihypertrophic microRNA levels in peripheral blood mononuclear cells and their relationship to left ventricular hypertrophy in patients with essential hypertension. *J Am Soc Hypertens*. 2015;9:802–810. doi: 10.1016/j.jash.2015.07.013

37. Syed M, Ball JP, Mathis KW, Hall ME, Ryan MJ, Rothenberg ME, Yanes Cardozo LL,

Romero DG. MicroRNA-21 ablation exacerbates aldosterone-mediated cardiac injury, remodeling, and dysfunction. *Am J Physiol Metab*. 2018;315:E1154–E1167. doi: 10.1152/ajpendo.00155.2018

38. Recamonde-Mendoza M, Werhli A V., Biolo A. Systems biology approach identifies key regulators and the interplay between miRNAs and transcription factors for pathological cardiac hypertrophy. *Gene*. 2019;698:157–169. doi: 10.1016/j.gene.2019.02.056

39. Huang W, Resch S, Oliveira RK, Cockrill BA, Systrom DM, Waxman AB. Invasive cardiopulmonary exercise testing in the evaluation of unexplained dyspnea: Insights from a multidisciplinary dyspnea center. *Eur J Prev Cardiol*. 2017;24:1190–1199. doi: 10.1177/2047487317709605

40. Han W, Han Y, Liu X, Shang X. Effect of miR-29a inhibition on ventricular hypertrophy induced by pressure overload. *Cell Biochem Biophys*. 2015;71:821–826. doi: 10.1007/s12013-014-0269-x

41. Shi J yu, Chen C, Xu X, Lu Q. miR-29a promotes pathological cardiac hypertrophy by targeting the PTEN/AKT/mTOR signalling pathway and suppressing autophagy. *Acta Physiol*. 2019;227:e13323. doi: 10.1111/apha.13323

42. Martinelli NC, Cohen CR, Santos KG, Castro MA, Biolo A, Frick L, Silvello D, Lopes A, Schneider S, Andrades ME, Clausell N, Matte U, Rohde LE. An analysis of the global expression of microRNAs in an experimental model of physiological left ventricular hypertrophy. *PLoS One*. 2014;9:e93271. doi: 10.1371/journal.pone.0093271

43. Seok HY, Chen J, Kataoka M, Huang Z-P, Ding J, Yan J, Hu X, Wang D-Z. Loss of microRNA-155 protects the heart from pathological cardiac hypertrophy. *Circ Res*.

2014;114:1585–1595. doi: 10.1161/CIRCRESAHA.114.303784

44. Kwong R, González A, Ph D, Colan SD, Seidman JGG, Ph D, Díez J, Ph D, Seidman CE, Ho CY, López B, Coelho-Filho OR, Lakdawala NK, Cirino AL, Jarolim P, Kwong R, González A, Colan SD, Seidman JGG, Díez J, Seidman CE. Myocardial fibrosis as an early manifestation of hypertrophic cardiomyopathy. *N Engl J Med*. 2010;363:552–563. doi: 10.1056/NEJMoa1002659

45. Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: Genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. *Circ Res*. 2017;121:749–770. doi: 10.1161/CIRCRESAHA.117.311059

46. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci*. 2008;105:13027–13032. doi: 10.1073/pnas.0805038105

47. Jafarinejad-Farsangi S, Gharibdoost F, Farazmand A, Kavosi H, Jamshidi A, Karimizadeh E, Noorbakhsh F, Mahmoudi M. MicroRNA-21 and microRNA-29a modulate the expression of collagen in dermal fibroblasts of patients with systemic sclerosis. *Autoimmunity*. 2019;52:108–116. doi: 10.1080/08916934.2019.1621856

48. Huang Y-HH, Yang Y-LL, Wang F-SS. The role of miR-29a in the regulation, function, and signaling of liver fibrosis. *Int J Mol Sci*. 2018;19:1889. doi: 10.3390/ijms19071889

49. Assmann TS, Recamonde-Mendoza M, Costa AR, Puñales M, Tschiedel B, Canani LH, Bauer AC, Crispim D. Circulating miRNAs in diabetic kidney disease: case–control study and in silico analyses. *Acta Diabetol*. 2019;56:55–65. doi: 10.1007/s00592-018-1216-x

50. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, Van Der Made I, Herias V, Van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE. MiR-133 and miR-30 Regulate connective tissue growth factor: Implications for a role of micrornas in myocardial matrix remodeling. *Circ Res*. 2009;104:170–178. doi: 10.1161/CIRCRESAHA.108.182535

51. Verjans R, Peters T, Beaumont FJ, Van Leeuwen R, Van Herwaarden T, Verhesen W, Munts C, Bijnen M, Henkens M, Diez J, De Windt LJ, Van Nieuwenhoven FA, Van Bilsen M, Goumans MJ, Heymans S, González A, Schroen B. MicroRNA-221/222 family counteracts myocardial fibrosis in pressure overload-induced heart failure. *Hypertension*. 2018;71:280–288. doi: 10.1161/HYPERTENSIONAHA.117.10094

52. Bargaje R, Gupta S, Sarkeshik A, Park R, Xu T, Sarkar M, Halimani M, Roy SS, Yates J, Pillai B. Identification of novel targets for miR-29a using miRNA proteomics. *PLoS One*. 2012;7. doi: 10.1371/journal.pone.0043243

53. Silverman MG, Yeri A, Moorthy MV, Camacho Garcia F, Chatterjee NA, Glinge CSA, Tfelt-Hansen J, Salvador AM, Pico AR, Shah R, Albert CM, Das S. Circulating miRNAs and risk of sudden death in patients with coronary heart disease. *JACC Clin Electrophysiol*. 2020;6:70–79. doi: 10.1016/j.jacep.2019.08.011

54. Liu Y, Afzal J, Vakrou S, Greenland G V., Talbot CC, Hebl VB, Guan Y, Karmali R, Tardiff JC, Leinwand LA, Olgin JE, Das S, Fukunaga R, Abraham MR. Differences in microRNA-29 and pro-fibrotic gene expression in mouse and human hypertrophic cardiomyopathy. *Front Cardiovasc Med*. 2019;6. doi: 10.3389/fcvm.2019.00170

FIGURE LEGENDS

Figure 1. Literature search findings and selection.

Figure 2. Heat-map representation of the miRNAs expression in HCM. The colors represent the expression values: green indicates down-regulated; red indicates up-regulated, and yellow represents no change in regulation. Studies are identified by numbers (that correspond to the reference number).

Table 1. Down and Black checklist for quality assessment.

Ref.	Reporting (11 points)	External validity (3 points)	Bias (7 points)	Selection bias (6 points)	Power (5 points)	Total (32 points)
Palacín 2011 [17]	7	0	5	4	2	18
Wang 2012 [18]	8	0	5	3	2	18
Leptidis 2013 [19]	9	0	5	2	3	19
Palacín 2013 [20]	7	0	5	1	5	18
Kuster 2013 [21]	6	0	5	1	3	15
Song 2014 [22]	8	0	5	4	4	21
Roncarati 2014 [13]	10	2	5	4	5	26
Derda 2015 [14]	9	1	5	2	5	22
Fang 2015 [15]	11	1	5	4	5	26
Ntelios 2017 [23]	7	0	5	2	5	19
Li 2018 [24]	9	0	5	2	5	21
Zhou 2019 [25]	6	0	5	3	5	19
Huang 2020 [26]	11	2	5	2	4	24

Table 2. Characteristics of selected articles and evaluated miRNAs.

Ref.	Num. of subjects	Patients characteristics	Control population	Origin/Sample	Method	miRNA
Roncarati 2014[13]	41	71% male, 50±13 years, MWT=21±5 mm, NYHA II (34.2%);	Healthy (n=41)	Blood/ Serum	RT-PCR	1, 16, 21, 26a, 27a, 29a, 30a, 126-3p, 126-5p, 133a, 143, 145, 155, 195, 199a-3p, 199a-5p, 208a, 208b, 214, 499-3p, 499-5p
Derda 2015[14]	51	HNCM (n=23); 74% male, 56 years, IVS=19.5 mm, HOCM (n=28); 32% male, 56 years, IVS=20.2 mm	Healthy (n=22), severe aortic stenosis (n=47), amyloidosis (n=9)	Blood/ Serum	RT-PCR	1, 21, 29a, 29b, 29c, 133a, 155, 499
Fang 2015[15]	55	$T_1 \geq 470$ ms (n=28); 49.1±14 years, IVS=20.3±5.8 mm; $T_1 < 470$ ms (n=27); 49.9±10.8 years, IVS=19.1±3.3 mm	Healthy (n=4)	Blood/ Serum	Microarray +RT-PCR	10b-5p, 15a-5p, 17-5p, 18a-5p, 19b-3p, 21-5p, 29a-3p, 30d-5p, 96-5p, 133a-3p, 146a-5p, 192-5p, 193-5p, 200a-3p, 296-5p, 373-3p
Zhou 2019[25]	69	Fibrosis positive (n=30): 66.7% male, 48.4±6.3 years; MWT>30 mm 10%; NYHA II (33.3%) Fibrosis negative (n=39): 69.2% male, 47.9±5.2 years, MWT>30 mm 12.8%, NYHA II 38.5%	no	Blood/ Serum	RT-PCR	21, 26a, 29a, 27a, 30a, 126-3p, 126-5p, 133a, 143, 145, 155, 195, 214, 199a-3p, 199a-5p, 499-5p,
Palacín 2013[20]	24	70% male, age range: 16 – 65 years. IVS=21.5 mm; all NYHA III-IV;	Healthy (n=25)	Blood/ Serum	RT-PCR	1, 30b, 92a, 133a, 133b, 199a-3p, 208a, 208b, 451, 483-5p

Huang 2020[26]	42	HOCM submitted to septal myectomy; 69% male, 46.2±15.4 years; IVS=23.1±15.4 mm	Healthy (n=30)	Surgical myectomy + Blood/Serum	RT-PCR	9, 15, 19b, 21, 31, 33, 93, 155, 221, 222, 433
Li 2018[24]	10	End-stage HCM submitted to heart transplant 39±14 years; 80% male; IVS=13.9±3.1 mm	Healthy (n=10) and end-stage DCM (n=10)	Transplanted heart/ myocardium	RT-PCR	1, 10b, 21, 23a, 27a, 29a, 133a, 142, 155, 199a-3p, 199a-5p, 214, 497
Palacín 2011[17]	2	End-stage HCM submitted to heart transplant MYH7 mutation	Non-failing donor hearts (n=5)	Explanted hearts/ Myocardium	RT-PCR	1, 30b, 92a, 93, 133a, 133b, 191, 19a-3p, 125a-3p, 208a, 208b, 218, 223, 374, 451, 454, 483-5p, 495, 590-5p
Wang 2012[18]	4	HOCM submitted to septal myectomy	Non-failing donor hearts (n=8)	Surgical myectomy/ Myocardium	RT-PCR	221
Leptidis 2013[19]	5	End-stage HCM 41±5.8 years; 2 men and 3 women;	Non-failing donor hearts (n=4), and DCM patients submitted to LVAD (n=7)	Explanted heart/ Myocardium	RT-PCR	1-3p, 23a-3p, 23b-3p, 24-3p, 29b-3p, 30d-5p, 125a-3p, 126-3p, 133a-3p, 143-3p, 145-5p, 193-3p, 197-3p, 331-3p, 342-3p, 361-5p, 365-3p, 455-3p, 1975-3p, 1978
Kuster 2013[21]	6	Septal myectomy; MYBPC3 mutation All male; 22–62 years; IVS range: 18-30 mm	Non-failing donor hearts (n=6)	Surgical myectomy/ Myocardium	RT-PCR	10a*, 10b, 10b*, 34*, 96, 181a-2*, 184, 204, 222*, 371-3p, 383, 497, 708,

Song 2014[22]	16	Septal myectomy 75% male; 15–65 years; IVS range: 18–35 mm;	no	Surgical myectomy/ Myocardium	Microarray+ RT-PCR	21, 130b, 132, 139-3p, 139-5p, 144, 144*, 150, 363, 451, 486-3p, 1246, 3141
Ntelios 2017[23]	4	Septal myectomy (n=4),	no	Surgical myectomy/ Myocardium	RT-PCR	29a

Abbreviations: HCM=hypertrophic cardiomyopathy; HNCM: hypertrophic non-obstructive cardiomyopathy; HOCM: hypertrophic obstructive; IVS: interventricular septum; LVAD: left ventricular assisted device; MWT=maximal wall thickness; NYHA=New York Heart Association; cardiomyopathy; Ref.=reference; RT-PCR: real-time polymerase chain reaction

*Overall characteristics of the cohort. Author did not provided specific data on patients submitted to miRNA analysis.

Table 3. Phenotype correlation of miRNAs in patients with HCM

Reference	miRNA(s)	Main findings
		Blood/serum tissue
Roncarati 2014[13]	mir-21 mir-27a mir-29a mir-155 mir-199a-5p	MWT on TTE: mir-29a (r=0.463); MWT on CMR: mir-29a (r=0.412); mir-21 (r=0.406); mir-155 (r=0.356); LVMI on CMR: mir-27a (r=0.380); mir-199-5p (r=0.421); Hypertrophy index on TTE: mir-29a (r=0.475); mir-21 (r=0.346); mir-155 (r=0.308); Fibrosis on CMR: mir-29a (r=0.691)
Derda 2015[14]	mir-29a mir-155	MWT on TTE: mir-29a (r=0.523) HOCM/MYH7 gene: mir-29a MYBPC3: mir-155
Fang 2015[15]	mir-18a-5p mir-146-5p mir-30d-5p mir-17-5p mir-200a-3p mir-19b-3p mir-21-5p mir-193a-5p mir-10b-5p mir-15a-5p	Correlation with T_1 time on CMR: mir-30d-5p (r=-0.599); mir-146a-5p (r=-0.658); mir-96-5p (r=-0.579). Detection of fibrosis: mir-10b=5p (AUC=0.701); mir-15a-5p (AUC=0.694); mir-17-5p (AUC=0.722); mir-18a-5p (AUC=0.742); mir-19b-3p (AUC=0.712); mir-21-5p (AUC=0.710); mir-29a-3p (AUC=0.717); mir-30d (AUC=0.729); mir-133a-3p (AUC=0.663); mir-192-5p (AUC=0.681); mir-193-5p (AUC=0.709); mir-146-5p (AUC=0.737); mir-200a-3p (AUC=0.721); mir-296-5p (AUC=0.681); 8 miRNAs together (mir-18a-5p, mir-30d-5p, mir-21-5p, mir-193-5p, mir-10b-5p, mir-15a-5p, mir-296-5p, and mir-29a-3p, AUC=0.870).

	mir-192-5p mir-296-5p mir-29a-3p mir-133-3p	
Zhou 2019[25]	mir-29a	Highly increased in fibrosis group
Huang 2020[26]	mir-19b mir-221	MWT on TTE and CMR: mir-221 (r=0.318 and r=0.342) LVMI on TTE: mir-221 (r=0.638) LVMI on CMR: mir-19b (r=0.214), mir-221 (r=0.725) LVEF on TTE: mir-221 (r=-0.557) LGE on CMR: mir-221 (r=0.630) Collagen volume fraction: mir-221 (r=0.459) Fibrosis on CMR: mir-221 (AUC=0.764); mir-19b (AUC=0.201)
Myocardium tissue		
Li 2018[24]	mir-1-3p	TTE: LVEDD (r=0.771) and LVEF (r=0.737)
Huang 2020[26]	mir-19b mir-221	Collagen volume fraction: mir-221 (r=0.516) LGE on CMR: mir-221 (r=0.307), mir-19b (r=-0.318)

Abbreviations: AUC, area under the curve; CMR, cardiac magnetic resonance; HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; LGE, late gadolinium enhancement; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MWT, maximal wall thickness; TTE, transthoracic echocardiogram.

FIGURES

Figure 1.

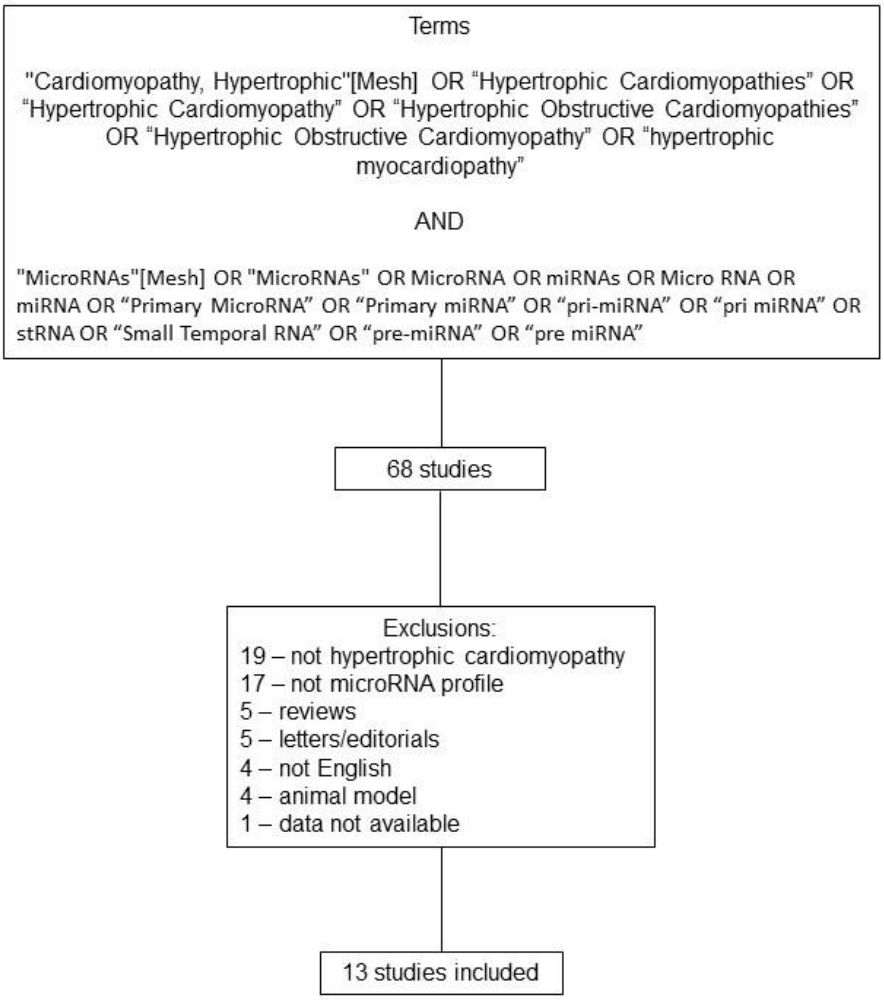
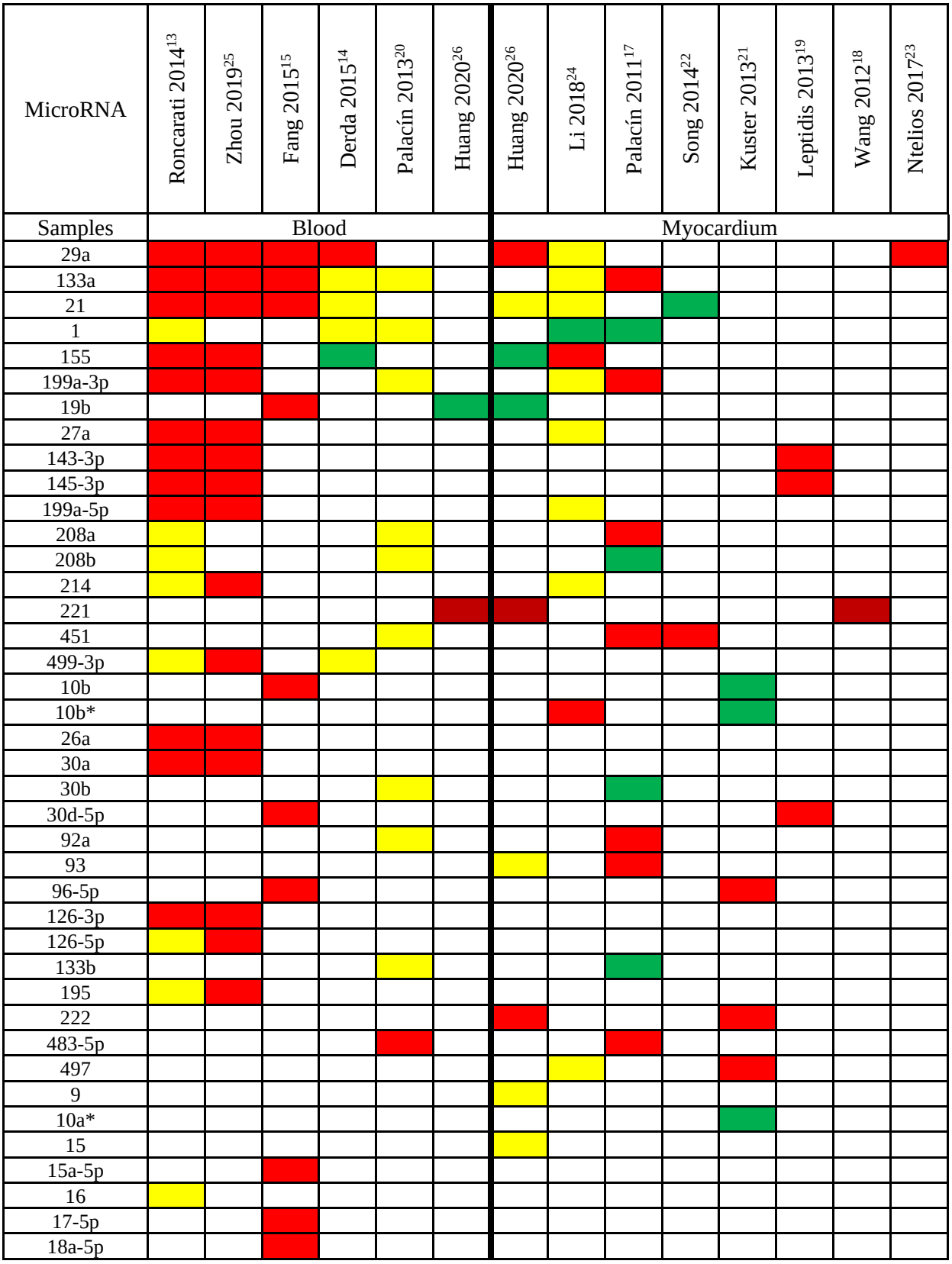
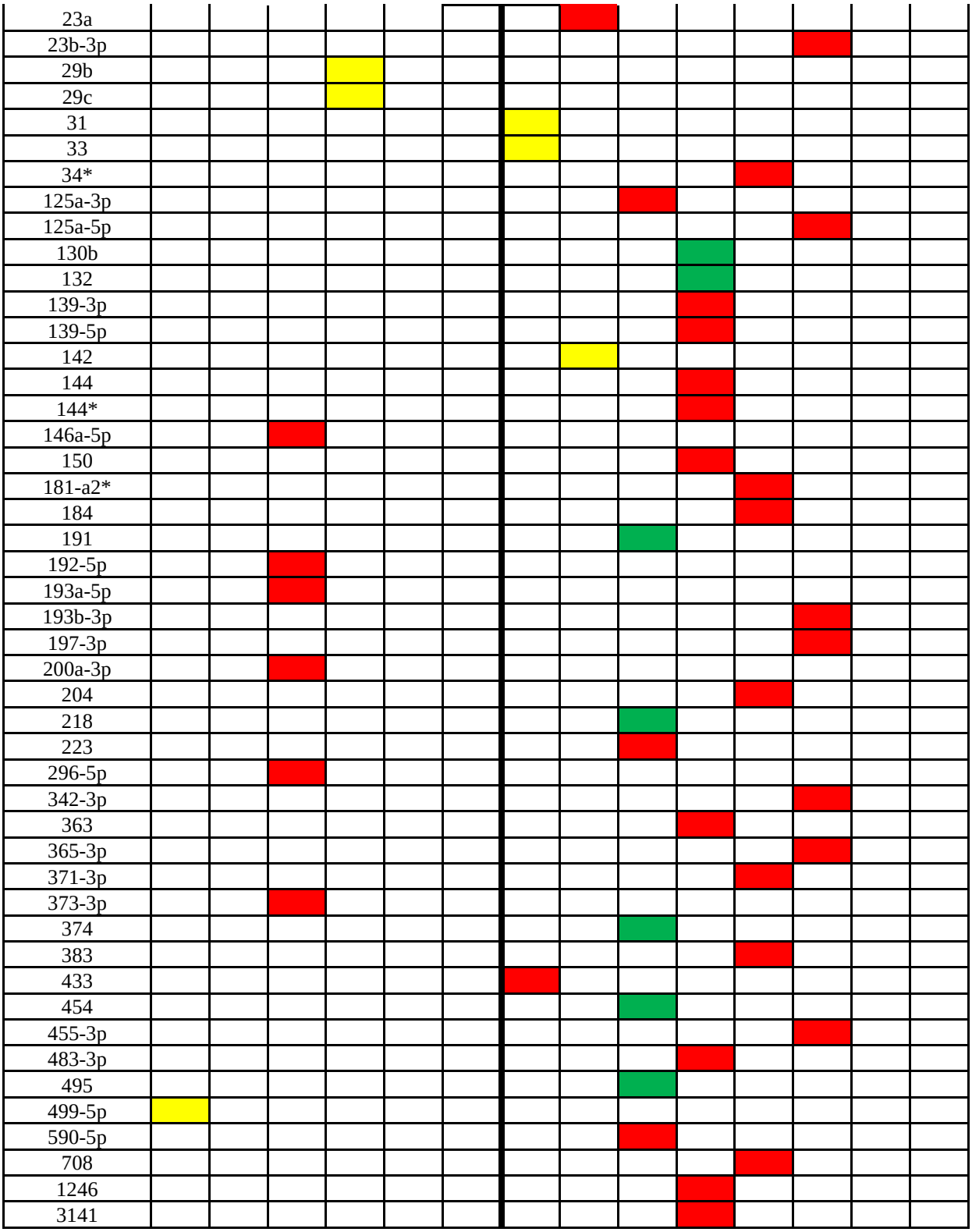


Figure 2.


MicroRNA
Roncarati 2014[13]
Zhou 2019[25]
Fang 2015[15]
Derda 2015[14]
Palacín 2013[20]
Huang 2020[26]
Huang 2020[26]
Li 2018[24]
Palacín 2011[17]
Song 2014[22]
Kuster 2013[21]
Leptidis 2013[19]
Wang 2012[18]
Ntelios 2017[23]
Samples
Blood
Myocardium
29a
133a
21
1
155
199a-3p
19b
27a
143-3p
145-3p
199a-5p
208a
208b
214
221
451
499-3p
10b
10b*
26a
30a
30b
30d-5p
92a
93
96-5p
126-3p
126-5p
133b
195
222
483-5p
497
9
10a*
15
15a-5p
16
17-5p
18a-5p


23a
23b-3p
29b
29c
31
33
34*
125a-3p
125a-5p
130b
132
139-3p
139-5p
142
144
144*
146a-5p
150
181-a2*
184
191
192-5p
193a-5p
193b-3p
197-3p
200a-3p
204
218
223
296-5p
342-3p
363
365-3p
371-3p
373-3p
374
383
433
454
455-3p
483-3p
495
499-5p
590-5p
708
1246
3141

7. CONCLUSÕES E CONSIDERAÇÕES FINAIS

O estudo demonstrou que a análise genético-molecular através de um painel de mutações é factível em nosso meio. Foram identificadas mutações patogênicas em 47% dos indivíduos testados, predominantemente nos genes sarcoméricos MYH7 e MYBPC3. Esses achados são consonantes com os referidos na literatura.

Estratégia para rastreamento de familiares em primeiro grau de pacientes com CMH em cenários de indisponibilidade de testagem genética foi empregada com elevada acurácia. A presença de marcadores clínicos ou ECG anormal isoladamente foram capazes de detectar os familiares em primeiro grau acometidos pela CMH. O ECG apresentou papel decisivo no rastreamento de famílias, de modo a sinalizar a presença de remodelamento elétrico em estágios precoces da doença. Um modelo modificado de ECG para detecção da doença foi aplicado com incremento da acurácia diagnóstica.

A revisão sistemática realizada permitiu identificar diversos microRNAs expressos na CMH, majoritariamente em *up-regulation.* Somente alguns avaliaram a correlação fenotípica com hipertrofia ventricular esquerda e fibrose miocárdica. Entre estes, os mir-21 e mir-29a apresentaram marcada correlação fenotípica. O mir-29a demonstrou achados mais consistentes, de modo a tornar-se um potencial biomarcador da CMH em seus diversos estágio evolutivos.

8. PERSPECTIVAS FUTURAS

Os familiares em primeiro grau dos pacientes com mutação detectada para CMH serão posteriormente genotipados através de método Sanger. Todos os indivíduos com teste genético positivo, probandos e familiares, serão avaliados para detecção de mir-21 e mir-29a em sangue periférico. Esta análise visa correlacionar a expressão dessas moléculas com o fenótipo da CMH, de modo a identificar sua associação com formas pré-clínicas da doença e seu potencial como biomarcador. Esta análise encontra-se em andamento. Contudo, devido às restrições impostas pela pandemia de SARS-Cov-2, não possui data prevista para término.

O rastreamento de familiares em primeiro grau encontra-se em fase de ampliação da amostra. O projeto auxiliar já se encontra aprovado no Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre. Está previsto o recrutamento adicional de 50 familiares em primeiro grau de pacientes com a doença em seguimento no respectivo ambulatório de nossa instituição. Os indivíduos selecionados serão submetidos a um questionário a ser testado de auto-avaliação de sintomas, ECG e ecocardiograma. Todos serão submetidos a seguimento pelo período de três anos, incluindo aqueles selecionados na primeira amostra de familiares submetidos a genotipagem. Esta análise tem como objetivo comparar a evolução sintomática, eletrocardiográfica e ecocardiográfica em 3 anos de indivíduos selecionados através de avaliação clínica isolada versus complementada por análise genético-molecular.

9. APÊNDICES

Termo de Consentimento Livre e Esclarecido

Título do estudo: "Estudo das formas familiares de cardiomiopatia hipertrófica através de indicadores clínicos e genético-moleculares"

Pesquisadores Responsáveis: Profa. Beatriz Piva e Mattos e Prof. Marco Antonio Rodrigues Torres

Pesquisadores: Profa. Úrsula Matte, Dr. Fernando Luís Scolari,

Local: Serviço de Cardiologia – Hospital de Clínicas de Porto Alegre

Convidamos você a participar deste estudo, cujo objetivo é avaliar pacientes portadores de uma doença cardíaca denominada cardiomiopatia hipertrófica através de consulta médica e exames. Serão também examinados os familiares em primeiro grau, pois esta doença pode afetar outros membros da família e produzir ou não sintomas.

Os pacientes e os familiares que desejarem participar do estudo serão avaliados através de:

1. Consulta médica única incluindo entrevista e exame físico
2. Eletrocardiograma em repouso
3. Ecocardiograma transtorácico uni e bidimensional com Doppler
4. Coleta de pequena amostra de sangue venoso periférico para extração do DNA

Os exames cardiológicos aos quais você será submetido são considerados de rotina, são externos, tem baixo risco e acarretam mínimo desconforto, exigindo apenas que você permaneça deitado. A amostra de sangue coletada será utilizada para estudo genético-molecular. O material extraído poderá ser armazenado por até 05 anos e será utilizado para confirmar a presença da doença.

Não são conhecidos riscos relacionados à sua participação neste projeto. Você também não terá benefícios diretos, entretanto sua colaboração auxiliará no melhor entendimento da cardiomiopatia hipertrófica. Você não terá despesas e nem qualquer compensação financeira por participar desta pesquisa. Você receberá uma via assinada deste documento e outra ficará guardada com o pesquisador.

Você poderá optar por não participar desta pesquisa ou retirar seu consentimento a qualquer momento sem que tenha qualquer prejuízo ao atendimento recebido no HCPA. Seus dados serão confidenciais, ou seja, seu nome não será divulgado. Este projeto foi aprovado pelo Comitê de Ética em Pesquisa e, se você tiver qualquer dúvida em relação aos seus direitos como participante de uma pesquisa, poderá entrar em contato com o Comitê pelo telefone 3359-7640. Se você tiver

qualquer dúvida sobre a pesquisa, poderá entrar em contato com um dos pesquisadores acima pelo telefone 3359-8344. Horário de atendimento do CEP: 08h à 17h – pelo telefone 3359-7640

Eu, _____, concordo em participar da presente pesquisa

Porto Alegre,_____

(data)

_____ _____

(Nome do Paciente) (Assinatura)

_____ _____

(Nome do Pesquisador) (Assinatura)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do Projeto: PESQUISA DE MUTAÇÕES CAUSADORAS EM PACIENTES PORTADORES DE CARDIOMIOPATIA HIPERTRÓFICA ATRAVÉS DE PAINEL GENÉTICO POR SEQUENCIAMENTO DE NOVA GERAÇÃO

Convidamos você a participar deste estudo, cujo objetivo é analisar a presença de mutações genéticas em pacientes portadores de uma doença cardíaca denominada cardiomiopatia hipertrófica. Esta pesquisa será baseada na amostra de sangue que você já coletou ao participar de outro estudo nº150607, denominado ESTUDO DAS FORMAS FAMILIARES DE CARDIOMIOPATIA HIPERTRÓFICA ATRAVÉS DE INDICADORES CLÍNICOS E GENÉTICO-MOLECULARES. As alterações, caso sejam encontradas e você deseje conhecer, serão informadas assim que estiverem disponíveis. Você não precisará realizar nova coleta de sangue, necessitará, apenas, autorizar a utilização deste material.

O atual projeto de pesquisa não apresenta riscos para você ou desconfortos. Você poderá, ou não, ter benefícios diretos ao participar do estudo. No entanto, sua colaboração possibilitará o melhor entendimento da cardiomiopatia hipertrófica. Caso você seja familiar de um paciente que apresenta a doença e seja diagnosticada alguma alteração, você será encaminhado para acompanhamento conforme sua necessidade.

Sua participação na pesquisa é voluntária, portanto, não é obrigatória. Caso você decida não participar ou desistir e retirar seu consentimento, não haverá nenhum prejuízo ao atendimento que você possa vir a receber da instituição. Não está previsto nenhum tipo de pagamento pela sua participação. Você não terá nenhum custo, mas poderá ser ressarcido por despesas decorrentes de sua participação. Caso ocorra alguma intercorrência resultante de sua participação, você receberá todo o atendimento necessário, sem nenhum custo pessoal. Os informações coletadas são confidenciais. Será realizada consulta de dados clínicos registrados no prontuário médico e no projeto nº 150607. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes. Portanto, seu nome não aparecerá na publicação dos resultados.

Caso você tenha dúvidas, poderá entrar em contato com a pesquisadora responsável Professora Beatriz Piva e Mattos ou a equipe de pesquisa pela telefone (51) 3359-8344 ou ainda, com o Comitê de ética em Pesquisa do Hospital de Clínicas de Porto Alegre (HCPA), situado no 2º andar, sala 2227, de segunda a sexta-feira das 08 horas às 17 horas, ou pelo telefone (51) 3359-7640.

( ) Aceito que o material coletado seja utilizado para nova análise.

Esse Termo é assinado em duas vias, uma para o participante e outra para os pesquisadores.

_____ _____

Nome do participante da pesquisa Assinatura

_____ _____

Nome do pesquisador que aplicou o Termo Assinatura

Local e Data: _____