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Prevalence and Significance of Rare Sarcomere Variants in Secondary Left Ventricular Hypertrophy due to Arterial Hypertension and Wild-Type Transthyretin-Related Cardiac Amyloidosis

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1. Background

1.1 Left ventricular hypertrophy

The term left ventricular hypertrophy (LVH) describes an augmentation of LV mass (LVM) caused by increased cardiomyocyte size. LVH can be physiological, due to an adaptation to strenuous physical exercise, or it can be a pathological condition, which is either primary (i.e., genetic) or secondary. While physiological LVH represents a benign and reversible adjustment of the heart to exercise, pathological LVH is a compensatory phenomenon, that may become maladaptive and eventually evolve toward heart failure[1,2].

Primary LVH is typically caused by cardiomyopathies (i.e., primary myocardial diseases), in the absence of pressure or volume overload of the heart. Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease, and is the main example of primary LVH[3].

On the contrary, secondary LVH typically occurs in the setting of pressure or volume overload of the heart. The heart compensates a hemodynamic overload in three ways: the Frank-Starling mechanism to increase crossbridge formation; augmentation of muscle mass; activation of neurohormonal pathways to increase contractility[2]. Augmentation of mass through cardiomyocyte hypertrophy – and eventually LVH – is the main mechanism. According to Laplace's law, wall stress is directly related to LV cavity size and intracavitary pressure, and inversely related to wall thickness. Thus, an increase in wall thickening counteracts the overload within the cavity. Secondary LVH may thus occur in the case of pressure overload, which may be caused by arterial hypertension or aortic stenosis, or volume overload, which may be caused by mitral regurgitation.

Overall, in both primary and secondary pathological LVH, several molecular mechanisms and environmental factors come into play in the development of LVH – apart from the genetic primary defect and the pressure-volume overload[1,2,4,5]. There is an incomplete understanding of how these mechanisms interact and of the relative contribution of each one.

As said, LVH is defined as an augmentation of LVM. Cardiac imaging techniques, especially echocardiography, are the gold standard tools for cardiac chamber quantification. According to American and European echocardiography guidelines[6], LVH is defined in the presence of either one of the following:

- LV septal wall thickness >0.9 cm for women and >1.0 cm for men;
- LVM >162 g for women and >224 g for men;
- LVM indexed (LVM/body surface area [BSA]; LVMi) >95 g/m^2 for women and >115 g/m^2 for men.

Notably, several studies have highlighted that in physiological LVH, LV wall thickness is seldom greater than 1.2 cm. Thus, this cut-off (i.e., ≥12 mm) is frequently used to define LVH according to LV wall thickness values[7–10].

By incorporating relative wall thickness (RWT) in the evaluation of LVMi, it is possible to describe LV geometry[6]. RWT is calculated as LV posterior wall thickness×2/LV end-diastolic diameter. LV geometry is defined as normal in the presence of normal LVMi and RWT ≤0.42; as concentric in the presence of normal LVMi and RWT >0.42; as concentric hypertrophy in the presence of increased LVMi and RWT >0.42; as eccentric hypertrophy in the presence of increased LVMi and RWT ≤0.42. LVMi is more precise than LVM, which is more precise than LV wall thickness in defining true LVH[11]. Indeed, it has been shown that LV wall thickness and LVM do not perfectly correlate[11]. In a large cohort of 2545 patients, LVH was found in about 53% when measured by LV wall thickness and in 47% when measured by LVMi; moreover, about 16% of patients with normal LV wall thickness met LVH criteria by LVMi, and 24% of those with normal LVMi met LVH criteria by LV wall thickness. Similarly, it has been shown that in HCM – which, by definition, is characterized by increased LV wall thickness – LVMi does not reach the threshold for LVH in about 20% of cases and is only mildly increased in another 15-20% of cases[12].

Irrespectively of its aetiology, the clinical relevance of LVH stands in the fact that it is associated with adverse outcomes in terms of mortality and heart failure. Pivotal reports from the Framingham Heart Study found LVH to be associated with cardiovascular (CV) morbidity and death, even after adjustment for the other major CV risk factors[13]. These results were further confirmed and remain valid up to these days[2,12,14–16].

Understanding the basis of LVH is a significant unmet need in medicine. Unravelling specific pathological mechanisms of LVH, independently from its aetiology, may provide potential therapeutical targets and thus contribute to prevention of CV disease.

1.2 Left ventricular hypertrophy in arterial hypertension

LVH in arterial hypertension, often referred to as ‘hypertensive heart disease’, is seen in up to one third of patients. In the PAMELA (Pressioni Monitorate E Loro Associazioni) study LVH was reported in about 18% of patients[17]. Similarly, in a large Korean study, the prevalence of LVH was of 19%[18]. A meta-analysis of 30 studies, including a total of 37700 patients with arterial hypertension, found a prevalence of LVH ranging from 36% to 41% of subjects[19].

The main driver of LVH development in AH is thought to be the haemodynamic burden, but patients with similar blood pressure values may differ in presentation of LVH. Even if it was shown that the prevalence of LVH increases with blood pressure values (from pre-hypertension to controlled

hypertension to uncontrolled hypertension)[4,17,18], a recent Framingham Heart Study analysis found LVH to be significantly present in each blood pressure category[14]. Moreover, LVH in arterial hypertension displays an extremely variable phenotype in terms of LV geometry (eccentric vs concentric LVH)[4,19].

This variability is likely explained by a number of other factors, only partially known, and whose mechanism of action is not fully understood. Non-haemodynamic variables, such as ethnicity, gender, neurohumoral, environmental and genetic factors, were shown to modulate the cardiac hypertrophic remodeling[4,20]. Genetic factors (mostly related to the renin-angiotensin-aldosterone system) have been investigated with only partial understanding of the genetic background of hypertensive heart disease.

LVH is one of the most important 'organ damages' caused by arterial hypertension. Its presence is associated with progressive development of diastolic and then systolic LV impairment and eventually heart failure[1,4,20].

1.3 Transthyretin-related cardiac amyloidosis

The term 'amyloidosis' refers to a large group of disorders caused by extracellular deposition of insoluble abnormal fibrils composed of misfolded proteins (i.e., amyloid), which alter tissue structure and impair organ function, including the heart. Cardiac amyloidosis typically presents as a restrictive cardiomyopathy, characterized by a hypertrophied and stiff LV. Immunoglobulin light-chain and transthyretin (TTR)-related amyloidosis are the two main types of cardiac amyloidosis. In particular, TTR-related cardiac amyloidosis may be hereditary (caused by mutations in the TTR gene) or due to degradation and infiltration of the wild-type TTR protein (wild-type or senile TTR-related cardiac amyloidosis, ATTRwt)[21–23]. LVH found in cardiac amyloidosis is therefore often referred to as a pseudo-LVH, in that it is not caused by cardiomyocyte hypertrophy, but it is due to extracellular volume increase secondary to amyloid infiltration. However, from a clinical-imaging standpoint, the disease is typically suspected in the presence of LVH in the context of heart failure with a preserved LV ejection fraction[24]. Moreover, it has been shown that increasing values of LV wall thickness associate with a worse prognosis in TTR-related cardiac amyloidosis[25,26].

ATTRwt is increasingly recognized due to the possibility of performing, in the majority of cases, a non-invasive diagnosis combining scintigraphy with bone tracers, blood tests for ruling out a monoclonal gammopathy and genetic analysis[24,27]. Despite the growing awareness and the increasing number of patients diagnosed, mechanisms causing development of the disease are still little known. Differently from the hereditary TTR-related disease, in which a genetic abnormality in the coding of the TTR protein is found, in the wild-type form the TTR genetic sequence is normal,

and the aging process is thought to play a key role in disease onset[28,29]. Nevertheless, it is still far from clear why in the elderly population, only some subjects develop ATTRwt, or why in the context of abnormal catabolism due to aging (an event virtually common to everyone), only in some subject the TTR protein misfolds, creates amyloid, and, most importantly, infiltrate the heart. Interestingly, though age surely plays a role in the development of the disease, in recent cohorts ATTRwt has been reported to be diagnosed at 'younger' ages, indicating that not only the eighth or ninth decade of life are affected, but also patients in their 60s[30].

1.4 Rare sarcomere variants

Sarcomere (SARC) genes encode for proteins involved in the structure and function of sarcomeres, which are the contractile units of the skeletal and cardiac muscle. Mutations (i.e., rare variants) of SARC genes are causative of several cardiomyopathies, and in particular play a major role in the development of HCM[5].

The history of SARC genes and HCM is strictly related. Pioneering studies in the early '90s paved the way to delineate a clear association of HCM with rare SARC variants in genes encoding β-myosin heavy chain (MYH7), myosin-binding protein C (MYBPC3), cardiac troponin I (TNNT2 and TNNI3), α-tropomyosin (TPM1), cardiac α-actin (ACTC1), myosin light chain (MYL2 and MYL3)[5].

In the following decades, however, two facts became clear. First, studies on familial screening showed that SARC gene variants exhibited an incomplete penetrance and variable expression. Family members carriers of the same variants may show different HCM phenotypes, and, most importantly, may not even develop the morphological features of the disease[31]. On the other hand, rare SARC variants were found in about 60% of HCM cases, and this group of patients with negative genetic testing (genotype-elusive) was increasingly identified over time[3,31]. Secondly, rare SARC variants started to be found in the general population, even in the absence of the typical HCM phenotype, with a frequency of up to 1:200 individuals[32–34]. Data from the Atherosclerosis in Risk Communities and UK Biobank cohorts have highlighted that SARC gene variants have a low penetrance in terms of HCM development[32,34]. Moreover, an analysis from the Framingham Heart Study among patients with LVH, found that carriers of rare SARC gene variants usually showed mild LVH, not typical of HCM[35].

Independently of being associated with the classic HCM phenotype, rare SARC variants have been found to be associated with adverse CV outcomes in the general population. Analysis from both the Framingham and Jackson cohorts, and more recently from the UK Biobank, found that carriers of

rare SARC variants, irrespectively of LVH, had an increased risk of cardiac events, in particularly heart failure-related[32,36].

2. Scope of the study

The aim of this project was to provide insights into the predisposing mechanisms to the development of secondary forms of LVH (in LVH due to arterial hypertension and in ATTRwt), by investigating the prevalence and the clinical correlates of rare SARC variants in these subsets of patients.

The degree of LVH is very heterogeneous within each condition associated with it, and in both primary and secondary LVH, the phenotype is influenced by several genetic and environmental modifiers[3,4,25]. Nevertheless, both models of LVH eventually share similar molecular aspects (i.e., altered calcium handling, abnormal energetics)[1,37].

Rare SARC variants have been found outside of the HCM phenotype (i.e., without overt HCM or with mild LVH not typical of HCM) with a prevalence in the general population of up to 1:200. Nevertheless, independently from the cardiac morphology, SARC variants have been associated with adverse CV outcomes[32,33,36], and it is thus possible that they play a role in pathological context not limited to HCM or other cardiomyopathies.

Arterial hypertension has been shown to be an environmental modifier underpinning susceptibility to HCM development[38]. Similarly, it may be hypothesized that SARC variants may represent a genetic background predisposing to development of LVH in arterial hypertension, at least in a subset of patients. The fact that subjects with LVH in the Framingham Heart Study, with or without SARC variants, showed a similar phenotype[35] might support this hypothesis.

Factors predisposing to ATTRwt development have been poorly investigated. It has been suggested that ATTRwt may occur in specific clinical scenarios where infiltration of the TTR misfolded protein is somehow favored. A significant prevalence of ATTRwt was reported in patients affected by severe aortic stenosis[39–41]. It was speculated that this high prevalence may be explained by the presence of a hemodynamically overloaded myocardium, which develops hypertrophy and fibrosis. These features might 'prime' the heart and predispose it to TTR infiltration[40]. SARC gene variants, even in the absence of a HCM phenotype, have been associated to LV remodeling with subclinical fibrosis[42]. The presence of mild LVH and/or subclinical fibrosis due to this genetic substrate might thus represent a similar scenario to that of aortic stenosis, favoring amyloid infiltration due to wild-type TTR.

This hypothesis, in both LVH due to arterial hypertension and ATTRwt, has never been tested to date. The detection of a specific genetic background favoring development of LVH across diverse conditions would allow identification of a specific risk factor for disease penetrance and ultimately contribute to precision medicine and tailored follow-up of patients. Moreover, as novel therapies targeted at HCM genetic-related dysfunction (i.e., myosin modulators) are entering the clinical

arena[43], the identification of rare SARC variants as predisposing factors to the development of either LVH due to arterial hypertension or ATTRwt would offer the chance to test the usefulness of these novel drugs also in these different scenarios, both for treatment and prevention.

3. Methods

This was a cross-sectional pilot study, investigating prevalence and clinical significance of rare SARC variants in two cohorts, a group of patients with LVH due to arterial hypertension and a group of patients with ATTRwt.

In both cohorts, LVH was defined based on echocardiography, as either LV wall thickness ≥12 mm (independently of sex) and/or LVMi >95 g/m^2 for women and >115 g/m^2 for men[6,9]. LVM was calculated by the formula[6]: 0,8 x [1,04 x (LV septal wall thickness + LV end diastolic diameter + LV posterior wall thickness)^3 – (LVID)^3] + 0,6 g. LVMi was calculated by dividing LVM by BSA.

All patients enrolled in the study provided written informed consent to participate, both for genetic analysis and for collection and treatment of clinical data for research purpose.

3.1 Arterial hypertension cohort

Patients with LVH due to arterial hypertension were recruited among those actively followed up at the Centre of Excellence for Arterial Hypertension Outpatient Clinic at Azienda Ospedaliera-Universitaria Sant'Andrea, Sapienza University of Rome.

Between April and November 2021, all patients evaluated at the outpatient clinic with a previous echocardiographic exam were screened for a history of LVH. Taking into consideration that regression of LVH with optimal medical therapy is known to happen in arterial hypertension[16], we limited our analysis only to patients with well-controlled blood pressure values. Well-controlled blood pressure values were defined as blood pressure lower than 140/90 mmHg in at least the two most recent clinical (i.e., office) evaluations, and/or 24-hour ambulatory blood pressure monitoring within the last 6 months prior to the index study evaluation showing mean 24-h blood pressure values lower than 130/80 mmHg[44]. Patients with secondary forms of arterial hypertension[45] were not included in the study.

Those with an echocardiographic history of LVH were offered to participate into the study; patients with moderate-to-severe mitral regurgitation and/or moderate-to-severe aortic stenosis were excluded. Of 33 patients identified, 10 refused to participate. All of the 23 remaining patients underwent a new echocardiographic examination to ascertain the presence of LVH. In 7 subjects LVH was not confirmed; 16 patients were therefore included in the arterial hypertension cohort.

For all patients we collected: anthropometric data; information regarding CV risk factors and events prior to our evaluation, functional status (NYHA functional class) and CV therapies; presence of ECG features of LVH (ascenrtained by Cornell and/or Sokolov-Lyon criteria[46]). Echocardiography was conducted according to international guidelines[6]; in addition to values needed to calculate

LVM (i.e., LV septal wall thickness, LV end diastolic diameter, LV posterior wall thickness), LV ejection fraction, grade of diastolic dysfunction and left atrium (LA) diameter were collected.

3.2 ATTRwt cohort

Patients with ATTRwt were recruited among those actively followed up at the Cardiomyopathy Outpatient Clinic at Azienda Ospedaliera-Universitaria Sant'Andrea, Sapienza University of Rome. Diagnosis of ATTRwt was done according to the Gillmore algorithm[27] (**Figure 1**). By definition, all patients with ATTRwt have LV wall thickness >12 mm[24,27].

Given that genetic analysis of the TTR gene is required to ascertain ATTRwt diagnosis; we retrospectively reviewed the archives of the Genetic Department at Azienda Ospedaliera-Universitaria Sant'Andrea in Rome, Sapienza University and identified patients with available samples to re-perform genetic testing. We selected those patients alive at the time of revision (November 2022).

Of 51 ATTRwt patients followed up at the Cardiomyopathy Clinic, 22 had available samples for genetic testing and were included in the ATTRwt cohort.

For all patients we collected: anthropometric data; information regarding CV risk factors and events prior to our evaluation, functional status (NYHA functional class) and CV therapies; presence of low voltages at ECG (typical of cardiac amyloidosis[24,47] due the infiltrative nature of the disease). Echocardiographic data from the first outpatient evaluation were retrieved; echocardiography was conducted according to international guidelines[6]. In addition to values needed to calculate LVM (i.e., LV septal wall thickness, LV end diastolic diameter, LV posterior wall thickness), LV ejection fraction, grade of diastolic dysfunction and LA diameter were collected.

3.3 Genetic testing

Genetic testing was performed at the Genetic Department at Azienda Ospedaliera-Universitaria Sant'Andrea in Rome, Sapienza University.

All patients from the arterial hypertension cohort received a venous blood sampling; samples were stored at -20° C. Samples from the ATTRwt cohort were already stored at the Department.

Genomic DNA was extracted from peripheral leucocytes using PureLink® Genomic DNA Mini Kit according to the manufacturer's instructions. Genetic sequencing was performed by next generation sequencing (NGS) using a focused panel including genes involved in HCM (Illumina TrueSight Cardio panel) on Illumina MiniSeq platform. Genes included in the panel are: ACTC1, ACTN2, BAG3, CSRP3, DES, DSC2, DSG2, DSP, GLA, JUP, JPH2, LAMA4, LAMP2, LMNA, MYBPC3,

MYH7, MYL2, MYL3, PKP2, PLN, PRKAG, RBM20, RYR2, SCNSA, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, VCL.

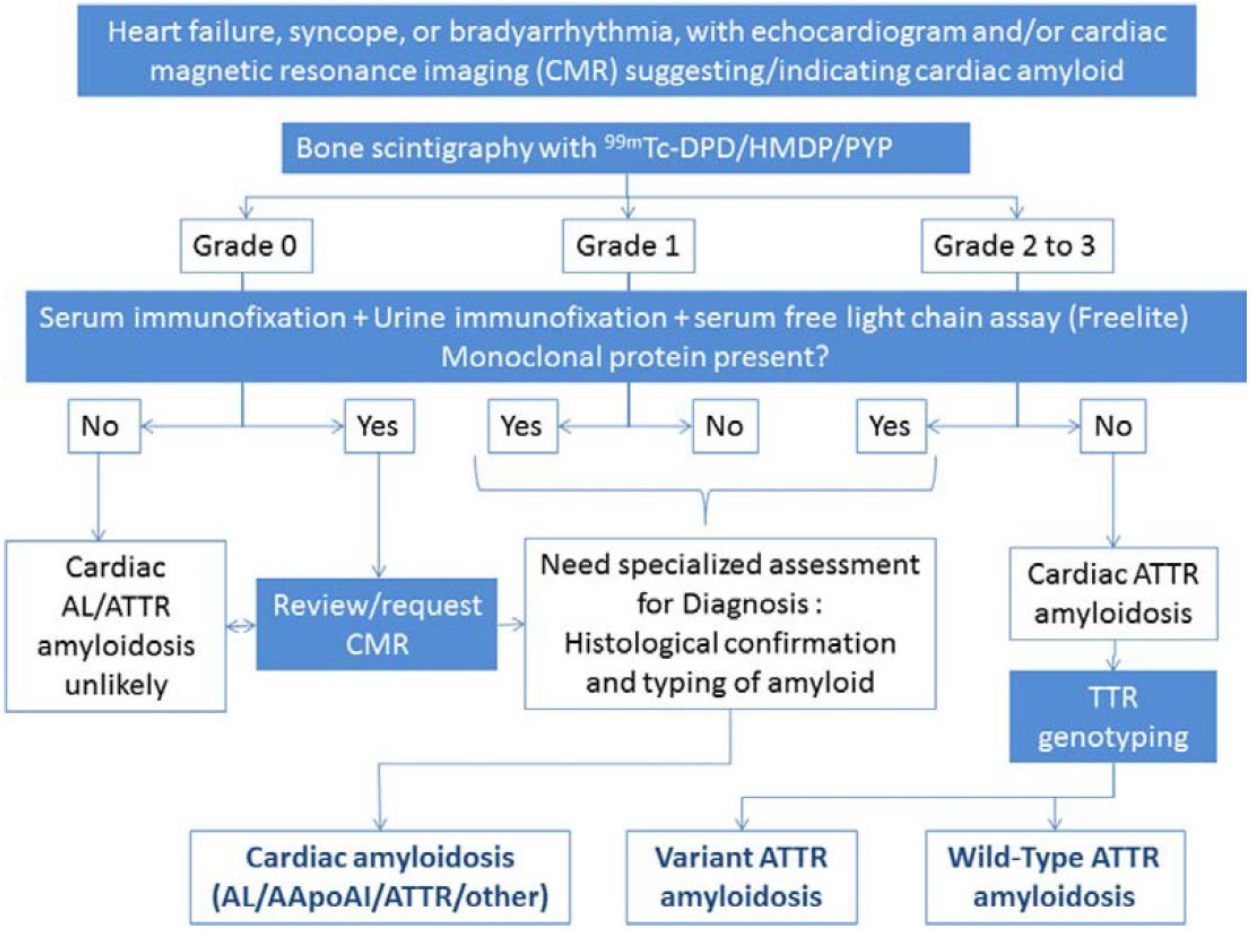
Only variants identified in SARC (ACTC1, ACTN2, MYBPC3, MYH7, MYL2, MYL3, TNNC1, TNNI3, TNNT2, TPM1) and in two non-SARC (CSRP3, JPH2) genes with established associated with HCM[5,48] were considered. Variants were classified according to the guidelines of American College of Medical Genetics[49]. When identified, pathogenic/likely pathogenic (P/LP) variants and variants of uncertain significance (VUS) were validated by Sanger sequencing. Benign/likely benign variants were not taken into consideration for the purpose of this analysis.

3.4 Statistical analysis

Analysis was conducted separately in the two cohorts. In both, carriers of rare SARC variants (SARC+) were compared with non-carriers (SARC-).

Statistical analysis was performed using SPSS software, version 25. Continuous variables were reported as mean ± standard deviation or as median with interquartile range (IQR) and compared by Kruskal-Wallis test. The standard chi-square test was used to compare proportions. A p-value ≤ 0.05 was considered statistically significant.

Figure 1. Diagnostic algorithm for the aetiologic diagnosis of cardiac amyloidosis (Gillmore algorithm). Reproduced from[27].



4. Results

4.1 Arterial hypertension cohort

Sixteen patients with LVH due to arterial hypertensin were included in this cohort; 14 males and 2 females, mean age was 58 ± 13 years (**Table 1**). All but 2 patients were of Caucasian ethnicity.
All patients displayed LVH by LV wall thickness (i.e., all had LV wall thickness ≥12 mm), whereas only 3 (19%) displayed LVH by LMVi.
One (6%) patient had chronic kidney disease; none had diabetes mellitus. All patients were in NYHA functional class I or II. In 5 (31%) patients ECG signs of LVH were observed. Mean LV interventricular septum wall thickness was 13 ± 1 mm, mean end-diastolic diameter 48 ± 4 mm, mean posterior wall thickness 10 ± 2 mm, mean LVM 203 ± 65 g and mean LVMi 99 ± 37 g/m^2. Mean RWT was 0.48 ± 0.04, all patients displayed concentric remodeling/LVH. Mean LV ejection fraction was 59 ± 7%, mean LA diameter was 41 ± 5 mm. Two (12%) patients showed pseudonormal or restrictive filling pattern. The main echocardiographic features of the cohort are reported in **Table 2**.
Three patients (19%) were carriers of rare SARC variants. The first SARC+ patient was a 62-year-old male carrier of the c.2389G>A (p.Ala797Thr) P variant on the MYH7 gene. His LV interventricular septum was 13 mm and LVM 185 g (LMVi 92 g/m^2). His anti-hypertensive therapy consisted of a beta blocker, an angiotensin receptor blocker and a calcium channel blocker.
The second SARC+ patient was a 62-year-old male carrier of the c.1224-80G>A P variant on the MYBPC3 gene. His LV interventricular septum was 14 mm and LVM 193 g (LMVi 88 g/m^2). His anti-hypertensive therapy consisted of a beta blocker and an angiotensin receptor blocker.
The third SARC+ patient was a 33-year-old male of African origin carrier of the c.1054C>G (p.Arg352Gly) VUS on the JPH2 gene. His LV interventricular septum was 13 mm and LVM 271 g (LMVi 121 g/m^2). His anti-hypertensive therapy consisted of angiotensin converting-enzyme inhibitor. **Figure 2** shows an echocardiographic comparison of a SARC+ and a SARC- patients with LVH due to arterial hypertension.
No statistically significant differences were observed between the 3 SARC+ and the 13 SARC- patients. ECG signs of LVH were observed in 2 (67%) SARC+ patients versus in 3 (23%) SARC- (p= 0.21). Mean values of interventricular septum wall thickness, end-diastolic diameter, posterior wall thickness, RWT, LVM, LVMi, LA diameter and LV ejection fraction were similar between SARC+ and SARC- patients.

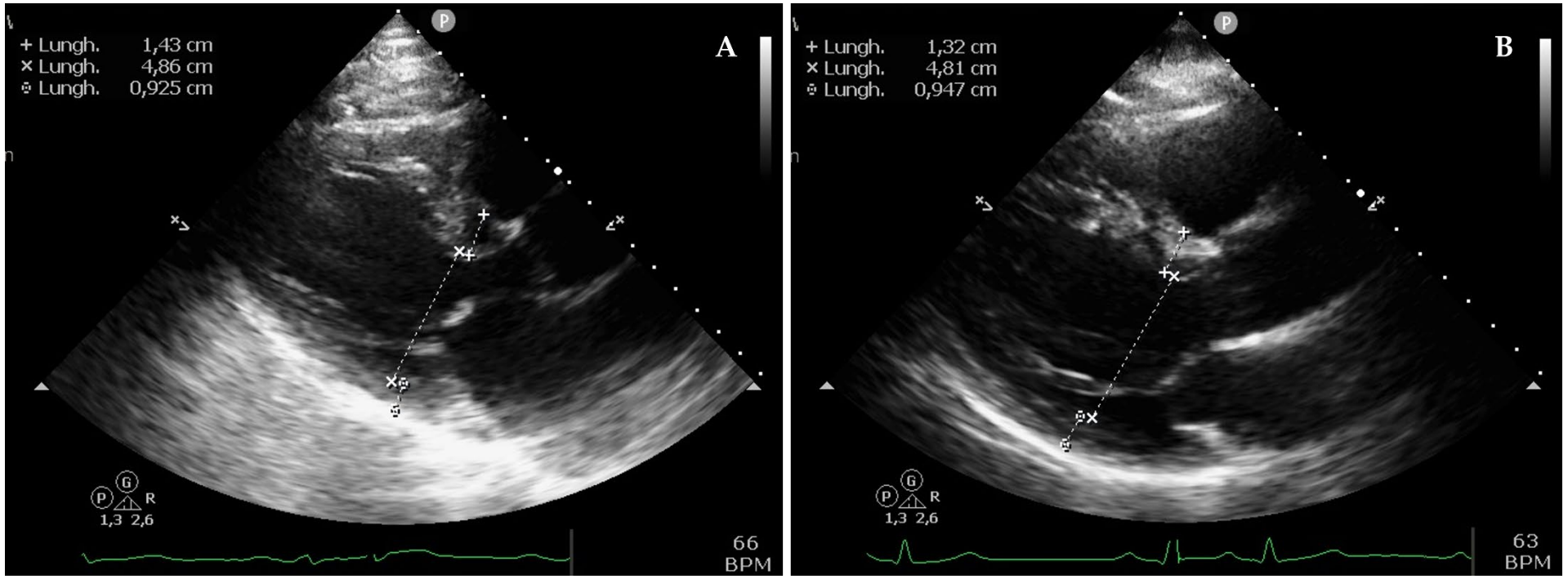
Table 1. Characteristics of patients with LVH due to arterial hypertension.

	Overall cohort n=16 (%)	SARC + n= 3 (%)	SARC – n= 13 (%)	p value
Gender *Males* *Females*	 14 (88) 2 (12)	 3 (100) 0	 11 (85) 2 (15)	1
Age *(years)*	58 ± 13	53 ± 17	59 ± 13	0.46
Caucasian ethnicity	14 (94)	2 (67)	12 (92)	1
Diabetes mellitus	0	-	-	-
Chronic kidney disease	1 (6)	0	1 (8)	1
Atrial fibrillation	1 (6)	0	1 (8)	1
Number of drugs for arterial hypertension treatment	3 ± 1	2 ± 1	3 ± 1	0.33
LVH at ECG	5 (31)	2 (67)	3 (23)	0.21
NYHA *I-II* *III-IV*	 15 (94) 1 (6)	 3 (100) 0	 12 (92) 1 (8)	1
Interventricular septum *(mm)*	13 ± 1	13 ± 1	13 ± 1	0.77
End-diastolic diameter *(mm)*	48 ± 4	50 ± 2	48 ± 5	0.18
Posterior wall *(mm)*	10 ± 2	11 ± 2	10 ± 2	0.40
RWT	0.48 ± 0.04	0.49 ± 0.02	0.48 ± 0.04	1
LVM *(g)*	203 ± 65	216 ± 48	199 ± 70	0.42
LVMi *(g/m^2)*	99 ± 37	100 ± 19	99 ± 41	0.50
LA diameter *(mm)*	41 ± 5	41 ± 9	41 ± 4	0.68
LV ejection fraction *(%)*	59 ± 7	62 ± 3	59 ± 7	0.53
Pseudonormal or restrictive filling pattern	2 (12)	0	2 (15)	1

Table 2. Main echocardiographic features of patients with LVH due to arterial hypertension.

Pt	Gender	Age	Altered gene	IVS	DTD	PP	RWT	LVM	LMVi	Diastolic dysfunction	LA diameter	LVEF
1	**M**	**62**	***MYH7***	**13**	**49**	**10**	**0,49**	**185**	**92**	**N**	**37**	**60**
2	F	45	-	13	40	7	0,5	116	65	N	41	61
3	M	42	-	13	46	8	0,46	153	73	N	33	69
4	M	64	-	17	50	9	0,52	241	107	N	41	58
5	M	53	-	13	48	10	0,48	188	94	Y	40	60
6	M	75	-	15	47	10	0,53	207	99	N	43	60
7	M	65	-	14	45	10	0,53	183	83	N	41	60
8	M	44	-	13	50	8	0,42	173	82	N	35	60
9	**M**	**62**	***MYBPC3***	**14**	**49**	**9**	**0,47**	**193**	**88**	**N**	**51**	**65**
10	M	54	-	12	47	10	0,47	170	74	N	44	50
11	M	67	-	13	46	10	0,5	193	115	N	40	60
12	F	74	-	12	46	12	0,54	201	92	N	43	58
13	M	49	-	12	47	8	0,43	147	69	N	41	65
14	**M**	**33**	***JPH2***	**13**	**52**	**13**	**0,5**	**271**	**121**	**N**	**35**	**60**
15	M	53	-	13	52	9	0,42	212	108	N	45	65
16	M	79	-	15	60	14	0,48	407	226	Y	49	40

Figure 2. Echocardiographic comparison of a SARC+ and a SARC- patient with LVH due to arterial hypertension. Long-axis parasternal view of the LV, with measures of the interventricular septum, the end-diastolic diameter and the posterior wall; on the left (panel A) a patient carrier of a rare SARC variant, on the right (panel B), a SARC- patient.



4.2 ATTRwt cohort

Twenty-two ATTRwt patients were included in this cohort; all males, with a mean age at ATTRwt diagnosis of 79 ± 7 years (**Table 3**). All patients were of Caucasian ethnicity.

All patients displayed LVH by LV wall thickness (i.e., all had LV wall thickness ≥12 mm), whereas 18 (82%) displayed LVH by LMVi.

Three (14%) patients had chronic kidney disease, 5 (23%) had diabetes mellitus, 15 (68%) had arterial hypertension. Fourteen (64%) patients were in NYHA functional class I or II. In 7 (32%) patients low voltages at ECG were observed. Mean LV interventricular septum wall thickness was 16 ± 2 mm, mean end-diastolic diameter 48 ± 5 mm, mean posterior wall thickness 14 ± 2 mm, mean LVM 306 ± 63 g and mean LVMi 167 ± 36 g/m^2. Mean RWT was 0.61 ± 0.12; 2 (9%) patients displayed eccentric LVH. Mean LV ejection fraction was 50 ± 9%, mean LA diameter was 49 ± 5 mm. Eighteen (82%) patients showed pseudonormal or restrictive filling pattern. The main echocardiographic features of the cohort are reported in **Table 4**.

Five patients (23%) were carriers of rare SARC variants. The first SARC+ patient was a 78-year-old male carrier of the c.19A>T (p.Arg146His) VUS on the ACTC1 gene. His LV interventricular septum was 16 mm and LVM 305 g (LMVi 175 g/m^2).

The second SARC+ patient was a 81-year-old male carrier of the c.1224-80G>A VUS on the CSPR3 gene. His LV interventricular septum was 19 mm and LVM 313 g (LMVi 181 g/m^2).

The third SARC+ patient was a 95-year-old male carrier of the c.422A>G (p.Ile148Val) LP variant on the TNNC1 gene. His LV interventricular septum was 13 mm and LVM 272 g (LMVi 147 g/m^2).

The fourth SARC+ patient was a 88-year-old male carrier of the c.3865C>T (p.Arg1289Trp) VUS on the MYH7 gene. His LV interventricular septum was 16 mm and LVM 303 g (LMVi 155 g/m^2).

The fifth SARC+ patient was a 82-year-old male carrier of the c.4817G>A (p.Arg1606His) VUS on the MYH7 gene. His LV interventricular septum was 17 mm and LVM 286 g (LMVi 165 g/m^2).

Figure 3 shows an echocardiographic comparison of a SARC+ and a SARC- patients with ATTRwt.

No statistically significant differences were observed between the 5 SARC+ and the 17 SARC- patients. Mean values of interventricular septum wall thickness, end-diastolic diameter, posterior wall thickness, RWT, LVM, LVMi, LA diameter and LV ejection fraction were similar between SARC+ and SARC- patients. LV ejection fraction appeared slightly higher in SARC+ patients.

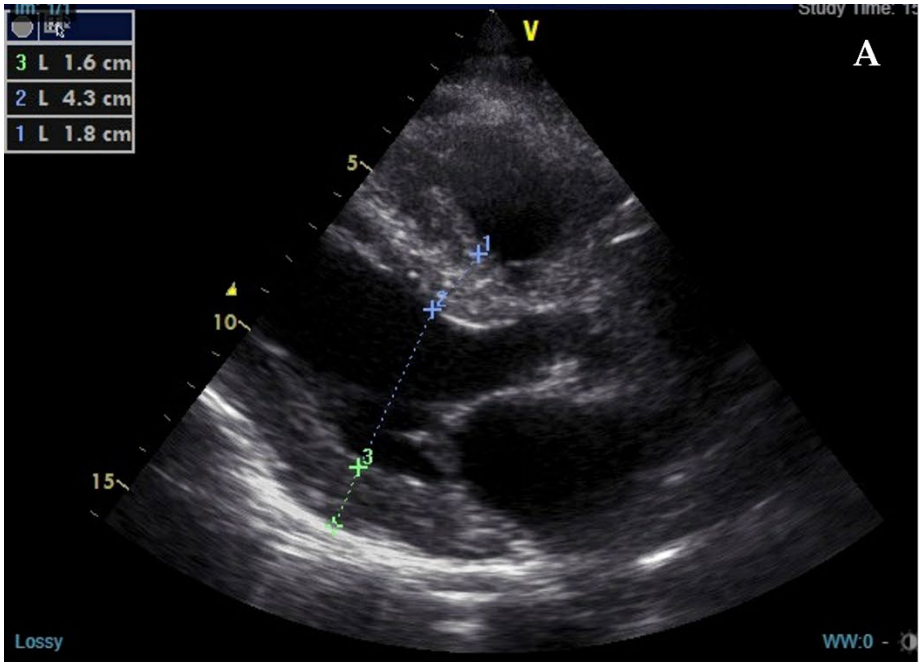
Table 3. Characteristics of ATTRwt patients.

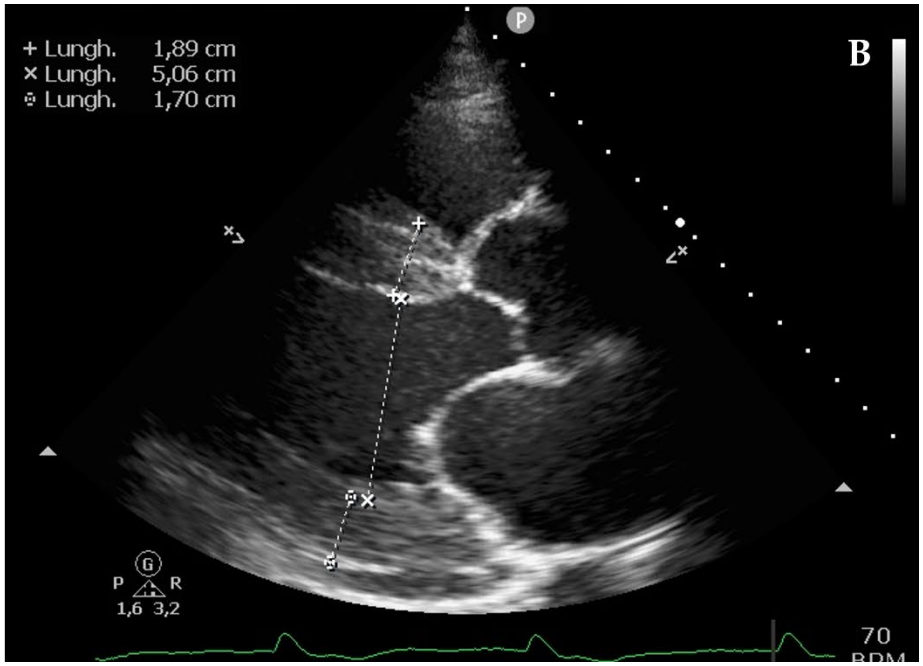
	Overall cohort n=22 (%)	SARC + n= 5 (%)	SARC – n= 17 (%)	p value
Gender *Males* *Females*	 22 (100) 0	 -	 -	 -
Age *(years)*	79 ± 7	85 ± 7	77 ± 7	0.08
Diabetes mellitus	5 (23)	1 (20)	4 (24)	1
Arterial hypertension	15 (68)	3 (60)	12 (71)	1
Chronic kidney disease	3 (14)	2 (40)	1 (6)	0.12
Atrial fibrillation	12 (55)	3 (60)	9 (53)	1
Low voltages at ECG	7 (32)	2 (40)	5 (29)	1
NYHA *I-II* *III-IV*	 14 (64) 8 (36)	 3 (60) 2 (40)	 11 (65) 6 (35)	0.15
Interventricular septum *(mm)*	16 ± 2	16 ± 2	16 ± 2	0.90
End-diastolic diameter *(mm)*	48 ± 5	46 ± 6	49 ± 5	0.33
Posterior wall *(mm)*	14 ± 2	15 ± 2	14 ± 2	0.47
RWT	0.61 ± 0.12	0.65 ± 0.17	0.59 ± 0.11	0.37
LVM *(g)*	306 ± 63	296 ± 17	309 ± 71	0.37
LVMi *(g/m^2)*	167 ± 36	165 ± 14	168 ± 40	0.48
LA diameter *(mm)*	49 ± 5	49 ± 4	49 ± 5	0.91
LV ejection fraction *(%)*	50 ± 9	55 ± 4	49 ± 10	0.19
Moderate-to-severe mitral regurgitation	5 (23)	2 (40)	3 (18)	0.55
Moderate-to-severe aortic stenosis	1 (5)	1 (20)	0	0.23
Pseudonormal or restrictive filling pattern	18 (82)	5 (100)	13 (77)	0.54

Table 4. Main ECG and echocardiographic features of ATTRwt patients.

Pt	Gender	Age	Altered gene	IVS	DTD	PP	RWT	LVM	LMVi	Diastolic dysfunction	LA diameter	LVEF
1	M	88	-	12	42	11	0.52	168	97	N	45	60
2	M	74	-	17	47	14	0.60	309	179	Y	59	60
3	M	81	-	16	44	9	0.41	203	104	N	45	60
4	**M**	**78**	***ACTC1***	**16**	**45**	**16**	**0.71**	**305**	**175**	**Y**	**44**	**60**
5	M	70	-	14	44	14	0.64	240	112	Y	52	50
6	M	85	-	13	52	12	0.64	240	112	Y	45	30
7	**M**	**81**	***CSPR3***	**19**	**40**	**17**	**0.85**	**313**	**181**	**Y**	**47**	**55**
8	M	64	-	18	54	14	0.52	399	204	Y	54	50
9	M	81	-	16	51	15	0.59	250	173	Y	50	50
10	M	80	-	13	54	12	0.44	280	160	Y	45	55
11	M	74	-	17	50	15	0.60	355	210	Y	50	50
12	M	78	-	17	49	13	0.53	313	175	Y	47	35
13	M	81	-	19	40	17	0.85	313	166	Y	46	30
14	M	77	-	17	45	16	0.71	320	156	Y	46	53
15	M	69	-	17	58	14	0.48	425	228	Y	54	45
16	**M**	**95**	***TNNC1***	**13**	**55**	**11**	**0.40**	**272**	**147**	**Y**	**53**	**55**
17	**M**	**88**	***MYH7***	**16**	**48**	**14**	**0.58**	**303**	**155**	**Y**	**51**	**50**
18	M	82	-	17	47	14	0.65	378	219	Y	59	60
19	M	68	-	18	49	16	0.65	378	186	N	39	45
20	**M**	**82**	***MYH7***	**17**	**43**	**15**	**0.70**	**286**	**165**	**Y**	**48**	**55**
21	M	84	-	15	49	17	0.69	345	178	Y	48	40
22	M	77	-	17	50	14	0.56	339	193	N	52	50

Figure 3. Echocardiographic comparison of a SARC+ and a SARC- patient with ATTRwt. Long-axis parasternal view of the LV, with measures of the interventricular septum, the end-diastolic diameter and the posterior wall; on the left (panel A) a patient carrier of a rare SARC variant, on the right (panel B), a SARC- patient.





5. Discussion

Rare SARC variants associated with HCM are found in general population with a prevalence that ranges from 1:200 to 1:1500 individuals[32,34,36,50]. However, an overt HCM phenotype is present together with the genetic variant in a minority of cases. Recently, an investigation from >200 000 participants in the UK Biobank found a prevalence of P/LP SARC variants ranging from 1:149 to 1:250, with overt HCM found in only 2% of carriers[50]. In another analysis from the UK Biobank, De Marvao and colleagues found P/LP SARC variants to be present in about 1:400 and SARC VUS in about 2:100 individuals[32]. Only 4% of carriers of rare SARC variants displayed LVH defined by a LV wall thickness greater than 12 mm. Moreover, independently from the presence of LVH, SARC+ patients had an increased risk over time of death and major cardiac events, including heart failure. Similarly, a study involving the Atherosclerosis in Risk Communities cohort and the UK Biobank found that carriers of variants associated with HCM and dilated cardiomyopathy (including rare SARC variants), despite a low penetrance of the cardiomyopathy phenotype, have an increased risk of death, heart failure and atrial fibrillation[34].

The fact that rare SARC variants are associated with adverse outcomes independently of the presence of overt HCM suggests that they may be contributors, rather than specific determinants, of HCM development[31]; but at the same time, that they may be determinants of metabolic, molecular and microstructural abnormalities – not always evolving into overt LVH – which favor adverse CV events[42,51,52].

Given the discordant prevalence of rare SARC variants and of HCM in the general population, it is possible to hypothesize a role for SARC variants in the development and/or prognosis of other CV conditions and/or diseases, as in the case of secondary LVH. This hypothesis is supported by the fact that primary and secondary LVH eventually display similar molecular and metabolic alterations (i.e., cytosolic calcium overload, energy depletion, microvascular coronary dysfunction[1,4,37,44,51,53–57]), or by the fact that pseudo-LVH, such as cardiac amyloidosis, may need (micro)structural myocardial alterations to develop[40]. A possible role for rare SARC variants is an intriguing hypothesis, especially when considering that pathophysiological mechanisms of secondary LVH and of ATTRwt development are largely unknown. Indeed, even if LVH due to arterial hypertension is associated with blood pressure values, the presence of LVH is variable at similar degree of pressure overload. In an analysis from the Framingham Heart Study, LVH was not found solely in those with uncontrolled blood pressure values. On the contrary, LVH in those with increased blood pressure values had a prevalence ranging 21% to 42%, and of about 11-12% in those with blood pressure values at target[14]. It is well accepted that other factors, beside a ‘pure’ pressure overload, influence LVH development in arterial hypertension[4,20]. Similarly, ATTRwt was

once believed to be a disease of the elderly and only related to aging – and in fact it was called *senile* TTR-related cardiac amyloidosis. However, it is far from clear why ATTRwt develops in only a proportion of the elderly population[58,59], when aging is a virtually universal process. Predisposing factors for ATTRwt development are unknown, and the disease has been found also at 'young' ages (i.e., in patients aged 60 years old)[30].

On this basis, we performed the present study, which was a cross-sectional pilot investigation involving two cohorts with secondary LVH from the Azienda Ospedaliera-Universitaria Sant'Andrea, Sapienza University of Rome. The first cohort included patients with LVH due to arterial hypertension, while the second cohort included patients with ATTRwt. In both cohorts, with the support of the Genetic Department, NGS genetic analysis dedicated to rare SARC variants associated with HCM was conducted.

In the arterial hypertension cohort, the prevalence of SARC+ was 19% (3 out of 16 patients), while in the ATTRwt cohort it was 23% (5 out of 22 patients). Given the small sample size of the two cohorts, it is not possible to draw definitive answers regarding a role of rare SARC variants in the development of secondary LVH. Nevertheless, this result deserves further investigations. Presence and significance of SARC variants in secondary forms of LVH, and in particular in 'hypertensive heart disease' and in ATTRwt were never evaluated.

A genetic background favoring LVH in arterial hypertension has been suggested and speculated. Previous analyses have investigated common genetic variants or polymorphisms, with only partial understanding of this genetic background[4,60,61]. For what concerns ATTRwt, to date only two studies investigated its genetic background. One study found that variants in the RBP4 gene might act as modifiers in the phenotypic expression of the disease[62]. Another study from a small cohort identified four polymorphisms with a possible role in ATTRwt susceptibility[63].

From a 'precision medicine' standpoint, unravelling specific pathophysiologic mechanisms of LVH across the spectrum of the diverse conditions associated with it appears of paramount importance, yet much remains to be defined. Identification of such predisposing factors may hold the potential for novel treatment and preventive strategies in CV medicine.

In our study, SARC+ patients from both cohorts did not show significant differences in terms of LV morphology as compared to SARC- ones. This was somehow expected, as the few previous data on this regard, even if performed without a 'pheno-clustering' of the diverse aetiologies of LVH, never highlighted a significantly worse LVH phenotype in patients carriers of rare SARC variants[32,35,36]. This funding – especially if confirmed in future, larger cohorts – may suggest that the role of rare SARC variant in secondary LVH is that of a susceptibility substrate, and not of a modifier of LVH traits. However, no differences in terms of other clinical and epidemiological variables were

observed in both our cohorts between SARC+ and SARC- patients. It is likely that each LVH conditions recognizes a number of predisposing factors to its development, not all acting together at the same time in each patient. Given this, it might be expected that rare SARC variants act as a substrate for LVH development in specific groups of patients, or if other specific conditions are present. Thus, the fact that no differences were highlighted between SARC+ and SARC- patients in both our cohorts should be interpreted cautiously and may be influenced by the small sample size of our study. It would be fascinating to speculate that, for instance, rare SARC variants might have a role in LVH due to arterial hypertension in those patients with a lower pressure overload, or in younger ATTRwt patients (where other predisposing factors related to aging are not yet present). Furthermore, we acknowledge that future investigations in this field should take into account at least two other aspects:

1. beside the need for larger cohorts, to define an enrichment of rare SARC variants in LVH due to arterial hypertension and ATTRwt, NGS genetic analysis on control groups should be performed. As a strength of the design of our study is the clustering of specific forms of secondary LVH, we anticipate that controls should be identified among peers exposed to a similar 'risk'. For example, in the arterial hypertension cohort, genetic analysis could be performed in those patients with known arterial hypertension, but without LVH, to ascertain whether rare SARC variants are more common in those with LVH.
2. to define whether the presence of rare SARC variants also translates into worse outcomes, as observed in the general population, a prospective follow-up to collect clinical data and event is needed.

In conclusion, results from this pilot study should be considered only hypothesis-generating. Nevertheless, we investigated a previously unexplored aspect in the field of secondary LVH, with highly selected and well-phenotyped patients. Preliminary findings from our analyses granted us two academinc fundings (Bandi di Ateneo 2021, Sapienza University of Rome; Finanziamento SEED – Programma Nazionale per la Ricerca 2022, Sapienza University of Rome) to proceed with the study.

6. Conclusions

In this pilot cross-sectional study, rare SARC variants were found in a non-negligible proportion of patients with secondary LVH, warranting further investigations. Definitive results regarding a possible role of rare SARC variants as predisposing factors for LVH development, outside the HCM phenotype, cannot be drawn to date. Larger cohorts, with control groups, are needed to ascertain their role and relevance within the different subsets of secondary LVH.

Pathophysiological mechanisms at the basis of LVH due to arterial hypertension and ATTRwt remain largely undefined. Unravelling specific predisposing factors in the two conditions may prove beneficial to develop targeted treatments.

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