FACULTAD DE MEDICINA
UNIVERSIDAD DE CANTABRIA

GRADO EN MEDICINA

TRABAJO FIN DE GRADO

Rendimiento diagnóstico del estudio genético en la Miocardiopatía Hipertrófica en Cantabria

Diagnostic yield of Hypertrophic Cardiomyopathy genetic test in Cantabria

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Santander, Junio 2020

AGRADECIMIENTOS

En primer lugar, quiero agradecer a los tutores de este trabajo, los doctores Jesús Zarauza y Luis Ruiz, del Servicio de Cardiología del Hospital Marqués de Valdecilla, el esfuerzo y dedicación a la hora de ayudarme a realizar este trabajo, y agradecerles también todo lo que me han enseñado sobre Cardiología a lo largo de este último curso de la carrera. No quiero olvidar mencionar que parte de este trabajo se ha realizado bajo condiciones extraordinarias marcadas por la pandemia de COVID-19, que no ha impedido que ambos se siguieran volcando en mi enseñanza.

Quiero agradecer también a la doctora Ana Fontalba, del Servicio de Genética del Hospital Marqués de Valdecilla, su disposición para ayudarme en la recopilación de los datos genéticos empleados en este estudio y en la correcta interpretación de los mismos. Y también hacer mención a los doctores Miguel Lafarga y Maite Berciano por ayudarme a comprender la histología de la Miocardiopatía Hipertrófica para poder realizar una adecuada selección de las imágenes.

Por último, agradecer también a mis compañeros y familia, que me han aconsejado y acompañado a lo largo de este último año de carrera.

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ABSTRACT:

BACKGROUND: Hypertrophic cardiomyopathy is the most frequent inheritable heart disease, affecting 1 in 500 people. The inheritance pattern is autosomal dominant, and it is caused by mutations in sarcomere protein genes, which induce ventricular hypertrophy that cannot be explained by loading conditions. Its way of manifestation is widely diverse, from asymptomatic patients to patients suffering from sudden cardiac death. The role of genetics is becoming increasingly important in studying the variants involved, their prognosis, and their role in family screening.

OBJECTIVES: making a review about HCM and studying the yield of the genetic test in HCM population in Cantabria.

MATERIALS AND METHODS: creation of a data base with clinical, imaging and genetic information of HCM proband cases in Cantabria who underwent a genetic test between January 2018 and January 2020,

RESULTS: 140 patients were included (60.71% were men; mean age was 61.33 ±1.25 years). 33 (23.57%) patients had positive test, 19 (13.57%) had VUS test and 88 studies (62.86%) were negative. The most frequent pathogenic variants were MYBPC3 (42.42%) and MYH7 (39.39%).

DISCUSSION AND CONCLUSION: the yield of genetic test was lower than the prevalence of pathogenic variants described in literature (23.57% versus about 60%). There were no data enough to assess definitive conclusions

KEY WORDS : Hypertrophic Cardiomyopathy, genetic test, pathogenic variants, diagnosis, clinical manifestations.

RESUMEN:

INTRODUCCIÓN: La miocardiopatía hipertrófica es la cardiopatía hereditaria más frecuente, afectando a 1 de cada 500 personas. Está producida por mutaciones en los genes sarcoméricos que se heredan de manera autosómica dominante, produciendo hipertrofia ventricular no explicable por otras causas, pudiendo no producir síntomas o incluso producir muerte súbita cardiaca. El estudio genético está cobrando cada vez más importancia a la hora de identificar las variantes, su pronóstico y su papel en el cribado familiar.

OBJETIVOS: hacer una revisión de la literatura sobre MCH y estudiar la rentabilidad del restudio genético de la enfermedad en Cantabria.

MATERIALES Y MÉTODOS: creación de una base de datos con las características clínicas, radiológicas y genéticas de los casos índice de Cantabria a los que se les realizó el estudio genético entre enero de 2018 y enero de 2020.

RESULTADOS: se incluyeron 140 casos índice (60.71% eran hombres; la media de edad fue 61.33 ±1.25 años). 33 (23.57%) estudios fueron positivos, 19 (13.57%) tuvieron un

resultado con significado incierto y 88 (62.86%) fueron negativos.

DISCUSIÓN Y CONCLUSIONES: la rentabilidad del estudio genético encontrando variantes patogénicas fue menor que en la literatura (23.57% frente a 60%). No hubo datos suficientes para extraer conclusiones definitivas.

PALABRAS CLAVE: Miocardiopatía Hipertrófica, estudio genético, variante patogénica, diagnóstico, manifestaciones clínicas.

1. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common monogenic inheritable cardiomyopathy, transmitted as an autosomal dominant pattern, which is clinically identified in 1 of 500 people. The disease is defined by the presence of increased left ventricular (LV) wall thickness explained by sarcomeric mutations, and not related to abnormal loading conditions.[1, 8, 11, 13, 20]

HCM is defined by the presence of increased left ventricular (LV) wall thickness (more than 15 mm wall thickness by cardiac magnetic resonance, computed tomography or echocardiography). [11, 20]

At present, pathogenic or likely pathogenic variants have been identified in 60% of patients with HCM, which are responsible of deficits in sarcomere relaxation and consequent clinical manifestations or even sudden death. Over 450 mutations in 20 sarcomere and myofilament-related proteins have been identified for HCM. However, most mutations affect genes encoding for the β-myosin heavy chain (*MYH7*) and myosin binding protein C (*MYBPC3*) of the cardiac sarcomere. [1, 7, 9, 11, 14, 20]

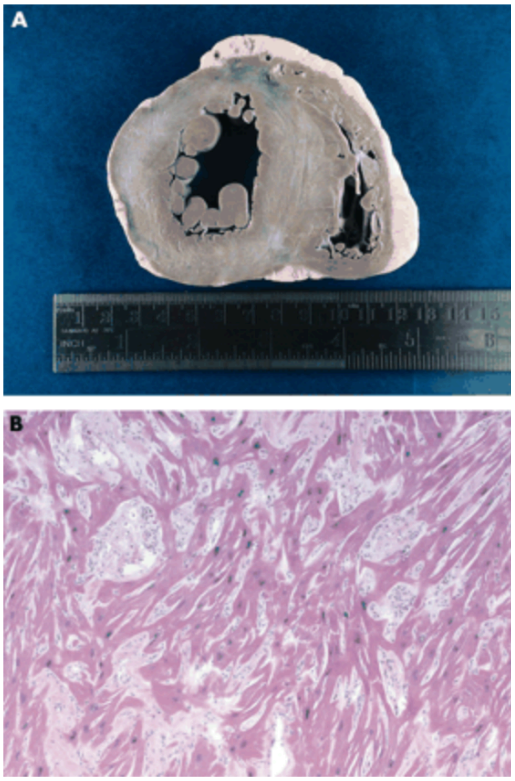

Figure 1. (A) Photograph of a necropsy heart at mid septal level from an individual with HCM. The hypertrophy involves both the free and septal walls of the left ventricle. (B) Haematoxylin and eosin stained section of myocyte disarray in HCM. Obtained from *Hughes SE, McKenna WJ. «New insights into the pathology of inherited cardiomyopathy.» Heart (2005): 257-264.*

Patients with HCM exhibit a variable phenotype with ventricular hypertrophy being the cardinal manifestation, myocyte hypertrophy and disarray (figure 1) as well as interstitial fibrosis as key pathological hallmarks, and impaired ventricular filling and dynamic left ventricular outflow tract (LVOT) obstruction as important pathophysiologic features. [2, 6]

Current annual disease related mortality is estimated at 0,5% in adolescence and adulthood, which means it is similar to general population. More than 60% of the survivors with HCM report normal heart function (NYHA class I). Although sudden cardiac death (SCD) is uncommon, it is a devastating complication. SCD results from ventricular arrhythmias caused by autonomic overactivity secondary to LVOT obstruction, microvascular ischemia, myocardial fibrosis, and myocyte disarray. It can be treated effectively with an implanted cardioverter/defibrillator (ICD), which is recommended in patients at a high risk of SCD based on multiple clinical factors . [2, 8, 11, 13,

It is important to highlight the fact that in less than 10% of cases, left ventricle hypertrophy not related to abnormal loading conditions is caused by mutations in genes related to metabolic disorders (Anderson-Fabry disease), mithocondrial cardiomyopathies, or neuromuscular diseases (Friedreich´s ataxia) and not directly related to sarcomeric mutations, which are considered as HCM phenocopies. [3, 4, 13, 20]

1.1. **PATHOGENESIS**

Mutations in the genes encoding sarcomeric proteins induce several alterations in the protein expression, morphology and function and influences sarcomere assembly, force generation, intracellular calcium homeostasis, and ATPase activity. Secondary to the gene defect, pro-hypertrophic and pro-fibrotic signaling (e.g., TGF-β, Periostin, MEF2, MAPK, RAS, NFAT) are activated, and there is also an altered gene expression, post-translational modifications, mitochondrial dysfunction and mitotic factors. All these modifications lead to the histopathological changes, provoking myocite disarray and myocardial fibrosis, which finally manifests as left ventricle hypertrophy (LVH). HCM pathogenesis and its consequences are summarized in Figure 3.[6, 8]

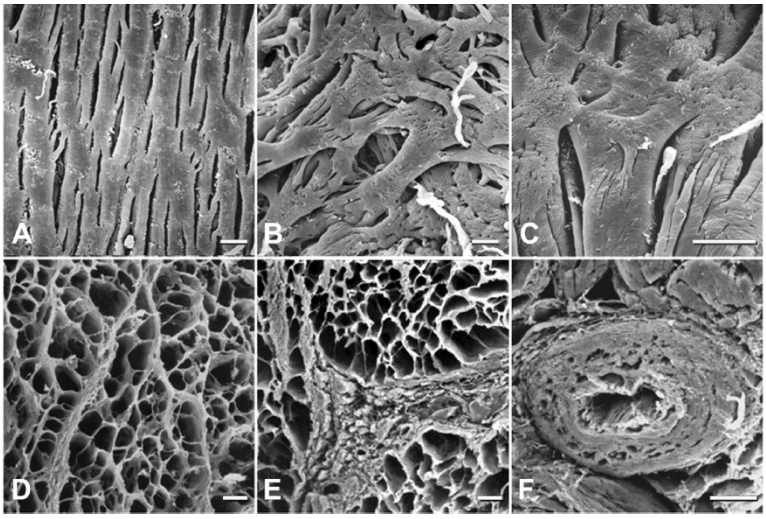

Figure 2. Scanning electron microscopy of cardiomyocytes from a normal subject (A, D) and a patient with HCM (B, C, E, F) are shown. Images were obtained after the removal of either connective tissue (A, B, C) or nonfibrous tissue (D, E, F). In the heart sample from the patient with HCM, myocardial disarray, abnormal branching, and bizarre myocardial hypertrophy (B, C) increased interstitial connective tissue elements (E) and thickened small intramural vessels, in which luminal narrowing (F) can be observed. Magnifications are x600 (A, B, F), x1000 (C), and x300 (D, E). Bar indicates 20 µm. Obtained from *Kanzaki Y, Yamauchi Y, Okabe M, Terasaki F, Ishizaka N. «Three-Dimensional Architercture of Cardiomyocytes and Connective Tissues in Hypertrophic Cardiomyopathi: A Scanning Electron Microscopic Observatio.» Images in Cardiovascular Medicine (2012): 738-739.*

The LVH and the myofibrillar disarray and fibrosis are responsible of hemodynamic derangements, as prolonged and impaired ventricular relaxation, with losing chamber compliance and abnormal intracellular calcium use, which finally provokes the diastolic dysfunction theoretically present in all patients with HCM (Figure 2).[8]

Narrowing of the LVOT from septal hypertrophy or abnormal subvalvular mitral apparatus, results in turbulent flow that “drags” the redundant mitral valve into the LVOT, resulting in decreased forward flow and systolic anterior motion (SAM)-mediated mitral regurgitation. One third of patients with HCM have LV outflow tract obstruction

(LVOTO), defined as LVOT gradient ≥30 mmHg as an instantaneous peak Doppler LV outflow tract pressure at rest or during physiological provocation such as Valsalva maneuver, standing and during exercise, and is dynamic in nature, a unique feature of HCM. The severity of the LVOTO is influenced by the ventricular volume, which in turn is a function of the interactions between myocardial contractility, ventricular preload and afterload. Increases in contractility, reductions in preload and afterload reduce ventricular volume and cause or intensify obstruction; one third part of HCM patients have that kind of obstruction remarkable only during maneuvers which disturb the loading conditions or the LV centrality.[8, 18, 20]

Mitral regurgitation is also related to high LV filling pressures. Mitral regurgitation finally leads into atrial enlargement and in final steps of HCM, it can be responsible of pulmonary hypertension.[8, 20] Furthermore, papillary muscle abnormalities such hypertrophy, anterior or lateral displacement and direct insertion into the anterior mitral valve leaflet and mitral valve alterations as elongation or tissue disturbances can also be responsible of increasing or helping the obstruction of the outflow tract. Those abnormalities in the mitral valve can also result in mitral regurgitation, which knowing the pathophysiology of LOVTO can make easier to understand why it is a condition closely related to LVOTO.[11, 18, 20]

Despite LVOTO is frequently found in HCM patients, it is not specific of the disease, since it can be developed in other circumstances, which can be very common in general population, like hypertension, hypovolemia, calcification of the mitral annulus and situations which makes the myocardial contractility to be increased, such those situations which induce reflex syncope. [10, 18, 20]

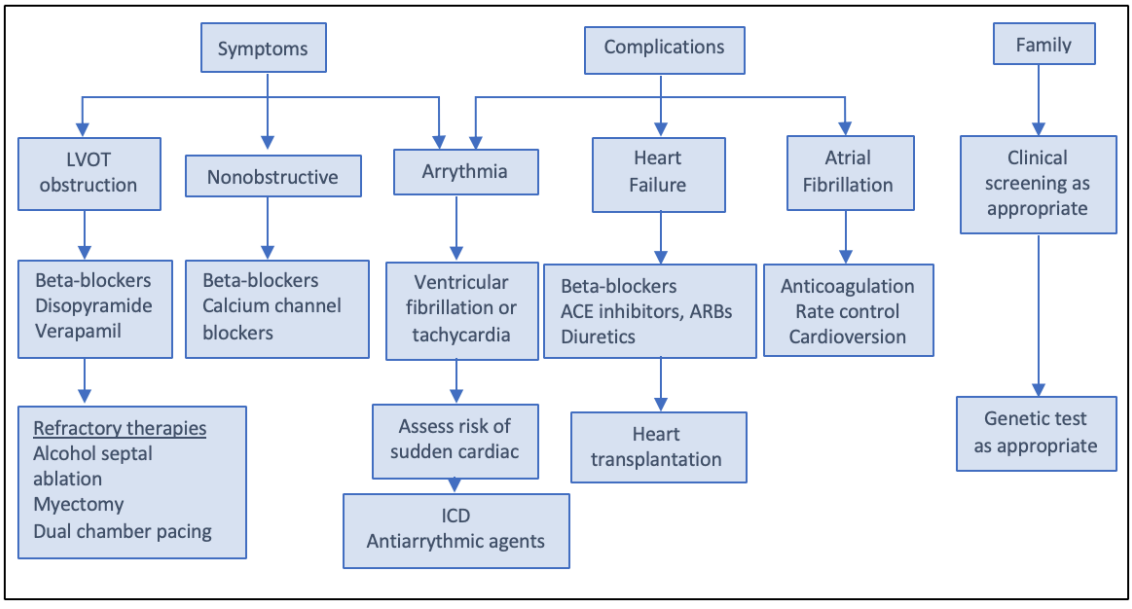

Figure 3. Schematic summarizing the pathogenesis of HCM. Modified from *CM, Wolf. «Hypertrophic cardiomyopathy: genetics and clinical perspectives.» Cardiovascular Diagnosis and Therapy (2019): 388-415*

1.2. **CLINICAL MANIFESTATIONS AND COMPLICATIONS**

Clinical manifestations of HCM are notably variable. There is a large range of symptomatology, as most patients are asymptomatic, whereas other suffer from sudden death as onset disease. Between these two possibilities, there is a wide variety of symptoms, which makes the symptomatology unespecified and may delay the diagnosis and the therapeutic intervention. Complications secondary to HCM can also be responsible of the clinical symptoms and signs which can make a patient stop being asymptomatic and beginning to develop clinical manifestations. Those complications are specially related to arrythmias and heart failure symptoms (Figure 4). [4, 8, 11, 18, 20]

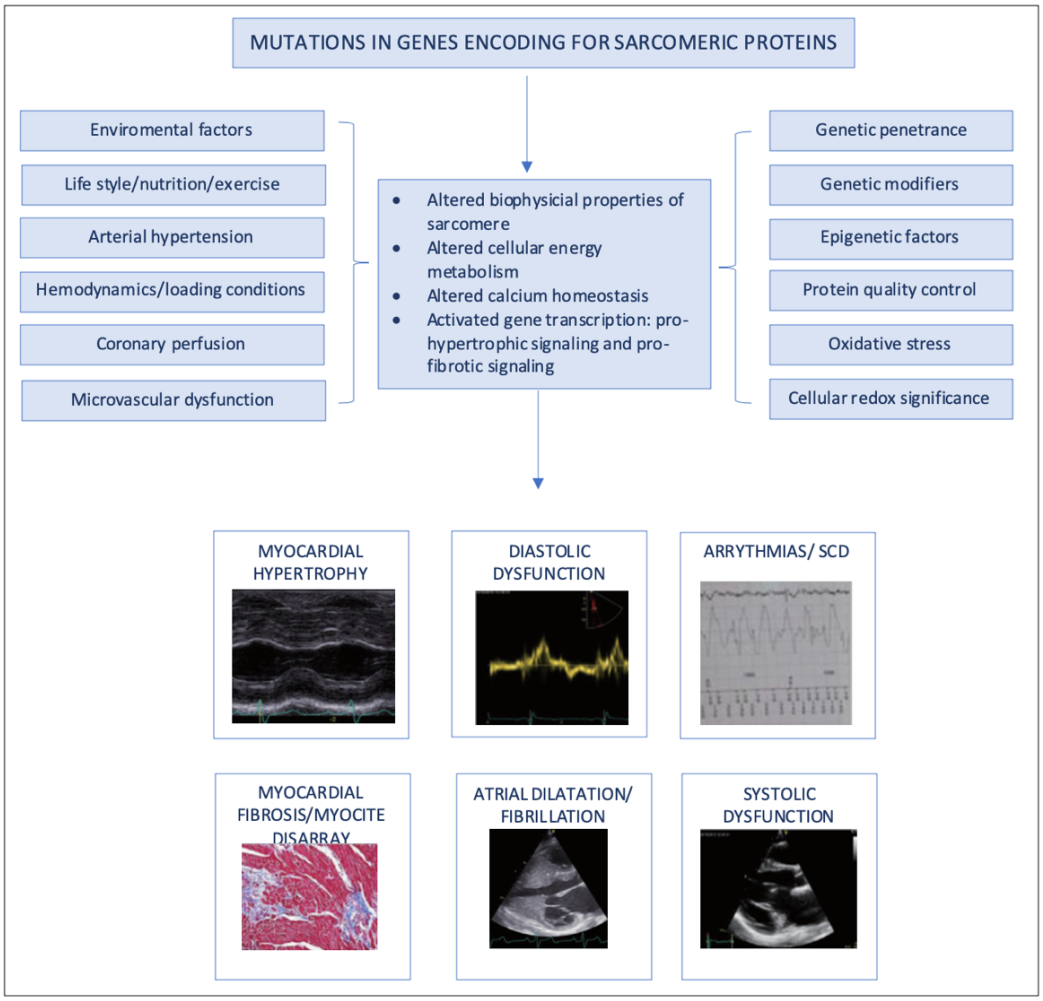

Figure 4. Schematic summarizing the main HCM symptoms, complications and management. *Took from Jacoby D, DePasquale E, McKenna W. 2013. «Hypertrophic cardiomyopathy: Diagnosis, risk stratification and treatment.» Canadian Medical Association Journal 127-134*

Sudden cardiac death is mainly developed due to ventricular arrythmias, as non-sustained ventricular tachycardia is suffered by 20-30% of HCM patients and they suppose a major risk factor for sudden cardiac death. The process responsible of arrythmias in HCM is not properly known yet; it may be implied the ventricular remodeling due to cardiac hypertrophy and fibrosis and myocardial ischemia and disarray. [2, 20]

People with HCM use to complain of exertional dyspnea or fatigue which may be induced by diastolic dysfunction and LVOTO, which is also associated to worse prognosis. Mitral regurgitation can be responsible of dyspnea too. Systolic function is usually preserved until final steps of the disease, so it is not so strongly related to dyspnea as the other parameters until HCM is really developed. LVOTO can be suspected due to a systolic ejection murmur which is best heard in the between the apex and the left sternal border of increasing intensity with decreasing preload maneuvers. [10, 18, 20]

Chest pain is a common symptom which takes place in HCM patients, most of the time due to injured coronary microvascular function caused by altered intramural vessels which induce an inadequate vasodilation. It could have typical features of angina or not. The chest pain can be limiting and supposes a challenge in order to differentiate it only through clinical data from classical obstructive atherosclerotic coronary disease. LVOTO can also be implied in chest pain.[2, 14, 20]

Palpitations suppose another HCM symptom, which can be caused by cardiac contractions or ventricular ectopy, and even due to non-sustained ventricular tachycardia. However, if palpitations episode is longer than a few minutes, atrial fibrillation and ventricular arrhythmia should be suspected, although other supraventricular arrhythmia could be the responsible, which also manifest with other unspecific symptoms such dyspnea, especially in case of atrial fibrillation and even, as has been said before, can provoke presyncope and syncope. Nevertheless, the risk entailed by atrial fibrillation and ventricular arrhythmia compels to exclude them by ECG monitoring, even with an implantable loop recorder if necessary. [10, 18, 20]

Syncope or pre-syncope can be suffered by patients with HCM due to LVOTO, as they can also be induced by hypovolaemia, complete heart block, sinus node dysfunction, ventricular tachycardia or abnormal vascular reflexes. [20]

Heart failure symptoms are frequent, but the clinical profile is so different among patients, as some of them can develop a systolic left ventricular dysfunction or left ventricular outflow tract obstruction. In other patients, diastolic dysfunction without reduced ejection fraction will be found. [4, 8, 18, 20]

It is important to remark the fact that atrial fibrillation and other supraventricular arrhythmias could exacerbate heart failure symptoms. In the majority of HCM patients, their myocardium develops during all their lives a progressive myocardial fibrosis and wall thinning remodeling process, which results in creeping diminished LV diastolic and systolic function, often accompanied by mild or moderate LV dilation and progressive decreased LV thickness, finally resulting in the decreasing ejection function, knowing this phase as the burn out or the hypokinetic-dilated phase. However, the outcome can be starring by LV diastolic dysfunction, sometimes associated to atrial dilation with absence or just little LV dilation. The mitral regurgitation can result in pulmonary hypertension in advanced stages. [10, 18, 20]

As has been said the chronic heart failure symptoms in HCM are similar indistinguishable from other heart failure causes, and acute heart failure precipitators are the same as general population, between the most remarkable atrial fibrillation, supraventricular

tachycardia, sustained ventricular tachycardia, acute mitral regurgitation due to acute chordal rupture or an infective endocarditis, myocardial ischemic attack and other comorbidity such anemia, hyperthyroidism and pregnancy. [20]

1.3 **IMAGING HCM**

The main imaging test when addressing a patient with hypertrophy will be echocardiography, although the suspicion may have been given by electrocardiographic patterns of left ventricular hypertrophy. Echocardiography is supported by other imaging tests such as Gadolinium-based contrast cardiac magnetic resonance (CMR) or computed tomography (CT) that also help rule out other possible etiologies that produce secondary ventricular hypertrophy. Once these possibilities have been ruled out and there is evidence of ventricular hypertrophy (more than 15 mm of ventricular wall), a genetic test will be carried out. [10, 14, 17, 20]

1.3.1. ECHOCARDIOGRAPHY

Echocardiography is the main image testing when diagnosing and monitoring HCM; maximum diastolic wall thickness should be measured using at least 2D short axis views in all LV segments, including base and apex (Figure 5), which should also include LV evaluation function, pulsed Doppler of mitral valve inflow, tissue Doppler velocities at the mitral annulus, pulmonary vein flow velocities, pulmonary artery systolic pressure and the LA size and volume. The ~~most~~ appropriate moment to measure the LV wall is the end-diastole, especially when studying short-axis views.[20]

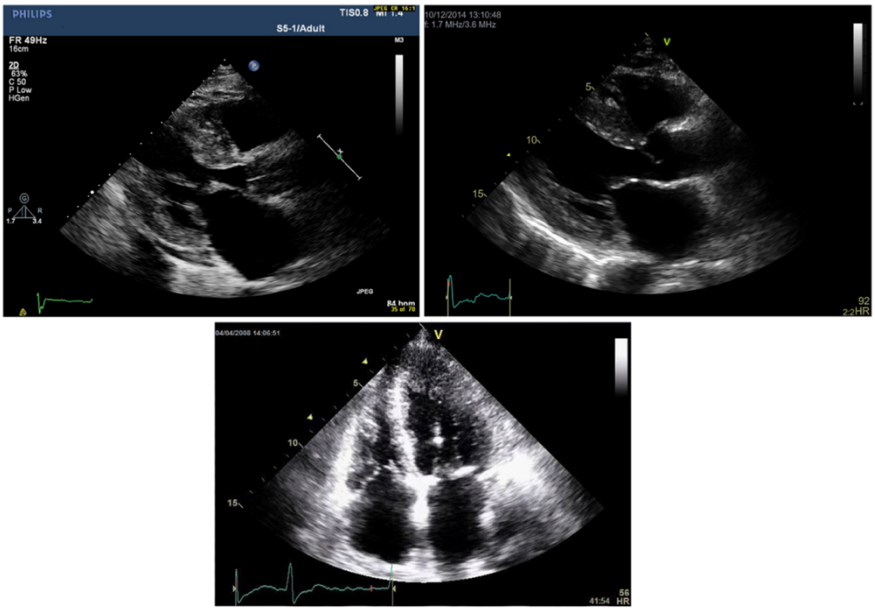

Figure 5. Asymmetric hypertrophy of the interventricular septum (A) and concentric (B) and apical (C) LV hypertrophy. Took from *Makavos G, Kairis C, Tselegkidi ME, Karamitsos T, Rigopoulos AG, Noutsias M, Ikonomidis I. «Hypertrophic cardiomyopathy: an updated review on diagnosis, prognosis and treatment.» Heart Failure*

Most important parameter when performing an echocardiography due to HCM suspicion is the LV wall thickness and its level. Once hypertrophy is detected, it is essential to examine all the segments from base to apex. Usually the most affected

myocardial segment basal interventricular septum but can be extended to other locations such lateral wall and posterior septum; apex and the right ventricle can also be affected, so parasternal and several apical views should be performed to diagnose apical HCM. The diagnose can be a challenging work when HCM is limited to one or two segments, especially if they are located in sites which being studied by echocardiography entails a difficult work.[18, 20]

The extension and the severity of the myocardial hypertrophy should be evaluated according to a standardized protocol using several echocardiography projections, which respects correct orientation and beam alignment along orthogonal planes in order to avoid false positives results due to overestimation of the wall thickness when oblique segments are studied (Figure 6). [18, 20]

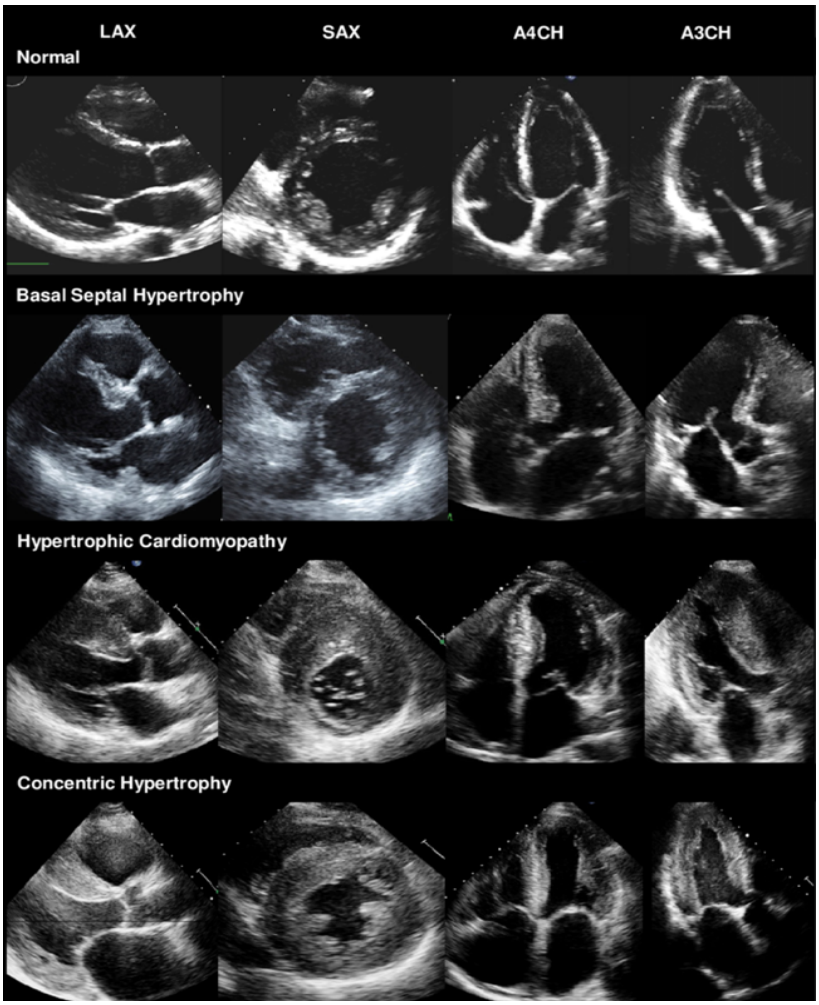

Figure 6. Comparison of normal myocardium, BSH, HCM, and concentric LVH on conventional two-dimensional echocardiography. PLAX = parasternal long axis view, SAX = short axis view, A4CH = apical 4-chamber view, A3CH= apical 3-chamber view. Took from *Kelshiker M, Mayet J, Unsworth B, Okonko D. 2013 «Basal Septal Hypetrophy.» Current Cardiology Reviews*

Echocardiography also is an important tool identifying LVOTO, which an important parameter in order to manage symptoms and making the sudden cardiac death risk stratification. LVOTO must be studied by 2D and Doppler echocardiography at rest and during a Valsalva maneuver, both with the patient sitting and then in semi-supine position. [11, 18, 20]

If there were no gradient measured after trying in those two positions, it would be recommended to trying it again on stand position. If the three positions fail in provoking LVOTO>50 mmHg, stress echocardiography should be performed but only in symptomatic patients, as in asymptomatic patients exercise provocation has not been properly evaluated, and the decision will be according to particular patients whose lifestyle and treatment depend on the presence of LVOTO gradient. Nevertheless, dobutamine echocardiography is not recommended since it could be poorly tolerated by the patient. Every patient whereas symptomatic or not must be studied in order to evaluate LVOTO gradient, as it is used to make the prognosis according to the sudden death risk. [20]

The next step in evaluating HCM by echocardiography should be the diastolic function by Doppler echocardiography as their sensitivity in measure the diastolic function is high although there is any diagnostic hallmark of disfunction (Figure 7). Loading conditions, heart rate and the patient age must be taken into account when studying the diastolic dysfunction as they are factors which can alter the test conclusions. In order to be complete and valuable, the study should include Doppler myocardial imaging, pulmonary vein flow velocities, pulmonary artery systolic pressure and LA size as part of the routine evaluation of a HCM patient. If the LV shows a restrictive filling pattern, which is defined by mitral peak velocity of early filling (E) and mitral peak velocity of late filling (A) ratio is at least bigger than 2, that patient would have a worse outcome without the ejection fraction having no role in that conclusion. [11,20]

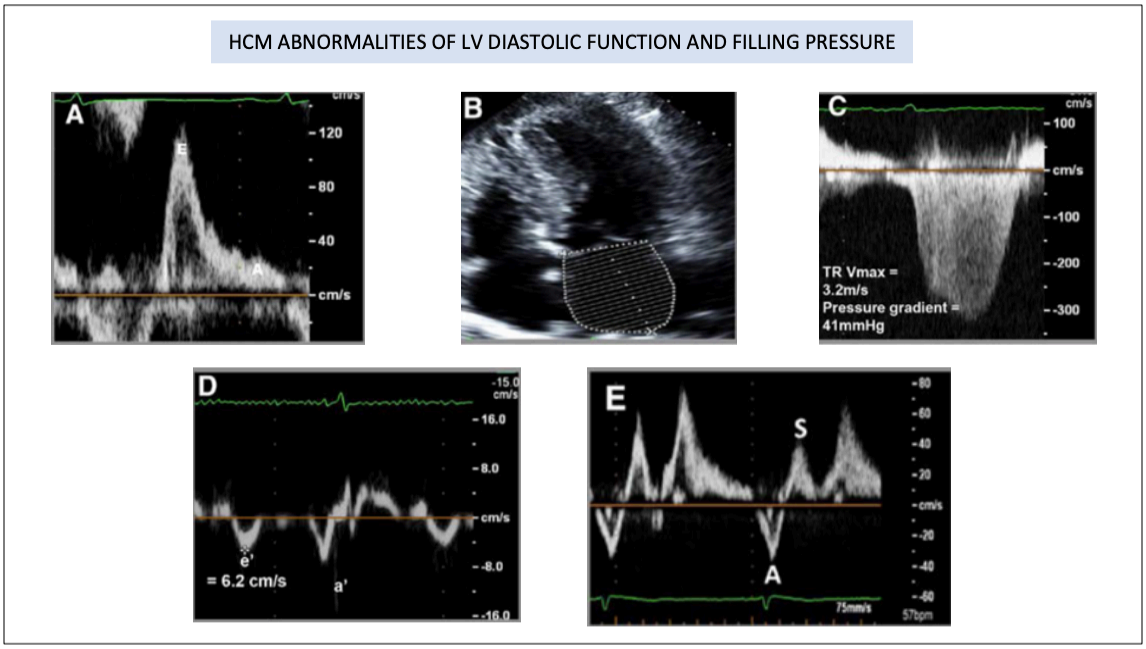

Figure 7. (A) Mitral inflow showing restrictive inflow pattern with elevated E/A ratio. (B) Four chamber view showing LA enlargement. (C) Tricuspid regurgitant jet velocity and right atrial pressure to estimate the higher pulmonary artery pressure. (D) Lateral annular tissue Doppler (TD) velocities, e´ reduced and impaired LV relaxation. (E) pulmonary venous flow velocity showing high left atrial pressures through blunted systolic atrial flow -S- and increased atrial velocitiy -A-. Took from Maron MS, Rowin EJ, Barry MJ. 2017. «How to image hypertrophic cardiomyopathy.» *Circulation: Cardiovascular Imaging* 1-15.

Although systolic contractile LV function is typically normal or increased in patients with HCM until very advanced remodeling phases, it should be studied by echocardiography,

but through myocardial longitudinal velocities and the strain and strain rate with Doppler myocardial imaging or speckle tracking techniques (Figure 8) inasmuch as the ejection fraction is poor measure in presence of myocardial hypertrophy. It is possible the ejection fraction to be normal, but the other parameters mentioned to be reduced, even before there is an observable wall thickening. The myocardial longitudinal deformation is usually reduced in the locations where observable hypertrophy is remarkable, as the longitudinal strain has become recently as the most promising parameter when evaluating the systolic function in HCM, since it can demonstrate subclinical LV systolic dysfunction due to impaired myocardial contraction even with preserved ejection fraction. [14, 20]

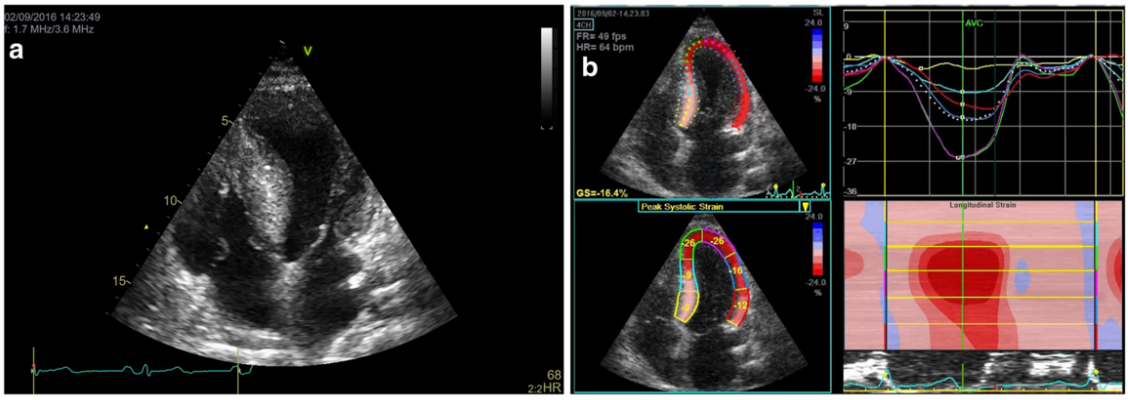

Figure 8. Hypertrophic cardiomyopathy with asymmetric hypertrophy of the interventricular septum (a) and impaired longitudinal strain − 16.4% particularly in the basal and midseptum segments (b). Obtained from *Makavos G, Kairis C, Tselegkidi ME, Karamitsos T, Rigopoulos AG, Noutsias M, Ikonomidis I. «Hypertrophic cardiomyopathy: an updated review on diagnosis, prognosis and treatment.» Heart Failure Reviews (2019).*

In conclusion, echocardiography is one of the first test which can help to approach a suspected HCM case. Furthermore, it can be useful to differentiate between HCM and other diseases responsible of LV hypertrophy, such phenocopies as Fabry disease. Information obtained like sparkling or granular myocardial tissue, soft pericardial effusion, interatrial septum or aortic valve thickening with ejection fraction reduction due to a restrictive pattern, it is highly suggestive of storage or infiltration heart disease. [13,18, 20]

Finally, transesophageal echocardiography is used to be performed when patient´s echocardiographic windows are considered poor, as it must also be performed before a surgical treatment as septal myectomy, both during the process and after in order to search for possible complications such ventricular septal defect, aortic regurgitation and residual LVOTO. [20]

If studying properly some segment is not possible with echocardiography, ultrasound contrast agents or a CMR should be performed. [20]

The 2014 ESC Guidelines recommendations for echocardiography in HCM patients are collected in Table 1.

2014 ESC GUIDELINES RECOMMENDATIONS FOR TRANSTHORACIC ECHOCARDIOGRAPHY IN HCM PATIENTS	EVIDENCE CLASS AND LEVEL
Initial evaluation in HCM patients: transthoracic 2D and Doppler echocardiography, both in rest a during the Valsalva maneuver and in sitting and semi-supine positions. Standing position echocardiography will be performed if any gradient has been provoked with the previous trials	Class I Level B
In order to provoke LVOTO through changing sitting, standing and semi supine position and mitral regurgitation induced by exercise, 2D and Doppler echocardiography should be performed in every symptomatic patient with resting or provoked instantaneous LVOTO <50 mmHg	Class I Level B
When a patient is going to be undergone to a septal alcohol ablation, a intracoronary contrast echocardiography is recommended in order to be sure of localization of alcohol	Class I Level B
Transthoracic 2D echocardiography is recommended to measure the maximum wall thickness during diastole in short axis views and in all segments from base to apex	Class I Level C
Pulsed Doppler of mitral valve inflow, tissue Doppler velocities at the mitral annulus the pulmonary vein flow velocities, pulmonary artery systolic pressure and the measure of LA volume with the aim of evaluating the LA enlargement is also recommended to be performed in all patients	Class I Level C
In case of suspicion of LV apical hypertrophy or aneurism, transthoracic echocardiography using intravenous contrast agents should be considered as CMR imaging alternative	Class IIa Level C
Patients without any symptoms but with a resting or provoked LVOTO <50 mmHg may be undergone a 2D and Doppler echocardiography in the three mentioned positions if the presence or absence of relevant LVOTO would condition the lifestyle patient´s decisions or treatment	Class IIb Level C

Table 1. Chart summarizing the 2014 ESC Guidelines recommendations in Echocardiography for HCM patients. Modified from *Task A, Elliot P, Uk C et al. «2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy.» European Heart Journal (2014): 2733-2779.*

1.3.2. CARDIAC MAGNETIC RESONANCE

Cardiac magnetic resonance (CMR) has become a very important routine resource in order to diagnose HCM (Figure 9), specially during this last decade, as it is helpful in diagnosing HCM in patients with poor acoustic windows for echocardiography and to make easier the visualization of some regions difficult to evaluate in other image test, such anterolateral left ventricle wall, the apex or the right ventricle. Despite the fact CMR is difficult to analyze, particularly when approaching a very heterogeneous disease as HCM, it is a fundamental step of the clinical diagnoses or suspicion of HCM. [8, 13, 14, 18, 20]

CMR is superior to TTE in the measurement of LV mass, but it is weakly correlated to maximum wall thickness, being normal in some HCM patients. [20]

CMR provides accurate measurements of maximal LV wall thickness and detection of apical aneurysms, thrombi, or myocardial crypts, which may represent a subtle feature of HCM in sarcomeric gene mutation-positive patient. (Figure 10). Other heart structures must be also studied, such the left atrial and the papillary muscles and trabeculations of the left ventricle wall. The sensitive of CMR is bigger than TTE in diagnosing papillary muscle abnormalities in patients with HCM. Both left and right ventricle ejection percentage should be also measured. [8, 13, 14, 18,

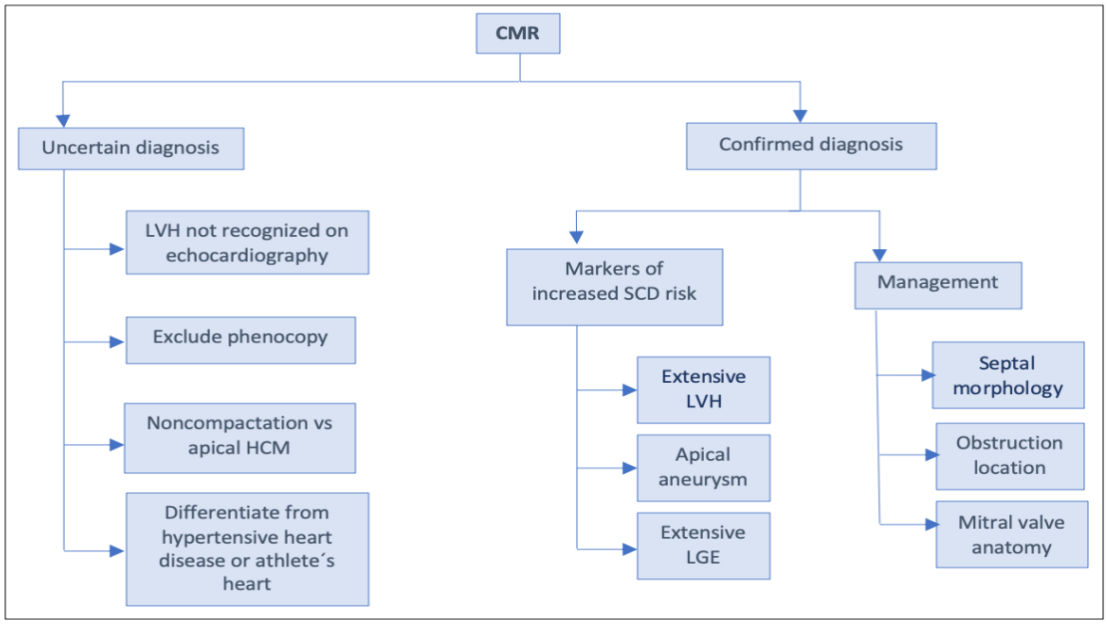

Figure 9. Clinical utility of CMR. *CMR= cardiac magnetic resonance; LVH= left ventricle hypertrophy; SCD= sudden cardiac death; LGE: late Gadolinium enhancement; HCM=* Hypertrophic Cardiomyopathy. Modified from *Geske JB, Ommen SR, Gersh BJ,. «Hyperthropic Cardiomyopathy. Clinical Update .» Heart Failure (2018): 364-375*

Late gadolinium enhancement (LGE) determines the presence and extent of myocardial fibrosis. The extent and the distribution of LGE which give more information about the risk of a certain patient, as it should be measured according to the percentage of the total left ventricle mass the enhancement occupies, since LGE is considered a modulator factor when assessing sudden cardiac death. That quantification has been developed in order to a standardized core laboratory study design, which make the measurement results reproducible. However, different in CMR hardware and diverse LGE protocols can make this protocol difficult to enforce in al centers, so qualitative estimation of the percentage of LGE by visual interpretation of an expert can be useful in the most cases. [14, 18, 20]

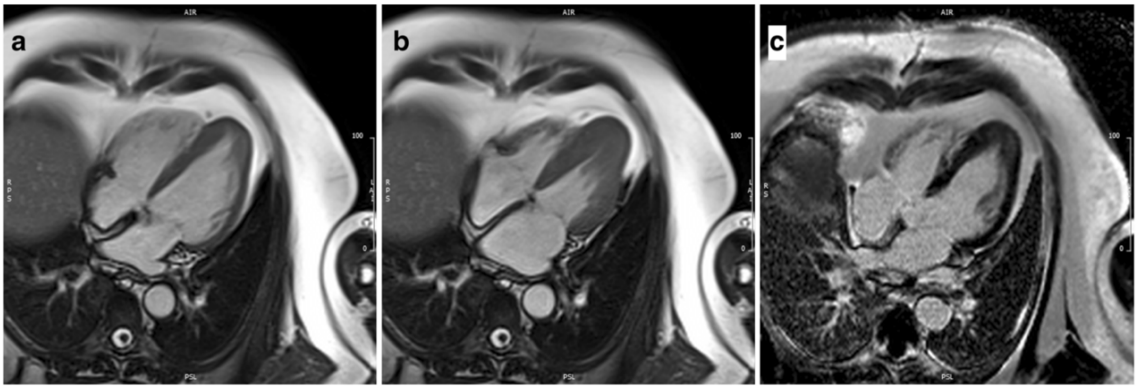

Figure 10. CMR in apical HCM. (A) End-diastolic frame of the four-chamber cine showing the apical hypertrophy. (B) End-systolic frame of the four- chamber cine showing the obliteration of the cavity at the apex and the apical displacement of papillary muscles. (C) Late gadolinium enhancement image at the four-chamber view. Obtained from *Makavos G, Kairis C, Tselegkidi ME, Karamitsos T, Rigopoulos AG, Noutsias M, Ikonomidis I. «Hypertrophic cardiomyopathy: an updated review on diagnosis, prognosis and treatment.» Heart Failure Reviews (2019)*

LGE is present in about 65% of HCM patients; the typical pattern distribution are mid-

wall hypertrophy areas and the anterior and posterior right ventricle insertion points. It could be said until HCM very advanced stages, when all the LV wall is thin and the LGE is distributed all around the wall thickness, LGE is not usual to be found in non-hypertrophied locations. Certain delay in the washout of the Gadolinium contrast is highly suggestive of space expansion, which can be caused by myocyte loss fibrosis or an infiltrative process. LGE quantification is based on an operator-defined threshold of signal intensity, which usually is from 2 to 6 standard deviations above normal myocardium. Other LGE quantification accepted are the assumption of full width at half maximal signal (FWHM) which is defined as pixels that are ≥50% the signal intensity of a hyperenhanced area or entail manual delineation of 'regions of interest' have also demonstrated high reproducibility. [14, 18, 20]

Furthermore, the LGE also have some paramagnetic properties, which show a shortening T1 relaxation times in the myocardial affected region, as LGE provides a myocardial dichotomization into bright (enhanced) versus dark (nulled), corresponding respectively to affected myocardium and normal tissue. [13,16] In this way, T1 imaging before Gadolinium contrast administration is a useful tool in order to make an estimation of myocardial volume of contrast distribution which is shown in extracellular volume fraction. Therefore, evaluation of focal and diffuse fibrosis using both LGE and T1 mapping techniques are useful tools in prognosing HCM. [14]

2014 ESC GUIDELINES RECOMMENDATIONS FOR CMR IN HCM PATIENTS	EVIDENCE CLASS AND LEVEL
In patients with poor echocardiographic windows CMR with LGE will be performed if there is HCM suspicion in order to confirm the diagnosis. There must be no contraindications	Class I Level B
CMR should always be performed and assessed by experienced teams in CMR and heart muscle diseases	Class I Level C
Patients fulfilling diagnostic crtieria for HCM should be considered to undergo a CMR with LGE to evaluate anatomy, ventricular function and myocardial fibrosis presence and extension. There must be no contraindications	Class IIa Level B
If there is suspicion of apical hypertrophy or aneurysm, CMR with LGE should be performed	Class IIa Level C
If cardiac amyloidosis is suspected, CMR with LGE should be performed	Class IIa Level C
Before performing septal alcohol ablation or myectomy, to evaluate the extent and distribution of hypertrophy and myocardial fibrosis	Class IIb Level C

Table 2. Chart summarizing the 2014 ESC Guidelines recommendations in CMR for HCM patients. Modified from *Task A, Elliot P, Uk C et al. «2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy.» European Heart Journal (2014): 2733-2779.*

The CMR utility is related to the possible association with higher incidence of myocardial stiffness and adverse LV remodeling, which includes the wall thinning, the end-systolic dimension and it is also related to increased risk of abnormalities in regional wall movement. All those events evolve in increasing heart failure with NYHA functional

classes III-IV and its consequences, as hospitalization, morbidity and all- cause heart failure related mortality. [20]

In absence of contraindications, ESC Guidelines recommend all patients with diagnosed or suspected HCM to be undergone a CMR with LGE as long as there are experienced teams in cardiac imaging and evaluating heart muscle disease as it is highly necessary the correct CMR interpretation (Table 2). [20]

CMR magnetic properties in isolation are useful to distinguish the different HCM phenocopies, since the distribution and severity of the Gadolinium contrast interstitial expansion can suggest a specific disease. In this way, Fabry´s disease typical CMR shows a reduced T1 signal without contrast at the same time there is presence of anterolateral LGE. Cardiac amyloidosis pattern are variable, as the global, subendocardial or segmental LGE, but a highly specific pattern consists of myocardial and blood-pool Gadolinium kinetics caused by myocardial and blood T1 signals. [14, 20]

It is important to remark in every patient who is suspected of cardiac amyloidosis should be undergone a CMR. Likewise, not only the distribution of gadolinium can help to make a suggestive diagnose different from HCM, but also the absence of fibrosis may be useful in order to differentiate HCM from physiological adaptation LV hypertrophy in athletes. However, it must be taken into account the fact that LGE can be absent in some HCM patients, especially in those who are young patient and have a mild disease. Comparison between CMR and echocardiography is shown in Figure 11. [14, 20]

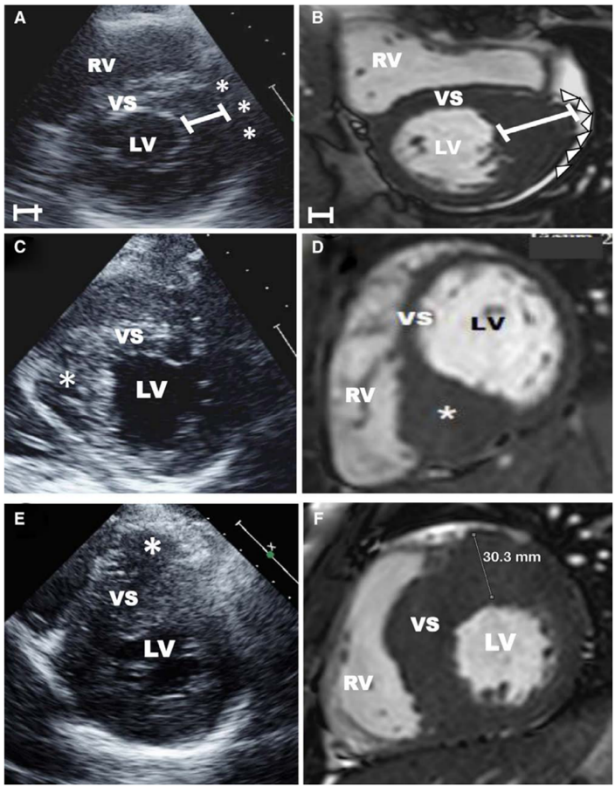

Figure 11. Cardiovascular magnetic resonance (CMR) and 2-dimensional echocardiography (2DE) imaging HCM. Took from Maron MS, Rowin EJ, Barry MJ. 2017. «How to image hypertrophic cardiomyopathy.» *Circulation: Cardiovascular Imaging* 1-15.

CMR is also useful when preparing the surgical planning for septal alcohol ablation or myectomy, especially in patients with multilevel LV obstruction, for example, both LVOTO and mid cavity HCM, or patients with RV outflow tract affected. Once the surgical procedure has been carried out, CMR offers information about the amount of tissue necrosis caused by the ablation as well as the regression of LV mass and the scarring location. [20]

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1.4. PROGNOSIS OF HCM

The next step after the confirmatory diagnosis of HCM is to make an approach of the sudden death risk as part of the routine evaluation. ACC/AHA guidelines recommend evaluating all HCM patients in order to identify the highly risk cases through non-invasive data presence. [13,18]The main factors to take into account to approach the sudden cardiac death risk are age of the patient at first evaluation, the left ventricle hypertrophy, the left atrial enlargement, the presence and severity of LVOTO, the family background of sudden cardiac death, personal history of non-sustained ventricular tachycardia and previous episodes of unexplained syncope (Figure 12). [6, 18]

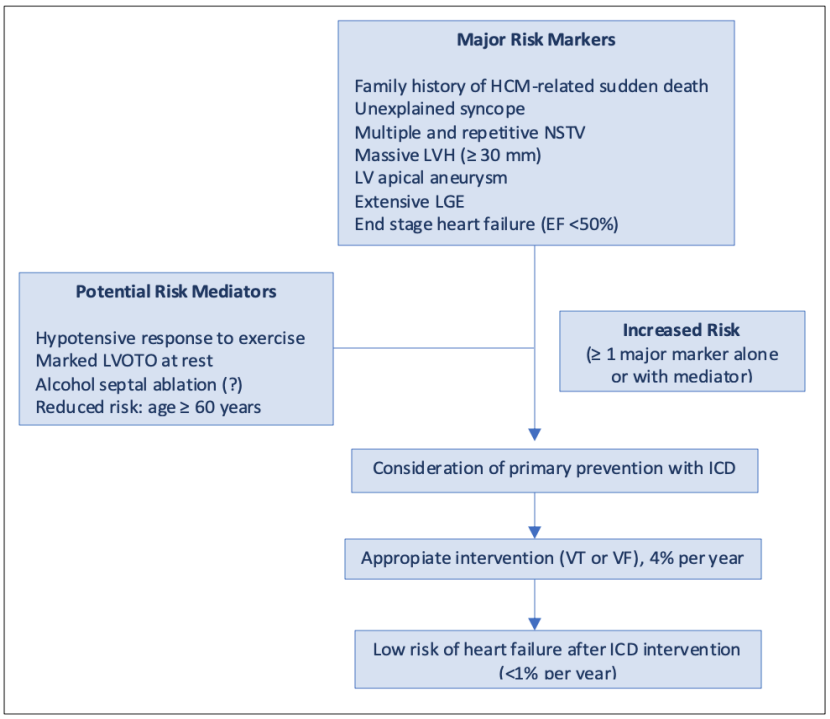

Figure 12. Risk Stratification and Primary Prevention of Sudden Death with Implantable Cardioverter–Defibrillators (ICDs) in Patients with HCM. Obtained from *Maron BJ. « Clinical Course and Management of Hypertrophic Cardiomyopathy.» The New England Journal of Medicine (2018): 655-668*

LV massive hypertrophy (about ≥30 mm) without any other risk factor, which carries more risk than is acceptable, so primary prevention with a prevention implantable cardioverter defibrillator therapy, should be offered to the patient. Altogether, it seems to be a linear correlation between wall thickness and sudden death risk in HCM, especially when talking about 30 mm measurements. Given these points, it is easy to understand the importance of correct wall thickness measurement, so any doubt should be stablished when considering CMR in the initial evaluation complementing echocardiography, particularly when hypertrophy is located in inaccessible with echocardiography. [14, 18]

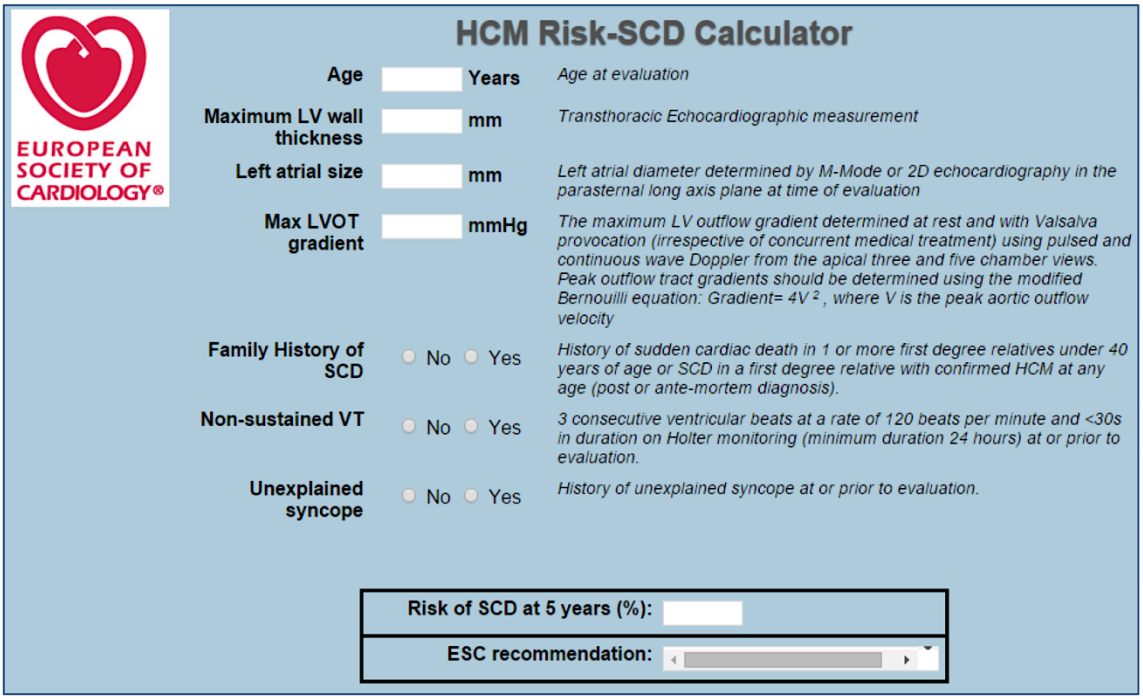

Figure 13. HCM risk sudden cardiac death calculator. *SCD= sudden cardiac death; LV=left ventricle; LVOT= left ventricle outflow tract; VT= ventricular tachycardia* Took from www.escardio.org

Although the European Society of Cardiology supports a risk stratification novel score which incorporates clinical variables (Figure 13), even those not included in ACC/AHA guidelines, as the evaluation of the LVOTO, the US/Canadian guidelines sustain LVOTO as an independent risk factor entails high difficulty to read into given the dynamic nature of gradients and the fact they can be treated. All in all, LVOTO is an important risk factor so it must be taking into account to score the patient´s sudden death risk.[13] In order to determine the absence of LVOTO and categorizing definitely the patient as non-obstructed, changing his prognosis related to adverse events and sudden death risk, exercise echocardiography may be useful. For the most part of asymptomatic patients without LVOTO, the likelihood of future development of limiting and progressive heart failure symptoms is low. What is more, most part of nonobstructive HCM patients will never need medical therapy, as drug treatment in symptomatic patients would just be limited to atrial-ventricle node blockers, improving myocardial blood flow and LV filling time. Into the small percent of the nonobstructive patients who will develop long-term symptoms, mostly related to diastolic dysfunction, the only definite treatment option will be heart transplant, like every advance diastolic heart failure patient. [14]

A relationship between LGE and long-term adverse events has been evaluated in several studies, but all of them are limited by incomplete risk assessment and differences in in scanning procedures and the absence of a protocol for LGE quantification. The analyzed data show there is an association between LGE and mortality due to cardiovascular issues, heart failure and all-cause death, although the increased risk of sudden cardiac death is not that high. However, when the distribution of LGE is extensive, about ≥15% of LV mass may identify HCM patients at increased risk for sudden death. Those patients would be benefited from primary prevention therapy. What is more, it seems the extension of LGE on CMR is consistently related to episodes of non-sustained ventricular tachyarrhythmia, but this evidence is less strong. However, non-sustained ventricular arrythmias are an independent risk factor taking into account at the time of evaluating the sudden cardiac death risk, not because of the demonstrated relation but due to the risk that arrythmia in isolation carries in any patient situation. Currently, LGE is not an independent predictor of sudden cardiac death, probably due to the recent incorporation of the CMR in the routine evaluation in HCM patients and the differences in follow-up duration and the differences when evaluating the quantity of LGE.[14,18, 20 ,]

Left atrial size measurement, although itself is not a direct parameter to make decisions in sudden death prevention, as it has not been demonstrated its independent relationship between the left atrial enlargement and the sudden death risk. However, the LA enlargement has been proved as a marker for heart failure death or atrial fibrillation; the measures from which the risk is consider are >48 mm in transverse linear dimension or ≥118 mL as chamber volume. [14, 20]

It is remarkable the importance of the HCM phenotypic subgroup of LV apical aneurysm formation, which sometimes is related to mid-cavity hypertrophy and LVOTO, which is not included in the traditional risk stratification algorithm, although aneurysms must be considered as a high risk profile owing to the more likeliness of adverse events related to the disease such thromboembolism apart from sudden death. Due to the possible echocardiography limitations in studying some patient´s LV chamber, CMR or contrast echocardiography if CMR is not available should be performed when there is suspicion of apical aneurysm or potential apical thrombus. [14, 20]

Not only sudden cardiac death is taken into account when talking about HCM prognosis, but progressive heart failure symptoms are also one of the most relevant issues when evaluating and studying the patient´s profile and long-term evolution.

Diastolic dysfunction has been studied by noninvasive test as a measure for intracardiac filling pressures. Regardless, the reality is there is any echocardiographic parameter considered as a reliable measure of filling pressures. In other words, diastolic function in HCM is very complex as it is the result of several facts including LV hypertrophy, abnormal myocardial blow flow at microvascular level, myocardial fibrosis and abnormal calcium handling. Together with this idea, it is explained why only a mild association between transmitral Doppler measures of mitral inflow (E/A) or tissue Doppler imaging from mitral annular velocities with simultaneous LA pressure measurement has been demonstrated. Furthermore, no relation has been showed between Doppler measures

of diastolic function at rest and then with exercise duration to predict future risk of progressive heart failure. [14]

Notwithstanding, HCM cases with restrictive mitral inflow patterns are an exception, as those correlations have been proved. Altogether, in every patient along with his profile, must be measured by echocardiography the filling pressures, which left atrial size, mitral deceleration time, estimated pulmonary artery pressures, pulmonary venous inflow, transmitral velocities and tissue Doppler. [14]

Figure 14 summarizes schematically the role of clinical manifestations and imaging in HCM prognosis in order to make a therapeutic approach.

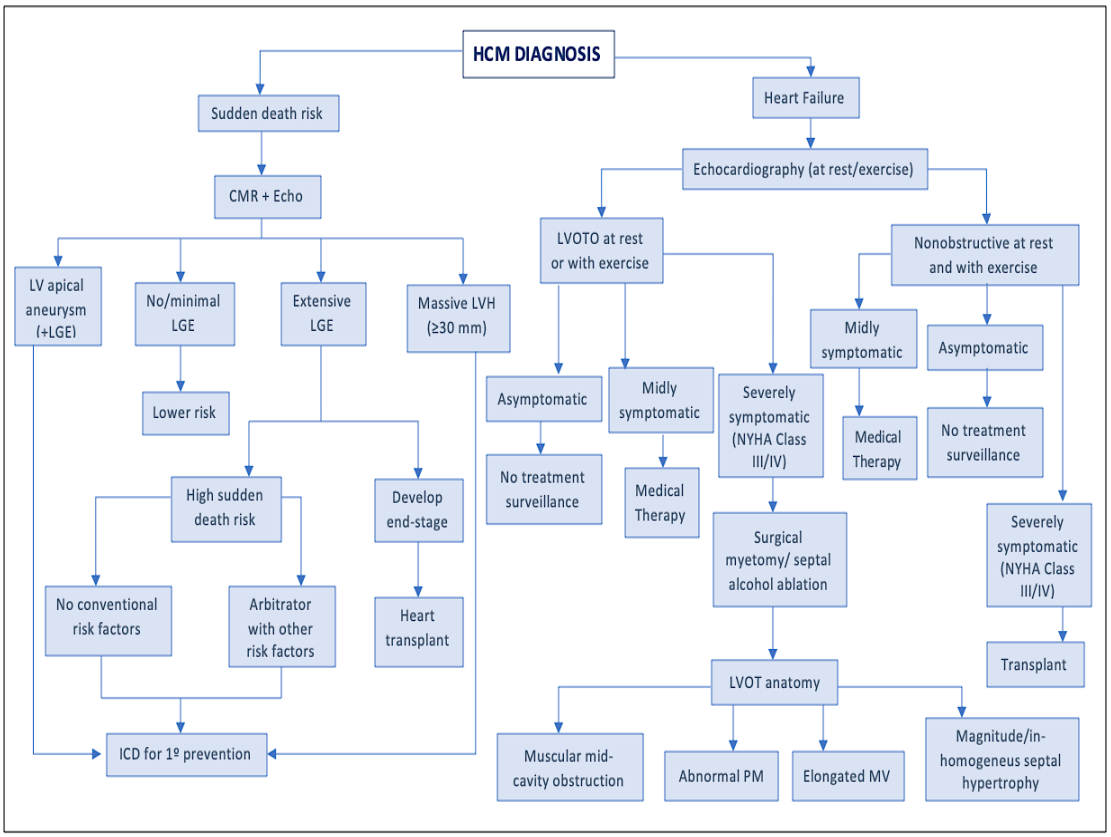

Figure 14. Flow diagram outlining the role of imaging in hypertrophic cardiomyopathy (HCM) management strategies. *HCM= hypertrophic cardiomyopathy CMR= Cardiac Magnetic Resonance; Echo= echocardiography; LV= left ventricle; LGE= late Gadolinium enhancement LVH= left ventricle hypertrophy; ICD= implantable cardiac defibrillator; LVOTO= left ventricle outflow tract obstruction; PM= papillary muscles; MV=mitral valve.* Modified from *Maron MS, Rowin EJ, Barry MJ. 2017. «How to image hypertrophic cardiomyopathy.» Circulation: Cardiovascular Imaging 1-15.*

Overall, systolic function in HCM patients is normal or even *supernormal* respecting the ejection fraction. As shown before in its corresponding section, the longitudinal strain is the most promising parameter when studying the systolic function HCM. That marker can indicate the subclinical LV dysfunction despite the ejection fraction is totally normal. Nevertheless, the prognostic correlation is unclear. In some studies, it has been shown the fact that about 10% of the nonobstructive patients develop end-stage HCM phase, which includes an ejection fraction under 50% by echocardiography or CMR; and those studies also show that percent has an increasing tendency. The only clinical markers whose correlation to end-stage HCM has been proved are the family history of end-stage

HCM and LGE ≥20%. End-stage HCM is also associated with higher likelihood of ventricular tachycardia or fibrillation and advanced heart failure symptoms, all of them supposing an important life-threatening event. Early diagnose of systolic dysfunction may allow to manage it with medical treatment and primary preventive ICD therapy. [14, 18, 20]

1.5. DIAGNOSTIC ALGORITHM

Diagnostic algorithm is represented in Figure 15. HCM diagnosis is based on the identification of increased LV wall thickness (more than 15mm or more than 3 standard deviations from predicted) which can be demonstrated by several image modalities, such echocardiography, cardiac MRI or cardiac CT. Although more than one cause can be responsible of increased wall thickness at the same time as HCM, such hypertensive cardiomyopathy, the suspicion must take place when the LV hypertrophy is more intense than expected for severity and time of evolution of the hypertension. [11, 20]

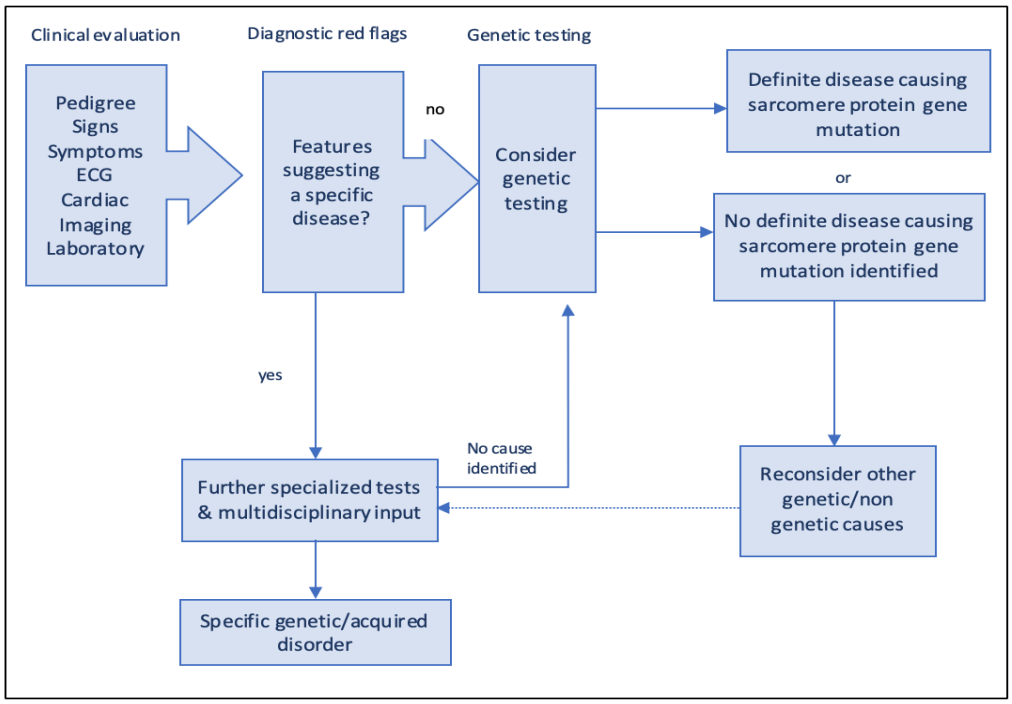

Figure 15. Schematic summarizing the general approach to the diagnosis of HCM. Took from 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy (*European Heart Journal, 2014)*

Characteristic HCM MRI shows LGE with evidence of scar tissue. However, the absence of the LGE does not discard the disease. Besides, HCM includes other traits as changes in the valvular apparatus or in the myocardial morphology or architecture, since the presence of myocardial fibrosis is not uncommon at the HCM diagnosis or during its evolution. [14,18, 20]

Sometimes HCM can be suspected by abnormalities in ECG, which must be performed in every patient with suggestive HCM symptoms or signs, although there is not a specific pattern related to HCM which can be considered pathognomonic. Every patient who shows unexplained symptoms or abnormalities in ECG or has family background of premature cardiac disease should be considered to discard HCM. [11,18,20]

Genetic test has incredibly changed the way of diagnosing HCM. The development of next-generation sequencing (NGS) allows to study thousands of genes and that is why all the guides include genetic test with the highest recommendation levels in order to diagnose HCM. Not only are they useful in identifying a pathogenic mutation responsible of HCM but also they have direct application in offering reproductive counselling and the follow-up of the first-degree familiars of a person with HCM. [1,2,3,4, 17, 20]

In summary, every patient whose symptoms or signs make us suspect of HCM will be undergone a cardiac imaging. Actually, echochardiography and cardiac MRI with Gadolinium are the two tests usually performed, and as it has been said before, an ECG will be also performed. Other tests could be made, but it is a clinical and individualized decision. Once specific syndromes mentioned before are discarded, it is time to study the likelihood of a mutation is responsible of the disease, and the prognosis of the patient and his family. In other words, a genetic test should be considered with the aim of identifying the mutation responsible of HCM. This order when proceeding with the diagnostic algorithm is more appropriate when it comes to a proband case. [1, 17, 18, 2]

1.6. GENETIC DIAGNOSE

When a patient is clinically diagnosed with HCM, it is important to take into account not only the familiar background, which must be investigated since the first suspicion of HCM, but also study the risk of the family members. [4]

Depending on the mutation implicated in each case, it would be necessary to make a screening of the first-degree relatives. [10] It is highly recommended to make a detailed family tree composed by at least 3 generations as part of the first evaluation made when HCM is suspected in a proband patient (Figure 16). They must include family background, geographic origin and symptoms related to heart diseases or sudden death (including unknown death cause and neonatal sudden infant death syndrome). It is considered to be the proband case the first patient studied in the family, and he can be dead or alive. The possibility of consanguinity must also be discarded.[4]

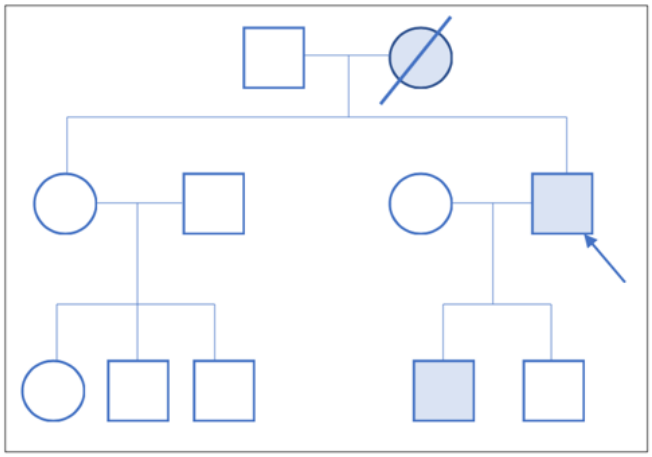

Figure 16. A genealogical tree showing autosomal inheritance in HCM. Circles represent women, rectangle men. Blue figures are patients affected from HCM, The arrow points the proband patient, as it is the first studied in the family; strikethrough circle means dead person. Modified from *Barriales R, Gimeno J, Zorio E. «Protocolo de actuación en las cardiopatías familiares: síntesis de recomendaciones y algoritmos de actuación.» Revista Española de Cardiología (2016): 300-309*

Monogenic diseases, such HCM, are caused by mutations or rare variants in some genes; most of the polymorphism are not pathogenic, but in some cases can act like modulating or modifier factors. As it has been said before, almost always HCM has been considered mainly an autosomal dominant disease, with a 50% risk of transmission to offspring. Old people and patients with non-classical features have lower likelihood of finding a mutation responsible of the HCM than people whose family is affected by the disease. [4,20] Once a pathogenic or likely pathogenic mutation is identified in the proband case, it is possible to confirm the HCM diagnosis and make a prognosis of the disease (Figure 17).

It would be recommended their relatives to be genetically tested, and if the result is positive (they carry the same mutation), they should be clinically evaluated; this combination is the best method to identified high and medium risk individuals and discharge a great number of non-carrier relatives. The genetic testing allows the screening of the patient´s relatives which facilitates the early diagnosis in pre-symptomatic phases and giving clinical follow-up and reproductive advice. The identification of a benign variant, usually a polymorphism in the proband case is not useful for the diagnosis or the relatives´ study, unless their function as modulating or modifiers factors is demonstrated in the presence of certain environmental factors. [4, 20]

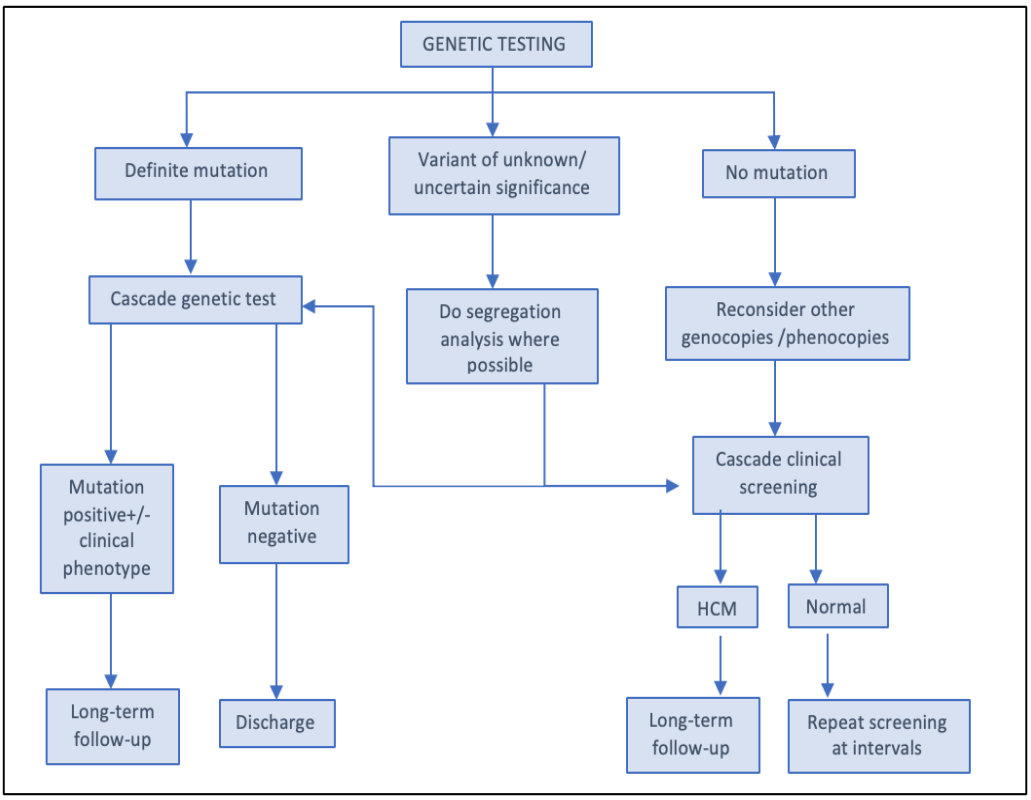

Figure 17. Schematic summarising the genetic procedure. Took from 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy (*European Heart Journal, 2014)*

The main recommendations about genetic testing in HCM by ESC 2014 guidelines are shown in Table 3.

2014 ESC GUIDELINES RECOMMENDATIONS FOR GENETIC TESTING IN HCM PATIENTS	Evidence Class and Level
Patients with diagnostic criteria of HCM, when its result allows to screen the rest of the family	Class I Level B
The genetic test must be carried out in certified laboratories with expertise in the interpretation of cardiomyopathy-related mutations	Class I Level C
Patients with clinical signs or symptoms which suggest HCM, as the genetic test would confirm the diagnosis	Class I Level B
Patients with borderline diagnosis of HCM, described as left ventricular wall thickness 12-13mm or hypertrophy in the presence of hypertension, athletic patient of valve diseases, once the patient has been studied by specialist teams	Class IIa Level C
In order to make a screening on relatives, it would be recommended to make a post-mortem genetic testing in stored tissue of deceased patients with confirmed HCM	Class IIa Level C

Table 3. Chart summarising the 2014 ESC Guidelines recommendations in CMR for HCM patients. Modified from *Task A, Elliot P, Uk C et al. «2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy.» European Heart Journal (2014): 2733-2779.*

Otherwise, variants of unknown significance (VUS), which are changes in the nucleotide sequence without current knowledge about its pathogenicity and are useless in supporting the diagnosis or stablishing the familiar disease risk. European Cardiology Society has recently recommended keeping up VUS porters, just in case the pathogenicity of the variant is discovered in the future, changing the patient´s prognosis.[4]

Despite genetic study is negative, what is, there has not been found any pathogenic or likely pathogenic mutation because there is no suspicious variant in the study, HCM and its familiar transmission must not be discarded if the patient has clinical diagnosis of the disease, as it is not known all the mutations and genes implicated in HCM, so we must consider that family affected by HCM. If the genetic testing is negative in relatives and they do not have developed any clinical sign or symptom suggesting HCM, they do not have to be followed-up, but must re-assest if they manifest any symptom or sign suggesting cardiac problems, or if new familiar information is revealed.[4,7, 20]

It is important to remark from an ethic point of view the results of studies which evidence there is not a relevant psychological adverse effect related to the clinical and genetic screening both in children and adults with risk of HCM, as long as they are managed in centers with professional physicians dedicated to Familiar Cardiopathies. [20]

2. HCM GENETICS

As previously mentioned, HCM-causing mutations are detected in about 60% of HCM probands, and they are characterized by locus and allelic heterogeneity; it is remarkable the fact that novel individual mutations are highly frequent. [1, 6, 7]

Before talking about specific HCM mutations, it is important to make a brief summary about the performance of the sarcomere to understand the level the most prevalent HCM mutations take place.

The cardiomyocyte basic motor unit is the sarcomere, which its fundamental structure are the thick and the thin filaments (Figure 18). The thick filament is basically composed by about 300 myosin molecules; each molecule is made up of 2 protein units of beta or alpha-myosin heavy chain and 4 myosin light chain molecules. The thin filament structure is a repetition of actin molecules which are closely related to the regulatory troponin complex, whose components are troponin T (TnT), troponin I (TnI) and troponin C (TnC)) and α-tropomyosin. The interaction between actin and myosin is performed by de cardiac myosin-binding protein C.[1]

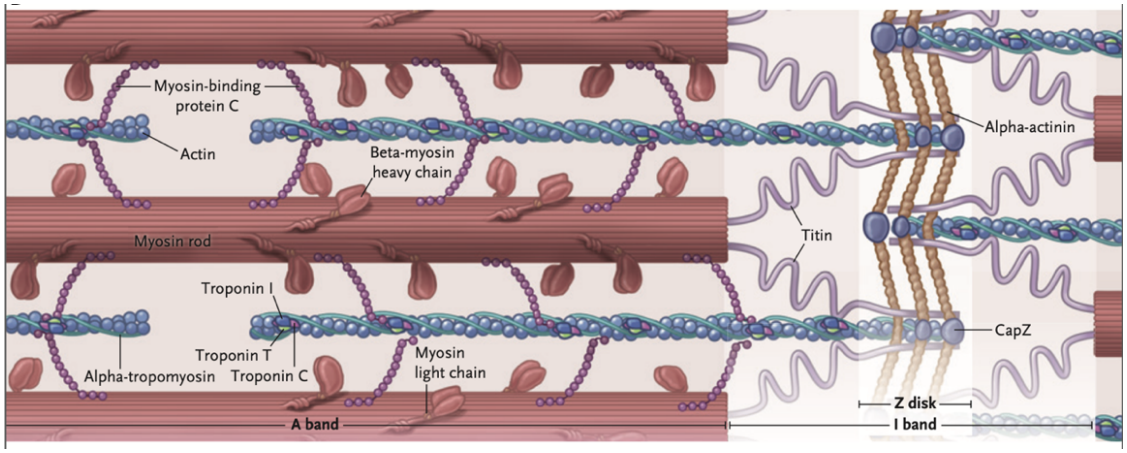

Figure 18. The sarcomere. It is composed of bands of highly organized thick and thin myofilaments. Each sarcomere is located between 2 Z bands, where titin and thin filaments are anchored. The thick filament fulfills motor and regulatory functions thanks to MYH7 and MYBPC3. Image took from Maron BJ. « Clinical Course and Management of Hypertrophic Cardiomyopathy.» The New England Journal of Medicine (2018): 655-668

Mutations in the genes encoding beta-myosin heavy chain (MYH7) and myosin-binding protein C (MYBPC3) account for the 75% of the cases a pathogenic variant is identified in a proband. MYH7 mutations are usually found in these four locations: the actin binding site, the nucleotide binding pocket, the hinge region adjacent to the binding site for two reactive thiols and in the alpha-helix close to the essential light chain interaction site. That is why the effect of the mutated protein depends on the mutation position too. [5, 20]

The majority of mutations in MYH7 are missense, which means a single nucleotide-base substitution results in a non-synonymous single amino acid substitution. On the other hand, MYBPC3 mutations use to be nonsense due to insertion or deletion and splicing variants or frameshifts causing premature stop codon, inducing a truncated protein transcript (truncating type of mutation). [1]

It is believed most missense mutations have a dominant negative effect, which makes the mutant protein to be incorporated into the sarcomere disrupting the normal sarcomere function and structure, or altering the ligand interaction in the kinase domains, or even changing the protein-protein interaction by affecting the surface-exposed portion. The changes induced in proteins by these mutations can also be related to folding process, which can trigger an early degradation. However, it is thought haploinsufficiency to be the mechanism responsible of the disease usually in truncating mutations. HCM phenotype is suspected to be resulted from a diminished contractile function due to altered actin-myosin interactions, and consequent inappropriate compensatory hypertrophic remodeling. [1]

On the other hand, about 10% of the HCM mutations are found in other sarcomere genes. These genes are basically related to myosin light chain 2 and 3 (MYL2, MYL3), tropomyosin alpha-1 chain (TPM1), cardiac troponin I, C and T (TNNT2, TNNC1, TNNI), and Actin Alpha Cardiac Muscle 1 (ACTC1). There are other mutations responsible of some cases of HCM, but they are not sarcomeric proteins genes. Less than 1% of the mutations are found in Z-disk proteins, such Filamin C (FLNC), Alpha-actinin-2 (ACTN2) and Vinculin (VCL). ZASP-LIM binding domain 3 (LBD3) is a Z-Disk protein whose mutations can be found in 1-5% HCM cases. Sarcomere-associated proteins as desmin (DES) and Four and a Half Lim Domain Protein-1 (FHL-1) are examples of other proteins which can be responsible of HCM development. They can be appreciated in Table 4. [1, 7, 20]

	Gene	Locus	Protein	Frequency
Myofilament mutation	MYBPC3	11p11.2	Cardiac myosin-binding protein C	15-25
	MYH7	14q11.2-q12	ß-Myosin heavy chain	15-25
	TNNI3	19p13.4	Cardiac troponin I	<5
	TNNT2	1q32	Cardiac troponin T	<5
	TPM1	15q22.1	ß -Tropomyosin	<5
	MYL2	12q23-q24.3	Ventricular regulatory myosin light chain	<2
	ACTC	15q14	-Cardiac actin	<1
	MYH6	14q11.2-q12	α -Myosin heavy chain	<1
	MYL3	3p21.2-p21.3	Ventricular essential myosin light chain	<1
	TNNC1	3p21.3-p14.3	Cardiac troponin C	<1
	TTN	2q24.3	Titin	<1
Z-disc mutation	LBD3	10q22.2-q23.3	LIM binding domain 3	1-5
	ACTN2	1q42-q43	α -Actinin 2	<1
	ANKRD1	10q23.33	Ankyrin repeat domain 1	<1
	CSRP3	11p15.1	Muscle LIM protein	<1
	MYOZ2	4q26-q27	Myozenin 2	<1
	TCAP	17q12-q21.1	Telethonin	<1
	VCL	10q22.1-q23	Vinculin/metavinculin	<1
Calcium-handling	JPH2	20q12	Junctophilin-2	<1
	PLN	6q22.1	Phospholamban	<1

Table 4. Genetic and Molecular Basis of Disease for HCM. Modified from *Maron BJ. « Clinical Course and Management of Hypertrophic Cardiomyopathy.» The New England Journal of Medicine (2018): 655-668*

A study developed in Spain among 2014 and 2017 revealed Formin homology 2 domain

containing 3 (FHOD3) is a novel disease-causing gene in HCM. Their pathogenic or likely pathogenic variants would account for about 2% of HCM cases, similar to the genes just mentioned. FHOD3 diaphanous autoregulatory and inhibitory domains interact so the protein is in an autoinhibited state, but its location and function are not well known yet, so functional studies are required to understand the mechanisms FHOD3 mutation carriers develop HCM. [16]

There are other genes whose role in HCM has been considered modifying rather than pathogenic, such RYR2, CAV3, and SCN5A. [20]

Actually, it is thought patients with sarcomere protein mutation suffer from earlier sudden cardiac death; and more important family background of sudden death is found when investigating a proband case with those mutations. It could be related to the fact that these patients also use to develop more severe myocardial hypertrophy and fibrosis and they are found microvascular disfunctions too. Moreover, patients with several sarcomeric proteins mutations, as we can imagine, develop a more aggressive phenotype which also shows up earlier in time. However, it is necessary to make more studies in order to improve the evidence of this theories. [7, 20]

3. METHODS

In this study, a data base has been made with the genetic test results of the proband HCM cases identified in Cantabria between January 2018 and the first week of January 2020. 291 studies of HCM have undergone a genetic test during those months. 140 patients were proband cases (48,1%) and the rest (151 cases) were first degree relatives who underwent a familial genetic and clinical screening. As the presence of a certain mutation is the same in each affected family, in the study we have only taken into account proband cases.

The cases have been diagnosed in the four hospitals in Cantabria (*Hospital Marqués de Valdecilla, Hospital de Sierrallana, Hospital Tres Mares y Hospital de Laredo*), as the different Cardiology Departments of those medical center are the responsible of clinical HCM diagnose and the doctors who apply for the genetic test.

The request of genetic test is processed by the *Hospital Marqués de Valdecilla Genetics* Department, and it is sent to an external laboratory located in Galicia (Spain) called Health in Code, which sent back the results to Genetic laboratory, who is responsible of reporting the Cardiologist the genetic information of the study.

Health in Code uses two different methods to carry out the genetic test: Next Generation Sequencing (NGS) and Sanger Sequencing Method from blood, saliva or tissues, previously subjected to extraction and purification processes in order to obtaining genomic DNA (QIAsymphony SP, Qiagen). Data sequencing analysis is performed by an own bioinformatic pipeline which includes both the demultiplexing sample and all the steps necessary to obtain the annotated variants together with their coverage and corresponding quality parameters.

The genes and capture probes library are Health in Code property. There are two panels developed in order to make de genetic diagnose of HCM.

The first of them is the basic panel which includes 17 genes related to HCM. These included genes have been selected according to a clinical criteria and its association to a particular phenotype. Two of the genes included in the basic panel (DES and FLNC) are related to Noonan Syndrome; PRKAG2 mutations trigger AMP gamma-2 subunit; GLA gene is responsible of Anderson-Fabry disease, and LAMP2 causes Dannon syndrome, as TTR is the gene related to altered protein which accrues in the amyloidosis heart disease. Apart from those genes, there have also been included the most frequent genes related to HCM.

This basic panel allows the Cardiologist to make a confirmatory test of the clinical suspicion of HCM with a single test, at the same time as ruling out the specific syndromes that we have been mentioning, which clinically could suppose a phenocopy of the disease, thus being able to discard these options and confirm others, or vice versa, through only one sample.

The results show those genetic variants which are selected taking into account its potential relation to the patient phenotype or important incidental founds. However, it must be considered the fact that the variant pathogenicity is not static anymore, as it changes over the time depending on the available evidence.

Sensitivity and specificity when analyzing the punctual substitutions and the small insertions or deletions ($\leq$ 20 pb) are more than 99%. But despite the higher specificity and sensitivity of the test, mistakes can occur in certain situations: already contaminated samples at the laboratory arrival, monosomies and trisomies, mosaicisms, troubles in paternal inheritance, presence of genetic variants which induces allelic dropouts, studies performed over DNA samples obtained of tissues with paraffin wax, homology locations, incorrect identification of locations with high GC content or homopolimeres, and reference sequencing mistakes.

The genetic test is not able neither to identify more than a variant affecting the same gene, so it is important to take this fact into account when studying patients who may have several recessive disease, which requires both mutated alleles to develop the clinical phenotype.

Each report on a patient's genetic study includes the clinical information of the variant, when identified, based on public databases that Health in Code manages with more than 1 million records from the existing literature on family heart disease and on the center's own research, also providing information on carriers and other mutations that may have been identified in the same gene. In addition, a bioinformatic study is also included on the sequence in which the variant (affected amino acid) is found and information on the region and residues, as well as information on the function of the protein that is encoded by the gene.

ACTC1	DES	FHL1	FHOD3	GLA	LAMP2	MYBPC3	MYH7	MYL2	MYL3
PRKAG2	PTPN11	TNNC1	TNNI3	TNNT2	TPM1	TRIM63	TTR	AARS2	ACAD9
ACADVL	ACTA1	ACTN2	AGK	AGL	AGPAT2	ALPK3	ATPAF2	BRAF	CAV3
COAS	COA6	COQ2	COX15	COX6B1	CSRP3	DLD	FAH	FHL2	FLNC
FOXRED1	GAA	GFM1	GLB1	GNPTAB	GUSB	GYG1	HRAS	JPH2	KLHL24
KRAS	LIAS	LZTR1	MAP2K1	MAP2K2	MLYCD	MRPL3	MRPL44	MRPS22	MTO1
MYOZ2	NF1	NRAS	PLN	PMM2	RAF1	SCO2	SHOC2	SLC22A5	SLC25A3
SLC25A4	SOS1	SURF1	TMEM70	AKT1	ANK2	ANKRD1	ATP5F1E	BAG3	BSCL2
C10orf71	CACNA1AC	CALR	CALR3	CASQ2	CAVIN4	CBL	CDH2	CRYAB	DSP
ELAC2	FXN	GATA6	KCNJ8	KLF10	LDB3	LMNA	MEF2C	MYH6	MYLK2
MYOM1	NEXN	PDHA1	PDLIM3	PHKA1	PPA2	PPP1CB	QRSL1	RIT1	SOS2
TAZ	TCAP	TMOD1	TRIMS4	TSFM	TTN	VCL	WISP1		

Figure 19. Basic and complete HCM genetic panel. Basic panel highlighted in bold writing. Took from www.HealthinCode.com

As previously mentioned, there are two panels in order to make the genetic HCM test. The complete HCM panel includes not only the main sarcomeric genes, but also all the disease phenocopies and secondary and candidate genes which are revealed in the systematic and current literature review, accounting for 118 genes in the panel (Figure 19) In bold print are marked the 17 genes included in the basic panel, which is the first approach when studying possibly variants related to the HCM pathogenesis. As can be seen, the panel is constituted by 13 genes in which have been found the most part of the pathogenic variants directly related to HCM (ACTC1, DES, FHL1, FHOD3, MYBPC3, MYH7, MYL2, MYL3, TNNC1, TNNI3, TNNT2, TPM1, TRIM63).

Usually when addressing a patient with clinical HCM the first genetic step is performing a genetic test beginning with the basic 17 genes panel. But once this first genetic test is negative and clinically the Cardiologist diagnoses a sure HCM phenotype it, it could be useful to perform the complete genetic panel, as this situation improves its profitability.

There are other situations, as the patient which is diagnosed with a very severe phenotype or there is a strong suspicion of hypertrophy myocardiopathy related to syndromes or other unusual genetic disease; or when the aim is to perform an exhaustive genetic study of this pathology.

Notwithstanding, in the basic panel it is also possible to find genes associated to other specific diseases; PTPN11 is one of the genes whose mutation is related to Noonan syndrome, but it is also associated to LEOPARD syndrome; GLA is a gene associated to the protein known as Alpha-Galactosidase A, which is the protein affected in Anderson-Fabry disease, as LAMP2 is the gene whose mutation is related to Danon´s syndrome.

Finally, PRKAG2 is related to AMP-gamma2 subunit, which is a protein related to other metabolic storage disorder so induces another HCM phenocopy. Finally, the panel also includes TTR gene, which is associated to wild-type TTR-related amyloidosis. [1]

It is important to take into account the fact that the proband case is considered to be the first family member to be studied, but it is possible he is not the first person in the family to be affected by HCM, as there can be family sudden death familiar background which was never investigated.

The study also included the first family member to have suffered sudden cardiac death and whose necropsy has shown left or septal ventricular hypertrophy, or another phenotype suggestive of HCM, what has led to a genetic study has been carried out from a tissue sample of the patient.

4. RESULTS

During the time interval between January 2018 and the first week of January 2020, a total of 291 genetic tests were performed in Cantabria in HCM patients. 140 of them (48.11%) were performed in proband patients (Figure 20).

The genetic test using the basic Health in Code panel results were 88 negative studies (62.86%) which means there were not identified any pathogenic, likely pathogenic or with unknown significance in the genes included in the basic panel. 33 studies resulted in pathogenic or likely pathogenic variants (23.57%), as 19 genetic tests showed variants of unknown significance (VUS), accounting for 13.57% of the studies.

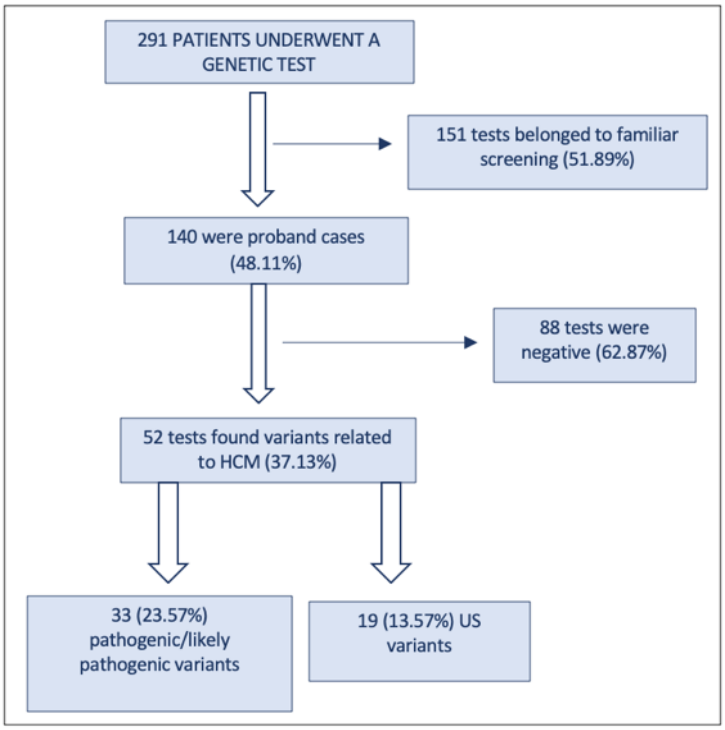

Figure 20. Flow chart of the patients´ genetic test performed. *US: uncertain significance*

Demographic and clinical characteristics of the patients in the study, classified depending on their genetic tests result are described in Table 5.

There were a majority of men (85), reaching the 60.71% of the probands studied. The mean age of the sample was 61.33 years (±1.25).

		Pathogenic variant (n=33)	VUS (n=19)	Negative test (n=88)	Total (n=140)
Mean age (years)		60.97±2.77	60.98±3.6	61.40± 1.52	61.33 ± 1.25
Sex	Male	20 (60.6%)	13 (68.42%)	52 (59.09%)	85 (60.71%)
	Women	13 (39.39%)	6 (31.58%)	36 (40.91%)	55 (39.29%)
LVEF	Normal (50-65%)	24 (72.72%)	13 (68.42%)	50 (56.82%)	87 (62.14%)
	Hyperdynamic (>65%)	6 (18.18%)	6 (31.58%)	34 (38.64%)	46 (32.86%)
	Systolic disfunction (<50%)	3 (9.09%)	0	4 (4.55%)	7 (5%)
Symptoms at 1st evaluation	Dyspnoea (NYHA II-III-IV)	12 (36.36%)	7 (36.84%)	29 (32.95%)	48 (34.29%)
	Chest pain	2 (6.06%	5 (26.32%)	8 (9.09%)	15 (10.71%)
	Palpitations	4 (12.12%)	1 (5.26%)	5 (5.68%)	10 (7.14%)
	Cardiac syncope	3 (9.09%)	0	0	3 (2.14%)
	Heart failure	2 (6.06%)	0	2 (2.27%)	4 (2.86%)
	Sudden cardiac death	0	0	1 (1.14%)	1 (0.71%)
Cardiovascular Risk Factors	Arterial Hypertension	15 (45.45%)	10 (52.63%)	65 (73.86%)	90 (64.29%)
	Smokers or former smokers	17 (51.51%)	4 (21.05%)	39 (44.32%)	60 (42.86%)
	Alcohol abuse	4 (12.12%)	3 (15.79%)	14 (15.91%)	21 (15%)
	Obesity (BMI>30)	6 (18.18%)	3 (15.79%)	24 (27.27%)	33 (23.57%)
	Dyslipemia	10 (30.30%)	8 (42.11%)	41 (46.59%)	59 (42.14%)
	Diabetes	1 (3.03%)	1 (5.26%)	10 (11.36%)	12 (8.57%)
Cardiovascular background	Ischemic heart disease	5 (15.15%)	4 (21.05%)	15 (17.05%)	34 (24.29%)
	History of sypraventricular arrythmia	7 (21.21%)	3 (15.79%)	16 (18.18%)	26 (18.57%)
SCD Family history	First-degree relatives	7 (21.21%)	1 (5.26%)	15 (17.05%)	23 (16.43%)
	Other relatives	4 (12.12%)	1 (5.26%)	7 (7.95%)	12 (8.57%)

Table 5. Demographic, clinical, personal and familiar background characteristics of the HCM population studied. *VUS= variant of uncertain significance; LVEF= left ventricular ejection fraction; BMI= body mass index; SCD= sudden cardiac death.*

Preserved or normal systolic function, defined as left ventricular ejection fraction (LVEF) measured in echocardiography or MRI study upper to 50% but lower than 65% was found in 24 patients (72.73%) with pathogenic variants. However, 6 patients (18.18%) had hyperdynamic LVEF, defined as an ejection fraction upper to 65%. Three patients (9.09%) with pathogenic variant identified had systolic disfunction due to a LVEF lower than 50%. The group of patients with VUS in their genetic test result showed 13 (68.42%) of them had preserved LVEF, as 6 (31.58%) had hyperdynamic LEVF. There were no patients with systolic dysfunction. Finally, 50 patients with non-identified variant in the genetic test (56.82%) resulted to have normal LEVF, since 34 (38.64%) even have hyperdynamic LVEF. Four patients (4.55%) showed systolic disfunction.

It is important to remark 50.71% of patients in the study population showed some kind of HCM-related symptom, including dyspnea, chest pain, cardiac syncope or palpitations. Nineteen of the 33 (57.57%) patients with pathogenic or likely pathogenic

variant identified in genetic test were symptomatic at first evaluation; 12 of them (36.36%) had dyspnea, defined as NYHA class upper than I2; 2 patients suffered from chest pain (6.06%); 4 patients occasionally perceived palpitations (12.12%); whereas 3 of the patients (9.09%) had a cardiac syncope. Twelve VUS patients also presented any kind of symptom (63.15%) related to HCM. 7 patients had any kind of dyspnea (21.21%); 5 patients had experimented chest pain (15.15%) and 1 patient had ever had palpitations (3.03%). In the negative genetic test sample, 29 patients suffered from dyspnea at first evaluation (32.95%); 8 had chest pain (9.09%) and 5 admitted having suffered palpitations (5.68%).
Meanwhile, 4 patients (2,86%) were directly first evaluated when they had been admitted due to heart failure, what is, when the HCM disease was developed and even nearly to end-stage HCM; 2 of them had a pathogenic variant responsible of their LVH. One patient (0.71%) included in this study suffered from sudden cardiac death and the genetic test was performed from a tissue sample obtained during the necropsy, due to the macroscopic confirmation of HCM when performing the autopsy, which resulted to be negative. What is more, other variants related to other diseases responsible of sudden cardiac death, such as long QT syndrome were not found in the genetic test of that patient.

Fourteen (42.42%) patients carrying a pathogenic or likely pathogenic variant in sarcomeric genes where totally asymptomatic. In the same way, 7 (36.84%) patients whose genetic test identified a VUS did not present symptoms at first evaluation.

Taking into account the cardiovascular risk factors the population study presented, it was showed 15 of the 33 (45.45%) patients with positive test had arterial hypertension, which was similar to the incidence in the VUS, in which 10 (52.63%) suffered from arterial hypertension. However, arterial hypertension was present in 65 patients (73.86%) with negative study, which means the incidence was higher in the patients with negative study. Dyslipemia incidence was similar in VUS and negative study groups, 8 (42.11%) in the VUS group and 46.59% in patients with negative study, as in the positive group was less frequent, accounting for 10 (30.3%) patients suffered from it. When talking about obesity, as 6 patients with positive study had a body mass index (BMI) upper to 30 (18.18%), and 3 patients (15.79%) from the VUS group. Nevertheless, obesity incidence was higher in the negative study group, accounting for 27.27% of the patients.

Ischemic heart disease was present in 5 patients (15.16%) with pathogenic variants, as 7 of the 33 (21.21%) patients of that sort also had history of supraventricular arrythmia, either paroxysmal or permanent atrial fibrillation or flutter. On the other hand, 4 patients with VUS (21.05%) suffered from ischemic heart disease, as 3 (15.79%) had supraventricular arrythmia background.

Fifteen and sixteen with negative test study had ischemic heart disease or supraventricular arrythmia respectively, representing both 17.05% and 18.18% of the sample with no variants identified in the genetic test. Sixty-five patients without variant related to HCM identified had arterial hypertension (73.86%), 39 were smokers or former smokers (44.32%) 24 suffered from obesity (27.27%), 41 had dyslipemia (46.59%). Only 10 patients were diabetic (11.36%).

It was shown that 7 (21.21%) of patients who had positive genetic study, also had a first-degree relative dead due to sudden cardiac death, and 4 had other relatives who had suffered sudden cardiac death (12.12%). One patient with VUS had a first degree relative and another patient had a non-first degree member (5.26%) dead due to sudden cardiac death. The incidence of sudden cardiac death in first degree member in patients with negative study (17.05%) was similar to the positive ones, and also in other relatives (7.95%).

First EKG characteristics		Pathogenic variant (n=33)	VUS (n=19)	Negative test (n=88)	Total (n=140)
Rythm	Sinus Rhythm	29 (87.88%)	18 (94.74%)	78 (55.71%)	125 (89.29%)
	Atrial Fibrillation/ Flutter (all types)	4 (12.12%)	1 (5.26%)	10 (11.36%)	15 (10.71%)
Atrioventricular block	1st degree	1 (3.03%)	0	2 (2.27%)	3 (2.14%)
	2nd and 3rd degree	0	0	0	0
QRS morphology	Normal	24 (72.73%)	17 (89.47%)	66 (75%)	107 (76.43%)
	LAH or LPH	5 (15.15%)	1 (5.26%)	3 (3.41%)	9 (6.43%)
	RBBB	1 (3.03%)	1(5.26%)	9 (10.23%)	11 (7.86%)
	LBBB	1 (3.03%)	0	6 (6.82%)	7 (5%)
	Bifascicular block	0	0	4 (4.55%)	4 (2.88%)
QRS voltages	Normal	20 (60.61%)	9 (47.37%)	55 (62.5%)	84 (60%)
	LVH criteria	12 (36.36%)	10 (52.63%)	33 (37.5%)	55 (39.29%)
	Low-voltages	1 (3.03%)	0	0	1 (0.71%)
Q waves (leads)	Right precordial (V1-V3)	0	0	1 (1.14%)	1 (0.71%)
	Left precordial (V4-V6)	1 (3.03%)	0	0	1 (0.71%)
	Inferior (II-III-aVF)	3 (9.09%)	0	1 (1.14%)	4 (2.88%)
	Inferolateral (V4-V6, II, III, aVF)	1 (3.03%)	0	1 (1.14%)	2 (1.43%)
	High lateral (I-aVL)	0	0	0	0
	Other	1 (3.03%)	0	1 (1.14%)	2 (1.43%)
T wave inversions (leads)	Right precordial (V1-V3)	1 (3.03%)	3 (15.79%)	4 (4.55%)	8 (5.71%)
	Left precordial (V4-V6)	4 (12.12%)	2 (10.53%)	10 (11.36%)	16 (11.43%)
	Precordial (V2-V6)	5 (15.15%)	2 (10.53%)	8 (9.09%)	15 (10.71%)
	Inferior (II-III-aVF)	1 (3.03%)	0	4 (4.55%)	5 (3.57%)
	Inferolateral (V4-V6, II, III, aVF)	1 (3.03%)	0	4 (4.55%)	5 (3.57%)
	High lateral (I-aVL)	1 (3.03%)	1 (5.26%)	8 (9.09%)	10 (7.14%)
	Other	3 (9.09%)3	1 (5.26%)	3 (2.41%)	7 (5%)

Table 6. Chart with the main characteristics of the first EKG performed in patients from the study

Characteristics of the first EKG performed in the patients once HCM is suspected are shown in Table 6.

During the follow-up, in the sample with pathogenic or likely pathogenic variants identified were recorded 6 patients with non-sustained or sustained ventricular tachycardia (18.18%); also, 6 patients were admitted due to heart failure (18.18%) and 4 patients suffered from ischemic events (12.12%). Nine patients (27.27%) were selected

to receive an implantable cardioverter-defibrillator, and 4 patients (12.12%) needed a pacemaker implant during their evolution.

All the events during the follow-up are shown in Table 7. Myectomy, known as surgical reduction or alcohol ablation, was performed to 2 patients with pathogenic variants (6.06%). Heart transplant was only performed to one patient, who also belonged to the sample with pathogenic variants identified.

Events during follow-up	Pathogenic variant (n=33)	VUS (n=19)	Negative test (n=88)	Total (n=140)
NSVT / SVT	6 (18.18%)	3 (15.79%)	7 (7.95%)	16 (11.43%)
ICD implant	9 (27.27%)	2 (10.53%)	6 (6.82%)	17 (12.14%)
Pacemaker implant	4 (12.12%)	0	4 (4.55%)	8 (5.71%)
Septal alcohol ablation/myectomy	2 (6.06%)	1 (5.26%)	3 (3.41%)	6 (4.29%)
Heart failure admission	6 (18.18%)	0	10 (11.36%)	16 (11.43%)
Heart transplant	1 (3.03%)	0	0	1 (0.71%)
Ischemic event	4 (12.12%)	0	3 (3.41%)	7 (5%)
Dead	1 (3.03%)	0	2 (2.27%)	3 (2.14%)

Table 7. Events during follow up in study population. *VUS= variant of uncertain significance; NSVT= non-sustained ventricular tachycardia; SVT= sustained ventricular tachycardia; ICD=implantable cardioverter-defibrillator*

Three patients with VUS suffered from a non-sustained or sustained ventricular tachycardia episode (15.79%). ICD implant was performed in 2 patients of that sample (10.53%), and one patient undergone a septal reduction (5.26%). Seven patients with non-sustained or sustained ventricular tachycardia episodes were recorded from the sample with negative genetic test (7.95%); 6 patients were treated with ICD (6.82%) and 4 with pacemaker (4.55%). 10 patients were admitted to the hospital due to heart failure (11.36%) and 3 patients suffered from ischemic events (3.41%).

At the time the data collection for this study ended, only 3 patients were dead. One of them belonged to the group with pathogenic or likely pathogenic variant identified, and the cause of death was a sustained ventricular tachycardia, provoking a sudden cardiac death. The other two people did not have any variant related to HCM identified. One of them died due to heart failure and the other suffered from sudden cardiac death without being identified sustained ventricular tachycardia.

4.1 GENETIC RESULTS

Table 8 summarizes the results obtained both in positive studies and the VUS studies. MYBPC3 mutation was the most prevalent in the study, present in 14 studies (10% of the total genetic test, 42.42% of the positive tests). There were found six missense

variants (42.85%), six truncating variants (42.85%) and two splicing variants (14.29%).

GENE	FULL NAME GENE	PATHOGENICITY	NUMBER OF PATIENTS
MYBPC3	Myosin binding protein C	Pathogenic or likely pathogenic	14 (42.42%)
		VUS	7 (36.84%)
MYH7	Myosin heavy chain 7	Pathogenic or likely pathogenic	13 (39.39%)
		VUS	1 (5.26%)
MYL3	Myosin light chain 3	Pathogenic or likely pathogenic	2 (6.06%)
		VUS	0
FHOD3	Formin Homology 2 Domain Containing 3	Pathogenic or likely pathogenic	1 (3.03%)
		VUS	5 (26.32%)
TPM1	Tropomyosin I	Pathogenic or likely pathogenic	1 (3.03%)
		VUS	0
TNNI3	Troponin I type 3	Pathogenic or likely pathogenic	1 (3.03%)
		VUS	0
PLN	Phospholamban	Pathogenic or likely pathogenic	1 (3.03%)
		VUS	0
GLA	Galactosidase alpha	Pathogenic or likely pathogenic	1 (3.03%)
		VUS	1 (5.26%)
PTPN11	Protein tyrosine phosphatase, non-receptor type 11	Pathogenic or likely pathogenic	0
		VUS	1 (5.26%)
TNNT2	Troponin T type 2	Pathogenic or likely pathogenic	0
		VUS	1 (5.26%)
PRKAG2	Protein kinase AMP-activated non-catalytic subunit gamma 2	Pathogenic or likely pathogenic	0
		VUS	1 (5.26%)
FLNC	Filamin C	Pathogenic or likely pathogenic	0
		VUS	2 (10.53%)

Table 8. Chart with the mutations identified in genetic study and number of patients carrying each.

MYH7 was the second most frequent mutation, as there were 13 studies with variants on it (9.28% of the total genetic test and 39.39% of the positive tests). All of the variants identified in MYH7 were missense variants (100%).

MYL3 pathogenic or likely pathogenic variants were also found in two patient's genetic test (1.43% of the total genetic test and 6.06% of the positive tests). Both the variants were missense variants (100%).

FHOD3, TNNI3 and TPM1 were found each in one patient´s results. That means the presence of each pathogenic or likely pathogenic variant in those genes supposed the 0.71% of the genetics test and 3.03% of the positive tests. All of the variants were missense variants (100%).

However, as PLN pathogenic or likely pathogenic variant was also found only in one patient, the type of variant that patient carried was a truncating variant. Frequencies of the pathogenic mutations found in the study are represented in Figure 21

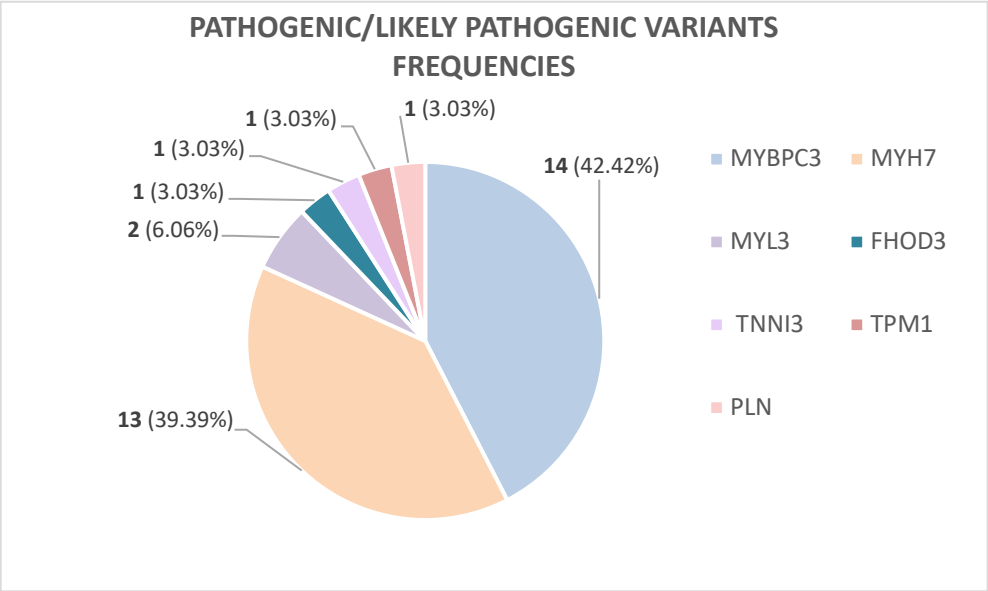

Figure 21. Graphic summarizing the frequencies of pathogenic or likely pathogenic variants found in the positive genetic test performed.

Equally important is the analysis of the variants of unknown significance found in the genetic test of 19 patients (Figure 22). Once again, MYBPC3 was the most frequent gene affected: 7 patients had MYBPC3 variants of uncertain significance (5%). There were found 5 patients with VUS in FHOD3 (26.32%). All of them were missense variants.

FLNC gene presented 2 variants of unknown significance (10.53%); one of the variants was a missense variant (50%), but the other type of variant was not conclusive with the information available (50%).

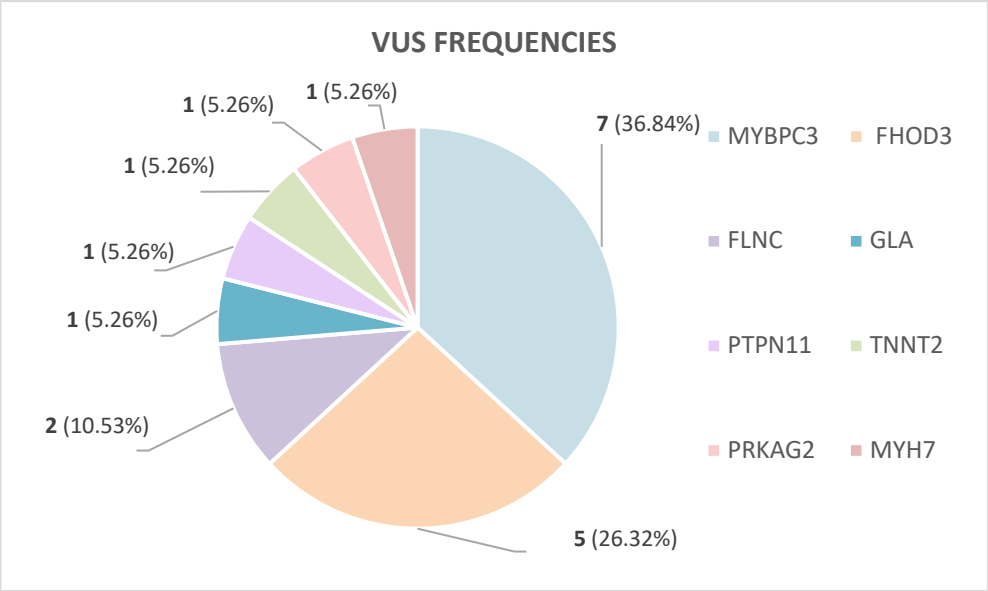

Figure 22. Graphic summarizing the frequencies of VUS found in the genetic test performed.

By last, there also were found VUS in GLA, PTPN1, TNNT2, PRKAG2 and MYH7 gene, one variant each gene, accounting for 0.71% of the total genetic tests, and 5.26% of the VUS genetic tests (each variant). All of them were missense variants.

5. DISCUSSION

HCM is the most common monogenic inheritable cardiomyopathy due to sarcomeric mutations, with an autosomal dominant pattern. Most cohorts in literature describe it is clinically present in 1 of 500 people, and it is defined by left ventricle wall thickness greater than 15 mm measured by echocardiography or CMR, which are the main imaging tools when diagnosing HCM. [1,8, 11,13, 20]

Pathogenic variants are identified in 60% of patients with HCM, related to abnormal myocyte hypertrophy and disarray with fibrosis, resulting in impaired ventricular filling, diastolic dysfunction and complications like LVOTO and arrythmias. This may result in a wide variety of symptoms, as exertional dyspnea, palpitations or chest pain, although many patients could be asymptomatic. Besides, sudden cardiac death is also a risk related to HCM, due to abnormalities in heart tissue which may be a substratum for arrythmias such ventricular tachycardia. [1, 7, 9 11, 14, 20]

Currently, about 450 mutations in 20 sarcomere and myofilament-related proteins have been identified as HCM causes; most of them are in MYH7 and MYBPC3. Genetic test is important in order to identify a pathogenic mutation which may confirm HCM and will give prognosis information taking into account clinical features too. The identification of a pathogenic mutation allows to screen the patient´s relatives before developing symptoms and complications. [6, 20]

In this context, this study aims to make an approach about the yield of genetic testing HCM patients in Cantabria.

Sex distribution in the study population corresponds to most of the bibliography in which it is accepted that the prevalence of men affected by HCM is approximately 60% of the study population. There were no differences between the mean age in the patients with positive study (60.97 ± 2.77 years) than the sample with negative genetic test (61.40 ± 1.52 years).

The patients with pathogenic or likely pathogenic variants found also had a lower prevalence of the main cardiovascular risk factors related to left ventricle hypertrophy, as the arterial hypertension was present in 45.45% of the patients, versus 73.86% of patients with no variants identified. This could be attributed to the genetic test was profitable when left ventricle hypertrophy studied by imaging test does not correlate to the severity or time the patient has been affected by arterial hypertension.

According to the important family background HCM has, another important fact about the proband patients in the study was the presence or absence of familiar past referred to sudden cardiac death, accepting also all those sudden unexplained deaths. The first-degree relative history of sudden cardiac death also was more prevalent in the group with pathogenic or likely pathogenic variants (30.43%) compared to the group with negative study (17.05%).

MYBPC3 and MYH7 were the most frequent genes affected in pathogenic or likely pathogenic variants, involving MYBPC3 the 42.42% of the positive test and MYH7 the

39.39%. This means the 81.81% of the genetic test which resulted in a pathogenic or likely pathogenic variant, were MYBPC3 and MYH7 the genes involved. It is a little higher prevalence than the information found in literature, where the most part of the studies support mutations in the MYH7 and MYBPC3 account for about the 75% of the cases. It could be explained by the small number of patients of this study comparing large cohorts used in the consulted studies. On the other hand, the prevalence isolated of MYBPC3 and MYH7 is very similar to most cohorts, which show the prevalence of both genes is approximately 40% each one.[6, 7, 20]

MYBPC3 was also the most frequent gene identified in VUS, and FHOD3 was the second most frequent gene, accounting for the 36.84% and 26.32% of the VUS, respectively. FHOD3 prevalence in the positive tests of this study (3.03%) was similar to prevalence described in literature (1-2%), contrary to FHOD3 VUS prevalence (26.32%), which was higher than expected.[1, 6, 16]

MYL3 prevalence in cohorts consulted in literature is about 1%. However, in this study a prevalence of 6.06% of the pathogenic variants was obtained. [1,6]

TNNT2 is considered in several cohorts as the third or fourth most frequent gene affected by mutations in HCM, with a frequency about 10% (5-15%), although in this study any pathogenic variant was identified in this gene. [1,6]

The prevalence of pathogenic variants in TPM1 and TNNI3 were similar to the cohorts in literature, as TPM1 use to be present in 1-3% of the genetic tests (in this study its frequency was 3.03%) and TNNI3 prevalence is about 5% and in this study was 3.03%.[1,6]

GLA gene, which is also related to HCM phenocopies such Anderson-Fabry disease accounted in the study for the 3.03% of the positive tests, as it is considered to have a frequency, together with other HCM phenocopies, of 5-10% of the studies.[1]

It is important to remark a pathogenic variant in PLN was identified in one patient, and the association between this gene and HCM is very unusual, as it is more frequently related to Dilated Cardiomyopathy.[2, 10]

Altogether, pathogenic or likely pathogenic variants were found in 7 sarcomere genes, as variants of uncertain significance were found in 7 sarcomere genes and one in GLA gene, related to Fabry´s disease. The only patient studied with sudden cardiac death episode as first manifestation of HCM, did not carry any pathogenic or likely variant, neither a variant of uncertain significance. [6]

All patients with positive test had ventricular hypertrophy confirmed by imaging tests (echocardiography or CMR). This means 100% of the patients with pathogenic variants had septum or some other point of the ventricle thickness ≥ 15 mm. On the other hand, 20 patients (22.72%) with negative study did not have hypertrophy of the left ventricle in the imaging studies. So, the yield of the genetic test could be higher if left ventricle hypertrophy has been confirmed at least by one imaging test, what is the genetic test should be performed once HCM has been clinically diagnosed in the patient.

The study has several limitations. The main one is there is a low number of positive studies, making it difficult to draw definitive conclusions. Other limitations in this study are the lack of information related to some patients due to the recent creation of a department dedicated to familiar Cardiomyopathies, so there are some data that could not be recorded, and some information about familiar background has been not collected properly. Must also be remembered some symptoms related to HCM could be suffered from other disease that provokes the same symptoms, like ischemic chest pain, heart failure due to other etiology, as they are certainly unspecific. If the patient suffers from cardiovascular disease different from HCM, such chronic ischemic cardiopathy or arterial peripheral vascular disease, it would be very challenging to distinguish which of the disease caused the symptoms. Nevertheless, the diagnose any heart disease will be almost be done simultaneously to HCM. Some clinic diagnoses of HCM were made several years ago, even decades, and the genetic test was performed in the interval of our data study because it could not be done before. It has been impossible to identify all the patients affected from HCM in Cantabria, only those who have been requested a genetic test has been taken into account in the study, so the percent of the clinical HCM that has been undergone a genetic test has not been possible to be calculated. Data collection has been prospective and with limitation in the access to all the information. Therefore, the results are only applicable to the HCM population in Cantabria clinically diagnosed.

6. **CONCLUSIONS**

HCM is the most common inheritable cardiomyopathy. The clinical diagnosis with image testing and the estimation of the risk of sudden cardiac death suppose an indispensable part of correct managing of the disease. Echocardiography and MRI are the best tests to study the patient both at first evaluation and also during the follow-up. It is remarkable the importance of making a detailed family tree composed by at least 3 generations as part of the first evaluation made when HCM is suspected in a proband patient, which means all the suspected HCM should be derived to the familiar Cardiomyopathies consultant.

Genetic testing has become one of the main tools in order to approach HCM. Currently pathogenic variants can explain about 60% of HCM cases. [1,11, 14, 20]

The overall yield of genetic testing using in Cantabria was low, as only 23.57% of the patients in the study were identified to carry a pathogenic or likely pathogenic mutations, which is lower than most cohorts in literature. The most involved genes were variants in MYBPC3 and MYH7. Genetic testing yield is higher when other causes of left ventricle hypertrophy, such arterial hypertension, are considered not strong enough to provoke such hypertrophy. This also occurs when those other causes of left ventricle hypertrophy are discarded studying the ventricle by imaging, such using CMR to differentiate athlete´s heart or HCM phenocopies. Familiar background of sudden

cardiac death must also be taken into account, as the yield may be higher if there are cases of sudden cardiac death in first- degree relatives.

The other pathogenic or likely pathogenic variants found in the study was similar to most HCM cohorts. Pathogenic variant in PLN was identified in a patient of the study, which is remarkable due to the higher prevalence of pathogenic variants in this gene more related to Dilated Cardiomyopathy than to HCM in literature. A variant of uncertain significance was identified in GLA gene, which encodes the α-Galactosidase A, being responsible of Fabry´s disease, one of the most common HCM phenocopies.

Besides, it is important to remark it seems there have been not found yet all the pathogenic or likely pathogenic variants related to HCM, which suggests some patients with clinical HCM but with no variants identified, may carry mutations in genes not discovered or studied as responsible of HCM yet.

In conclusion, the prevalence of pathogenic variants found in the genetic test was lower than the prevalence described in literature (23.57% versus 60% approximately). All the genetic test had ventricle hypertrophy demonstrated by cardiac imaging, but not all the patients with negative study (22.73% of the population with negative study) had hypertrophy, which means the yield is bigger when HCM is clinically diagnosed with hypertrophy developed. However, the conclusions cannot be definitive and extrapolated due to the low quantity of data available.

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