The p.(Cys150Tyr) variant in *CSRP3* is associated with late-onset hypertrophic cardiomyopathy in heterozygous individuals

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Abstract

Introduction and objectives

Up to 50% of patients with hypertrophic cardiomyopathy (HCM) show no disease-causing variants in genetic studies. Mutations in *CSRP3* have been associated with HCM, but evidence supporting pathogenicity is inconclusive. In this study, we describe an HCM cohort with a missense variant in *CSRP3* (p.Cys150Tyr) with supporting evidence for pathogenicity and a description of the associated phenotype.

Methods

CSRP3 was sequenced in 6,456 index cases with a diagnosis of HCM and in 5,012 probands with other cardiomyopathies. In addition, 3,372 index cases with hereditary cardiovascular disorders other than cardiomyopathies (mainly channelopathies and aortopathies) were used as controls.

Results

The p.(Cys150Tyr) variant was identified in 11 unrelated individuals of the 6,456 HCM probands, and it was not identified in patients with other cardiomyopathies ($p<0.0001$) or in our control population ($p<0.0001$). Ten of the index cases were heterozygous and one was homozygous. Homozygous had a more severe phenotype. Family screening identified 17 other carriers. Wild-type individuals showed no signs of disease. The mean age at diagnosis of affected individuals was 55 ± 13 years, and the mean left ventricular wall thickness was 18 ± 3 mm. The variant showed highly age-dependent penetrance. After a mean follow-up of 11 (±8) years, no adverse events were reported in any of the HCM patients.

Conclusions

The p.(Cys150Tyr) variant in *CSRP3* causes late-onset and low risk form of hypertrophic cardiomyopathy in heterozygous carriers.

Keywords:

hypertrophic cardiomyopathy, genetics, CSRP3, z-disk; cardiomyopathies

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disorder, with an estimated prevalence of at least 1:500 (1). The majority of cases are inherited as an autosomal dominant trait, and clinical expression is variable even within families (2). Genetic testing in HCM is recommended according to current clinical guidelines in order to facilitate cascade screening of at-risk relatives (3), and to provide information on prognosis (4,5). However, despite advances in genetic sequencing methods, up to 50% of cases show no disease-causing variants after extensive study (5,6).

CSRP3 encodes cysteine-rich protein 3, a muscle LIM protein which belongs to the family of CRP proteins (7). These proteins, predominantly localized to the Z-disk, mediate protein-protein interactions. Moreover, they are important for the maintenance of the myocyte cytoskeleton, have mechanosensory functions, and participate in actin cytoskeleton assembly (8,9). To date, only a few variants in this gene (mainly missense) have been associated with the development of HCM (10-13), and most of the available descriptions focus on index cases without familial cosegregation studies or belong to small, uninformative families (14-17). For this reason, more evidence is required to establish the association between *CSRP3* and HCM (17,18).

In this study, we describe a cohort with a missense variant in *CSRP3* (p.Cys150Tyr) and provide supporting evidence for pathogenicity and a description of the associated phenotype.

METHODS

From June 2014 to November 2019, *CSRP3* was sequenced using next generation sequencing (NGS) in 6,456 index cases with fulfilling a diagnosis of HCM according to current criteria (3) and in 5,012 probands with other cardiomyopathies referred to Health in Code (A Coruña, Spain) for genetic testing. Additionally, 3,372 index cases with a suspicion of an inherited

cardiovascular disorder other than cardiomyopathies (mainly channelopathies and aortic diseases) were used as controls.

Clinical phenotypes had been established by the referring centers prior to the genetic study. Patient samples were mainly received from hospitals in Spain and the United Kingdom, and the predominant ethnicity was European (>90%). The frequencies of variants in the general population were determined by using the gnomAD database (http://gnomad.broadinstitute.org; version v2.1.1, March/2020). Clinical and genetic familial cascade screening was performed when possible, following written informed consent. As part of the evaluation, all first-degree family members were offered clinical, echocardiographic and genetic studies to test for cosegregation of the variant with disease. Our study was conducted according to the Declaration of Helsinki. All participants signed an informed consent prior to the study.

Genetic studies, variant filtering and variant classification

Coding exons and intronic boundaries of 219 genes related to inherited cardiovascular diseases and sudden cardiac death (Supplementary Table 1) were captured using a custom probe library (SureSelect Target Enrichment Kit for Illumina paired-end multiplexed sequencing method; Agilent Technologies, Santa Clara, California, USA) and sequenced on the HiSeq1500 platform (Illumina, San Diego, California, USA) following lllumina protocols. The read depth of every nucleotide of genes related to the referred phenotype (including *CSRP3*) was >30 (mean 250-400). Exons that did not fulfill this standard were complementary sequenced using the Sanger method. Bioinformatic analysis was performed by means of a custom pipeline including software for variant calling, genotyping and annotation. To determine the pathogenicity of identified variants, we used the American College of Medical Genetics and Genomics classification (19).

Statistical Analysis

Continuous variables were expressed as mean ± SD, and comparison between groups was performed using Student's t test or the Mann-Whitney U test, depending on values distribution. Non-continuous variables were expressed as an integer number (percent of total) and compared using the chi-square test or Fisher exact test, as appropriate. A 2-sided p value <0.05 was considered to indicate statistical significance.

The analysis was performed using the R version 3.4.3 (The R Foundation for Statistical Computing Platform).

RESULTS

Eleven of the 6,456 HCM index cases harbored the p.(Cys150Tyr) variant in *CSRP3*. The variant has been submitted to ClinVar (accession SCV001423823). All of these patients were apparently unrelated. Patients came from three hospitals in Spain and one in the United Kingdom and were all of European (non-Finnish) descent. The variant was not identified in any of the individuals with other cardiomyopathies ($p<0.0001$) or in our control population ($p<0.0001$). Moreover, this variant is clearly enriched in the HCM population when compared with gnomAD (11/6,456 vs 4/141,417; $p<0.0001$). Ten of the index cases were heterozygous and one was homozygous for the variant. All carriers agreed to participate in the study. Importantly, none of the probands had other genetic variants that would explain the disease (Supplementary Table 2).

Familial Cosegregation

Seven families underwent clinical and genetic study of at least one at-risk family member. In four of the remaining families, the family members did not agree to undergo further studies or could not be reached for evaluation. A total of 32 family members were included, leading to the identification of another 17 heterozygous subjects. None of the wild-type family members had signs of HCM. Supplementary Figure 1 shows the family pedigrees.

Clinical characteristics

Of the 28 individuals harboring the p.(Cys150Tyr) variant (index cases and family members), sixteen (57%) fulfilled the criteria for HCM. Importantly, a marked age-dependent penetrance was observed (Figure 1).

Table 1 shows the baseline characteristics of individuals fulfilling HCM criteria. The mean follow-up duration of these individuals was 11 (±8) years. The mean age at diagnosis was 55 (±13) years, and 75% were males. The mean maximal left ventricular wall thickness (MLVWT) was 18 (±3 mm), and in 81% of cases the hypertrophy was asymmetric septal. No cases of extreme (i.e. >30 mm) left ventricular hypertrophy were observed. Figure 1 shows the relationship between age and MLVWT according to gender. Although a trend towards more severe degrees of hypertrophy is observed in males, this difference is not statistically significant.

The main electrocardiographic finding was the presence of an increased left ventricular voltage criteria (i.e. Sokolow-Lyon criteria >35 mm), which was present in 17 carriers (61%). The presence of pathological Q waves was reported only in 21% of patients. No cases of short PR, preexcitation or advanced atrioventricular block were reported.

Table 1. Baseline characteristics of individuals harboring the p.(Cys150Tyr) variants in *CSRP3* and fulfilling HCM criteria

	n=16
Male sex (%)	12 (75)
Age at diagnosis, years (±)	55 (13)
Hypertension (%)	5 (31)
Diabetes mellitus (%)	2 (7)
Dyslipidemia (%)	2 (7)
Family history of SCD	1 (4)
Reason for diagnosis (%) Chest pain Syncope Dyspnea Abnormal ECG Family study	 1 (6) 3 (19) 6 (38) 1 (6) 5 (31)
NYHA functional class (%) I-II III-IV	 15 (94) 1 (6)
AF (%)	3 (19)
LVEDD mm (%)	43 (5)
IVS mm (±) Echocardiogram MRI	 18 (3) 19 (3)
PW thickness	11 (3)

LA mm (±)	42 (5)
EF % (±)	70 (8)
LV morphology affected subjects (%) ASH Concentric Apical	 13 (81) 2 (13) 1 (6)
LVOTO (%)	7 (47)
Holter NSVT (%)	2 (13)
ICD (%)	1 (6)
Treatment (%) Betablockers Oral anticoagulants	 14 (88) 3 (19)
HCM-risk score (±)	2.2 (1.6)

ASH: asymmetric septal hypertrophy; ECG: electrocardiogram; EF: ejection fraction; ICD: implantable cardioverter-defibrillator; IVS: interventricular septum; LA: left atrium; LVOTO: left ventricular outflow tract obstruction; LV: left ventricle; LVEDD: left ventricular end-diastolic diameter; MRI: magnetic resonance imaging; NSVT: non-sustained ventricular tachycardia; PW: posterior wall; SCD: sudden cardiac death

Table 2 shows the specific clinical and echocardiographic characteristics of all evaluated individuals. The most severe case was observed in a homozygous male, who had 23 mm LV hypertrophy and was the youngest individual at the time of diagnosis (22 years old). Likewise, this subject was the only one who had an ICD implanted for primary prevention.

The ESC risk score calculator (3) showed a low risk of adverse events, with a mean value of 2% (±1.4). This result reflected the characteristics of these patients, as none of the carriers had an adverse event.

Table 2. Clinical characteristics of individuals harboring the p.(Cys150Tyr) variant

Family	**Case**	**Gender**	**Age at diagnosis/ last follow/up**	**Diagnosis**	**Reason for diagnosis**	**LVEDD (mm)**	**IVS (mm)**	**LA (mm)**	**EF (%)**	**LVOTO**	**HCM-risk score %**	**Others**
H350	II:2	M	47/74	HCM	Dyspnea	44	17	45	74	Yes (88)	1.6	
	II:4	M	55/65	HCM	Family study	42	17	41	72	No	1.2	Apical hypertrabeculation
	III:2	M	35		Family study	48	9	40	78	No	N/A	LVH criteria on ECG
	III:3	M	34		Family study	48	9	36	65	No	N/A	
	III:4	M	32		Family study	41	10	34	57	No	N/A	Apical hypertrabeculation
H15645	II:4	M	54/64	HCM	Syncope	45	19	46	86	Yes (100)	2.3	
	III:3	M	34		Family study	43	11	39	60	No	N/A	

	II:2	F	65/72	HCM	Family study	40	14	34	64	No	0.7	Apical hypertrabeculation
	III:1	F	41		Family study	44	10	34	68	No	N/A	
H28251	II:3	M	53/63	HCM	Syncope	38	17	35	75	No	1	
	I:2	F	78/88	HCM	Family study	37	16	40	66	No	1.8	
	III:3	F	40		Family study	40	10	35	70	No	N/A	
	III:4	M	37		Family study	41	11	34	64	No	N/A	
	II:7	F	47		Family study	46	10	39	60	No	N/A	
	II:8	F	46		Family study	39	10	35	61	No	N/A	
H67225	II:6	M	50/54	HCM	Dyspnea	49	20	46	78	No	2.2	
	II:5	M	63	HCM	Family study	45	14	40	58	No	0.9	

	II:2	F	68		Family study	45	12	35	65	No	N/A	
H67241	II:2	M	48/60	HCM	Chest pain	40	19	47	80	No	1.7	
H75094	II:3	F	45/75	HCM	Abnormal ECG	42	20	48	65	Yes (80)	2.1	
H46872	II:1	M	22/34	HCM	Dyspnea	35	23	36	74	Yes (90)	6.4	Homozygous. ICD primary prevention
	I:2	F	65		Family study	42	12	40	64	No	N/A	
H94882	II:3	M	79	HCM	Dyspnea	50	20		78	No		
H141	II:3	M	60/71	HCM	Dyspnea	48	19	42	65	Yes (77)	3.3	
	II:2	F	64/69	HCM	Family study	42	16	39	68	Yes (43)	1,1	
	III:4	F	45		Family study	45	12	38	68	No	N/A	
H114253	II:2	M	51/62	HCM	Dyspnea	41	19	37	65	No	1.3	
H114259	II:2	M	51/63	HCM	Syncope	48	22	50	60	Yes (117)	3.1	

Characteristics of the p.(Cys150Tyr) *variant*

The p.(Cys150Tyr) variant in *CSRP3* is present with a low frequency in the gnomAD database (4/141,417 individuals *vs* 11/6,456 index cases in our HCM population; $p<0.0001$), and only in heterozygosity. In the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/219444; accessed on April, 2020), there are conflicting interpretations about this variant's pathogenicity; one submitter classified it as likely pathogenic, whereas four as of unknown clinical significance. The Cys150 residue belongs to the second LIM domain; these domains are cysteine-rich and important for the maintenance of the structure and stability of the protein's domain. Finally, this is a highly conserved region between species, highlighting its importance (Figure 2).

On the basis of the ACMG criteria and given its characteristics, we consider that this variant is likely pathogenic. Specifically, its prevalence in affected individuals exceeds that in the control population; we observed familial cosegregation with the disease; and multiple bioinformatics tools that suggest a deleterious effect (Supplementary Table 3).

DISCUSSION

To the best of our knowledge, this study is the largest evaluating the association of a genetic variant in *CSRP3* and the development of hypertrophic cardiomyopathy in multiple families (Table 3).

Table 3. Genetic variants in *CSRP3* previously reported to be associated with HCM*

Variant	**Zygosity**	**Number of families**	**Number of patients**	**Reference**	**gnomAD frequency**	**ClinVar (# submitters)**	**Identified index cases HiC**	**Others**
c.50insGCAGATTTCTT p.(Tyr18Glnfs*194)	Het	1	4	van Rijsingen et al. (14)	1/251 444	N/A	N/A	This family also harbored the pathogenic variant p.(Arg633His) in *MYH7*. Double heterozygotes had an apparent more severe phenotype.
c.122_123dupGG p.(Lys42Glyfs*167)	Het	1	1	Bos et al. (11) Theis et al. (14)	N/D	Pathogenic (1)	N/A	This case had the p.(Asn468Ser) variant of unknown significance in *LDB3*.
c.131T>C p.(Leu44Pro)	Het	3	6	Geier et al. (10) Bos et al. (11) Geier et al., (12) Santos et al. (13)	4/282 276	Pathogenic (1) Likely pathogenic (1) VUS (3)	5 HCM	One of the patients also carried the pathogenic variant p.(Thr1042Lysfs*5) in *MYBPC3*.
c.136A>C p.(Ser46Arg)	Het	2	8	Geier et al. (12)	8/282 324	Pathogenic (1) VUS (3)	4 HCM 1 DCM	

c.160_164delTCGGAinsAGGGG p.(Ser54_Glu55delinsArgGly)	Het	1	3	Geier et al. (12)	N/D	Pathogenic (1)	N/A	
c.172T>G p.(Cys58Gly)	Het	1	11	Geier et al. (10) Geier et al. (12)	N/D	Pathogenic (1)	N/A	Functional studies showed a less stable mutant protein compared to wild-type.
c.190C>T p.(Arg64Cys)	Het	1	1	Bos et al. (11)	11/ 282 630	VUS (2)	N/A	Patient harbored the likely benign variant p.(Ile1131Thr) in *MYBPC3*.
c.197A>G p.(Tyr66Cys)	Het	1	1	Bos et al. (11)	3/282 690	VUS (3)	N/A	The patient harbored the pathogenic variant p.(Arg162Gln) in *TNNI3*.
c.272A>T p.(Gln91Leu)	Het	1	1	Bos et al. (11)	4/282 382	VUS (2)	1 HCM 1 DCM	
c.364C>T p.(Arg122*)	Hom	1	2	Lipari et al. (16)	N/D	Likely pathogenic (2)	N/A	
c.369T>A p.(Cys123*)	Hom	1	1	Janin et al. (17)	1/251 368	N/A	N/A	Familial cosegregation analysis not performed.
c.483_484insC p.(Lys162Glnfs*52)	Hom	1	1	Janin et al. (17)	1/251 460	N/A	N/A	Familial cosegregation analysis not performed.

Het=heterozygous; hom=homozygous; HiC= Health in Code; N/A=Not available; VUS: variant of unknown significance

*The p.(Arg100His) and p.(Trp4Arg) variants have been excluded, as nowadays they are considered polymorphisms and not disease-associated.

In our cohort, the p.(Cys150Tyr) variant was associated with mild late-onset HCM in heterozygous individuals, with an apparently good prognosis. The relationship between heterozygous rare variants in *CSRP3* and the development of mild degrees of LVH has also been suggested by other groups (2).

The age-dependent penetrance observed in our cohort is consistent with the current knowledge that non-genetic factors (such as age and gender) influence disease risk amongst pathogenic variant carriers and that the genetic architecture for many cardiovascular phenotypes should be viewed as a spectrum, rather than a simple dichotomy (20).

Moreover, we report for the first time a homozygous individual with a missense variant in *CSRP3* who presented an earlier disease onset and a more severe phenotype when compared to heterozygous subjects, thereby supporting the pathogenicity of this variant. Two groups have previously reported the presence of truncating biallelic variants in *CSRP3* as a potential cause of HCM (16,17), identifying three unrelated subjects (Table 3). Familial cosegregation was not reported in these studies. In light of our results, it is important to consider the possibility that the aforementioned variants could be also associated with mild phenotypes in heterozygous carriers, as it is the case of the p.(Cys150Tyr) variant, and this should be clarified in further studies. The importance of biallelic variants (truncating and missense) in *CSRP3* as a cause of HCM is supported by population genetic data, as only one homozygous individual with a rare variant (MAF <0.001) in *CSRP3* is identified in the gnomAD database.

Since the advent of next-generation sequencing techniques, the number of genes potentially associated with inherited cardiovascular disorders has greatly increased, although the level of evidence is variable (21). Recently, Ingles et al. (18) evaluated the validity of 57 genes reported to be associated with HCM. This group identified that only

eight of these genes were definitively associated with the disease (*MYBPC3, MYH7, TNNT2, TNNI3, TPM1, ACTC1, MYL2* and *MYL3*). According to these authors, the level of evidence between *CSRP3* and HCM is moderate, and more studies are required in order to fully establish this association.

The relationship between *CSRP3* and the development of cardiac hypertrophy has been studied by several groups, mainly in animal models. Arber et al. (8) reported that knockout mice models showed a significant activation of several hypertrophy signaling cascades, which led to severe hypertrophy in mice, cytoskeleton disturbances and ventricular dilatation and dysfunction. Moreover, loss of function of CSRP3 leads to significant calcium handling disturbances (22) and mechanical stress in cardiomyocytes, which also favors the development of hypertrophy (23).

The importance of assigning appropriate pathogenicity to genetic variants identified in individuals with hereditary cardiovascular disorders has been highlighted by several groups (24-26). Walsh et al. (24) showed that 11.7% of the individuals included in the ExAC database carried genetic variants that had been previously associated with hypertrophic cardiomyopathy. This frequency exceeds that expected for disease-causing variants (1), suggesting an incorrect classification of many of these variants. Therefore, accurate and comprehensive interpretation of rare variants remains one of the main challenges in clinical genetics (27). A more precise stratification of the risk associated with specific genetic variants is of extreme importance and may have an impact on the follow-up of both patients and family members (20). Studies such as this one, evaluating specific variants affecting several families with careful cosegregation analysis and demonstrating enrichment in HCM population compared with controls, are useful to assign an appropriate pathogenicity to the identified genetic variants. Our paper contributes to a better knowledge of the phenotype associated with *CSRP3*

variants, through the evaluation of a disease-causing variant in several families with HCM.

The role of the p.(Cys150Tyr) variant in CSPR3 in pediatric population is unknown. Our work suggests that it is associated with late-onset HCM in heterozygous individuals. A less stringent follow-up may be considered in pediatric heterozygous carriers without the phenotype, although more evidence is needed to fully establish this hypothesis. Recently, Lorenzini et al. (28) showed that following a first negative screening, approximately 50% of individuals carrying a pathogenic sarcomeric variant (both in adult and pediatric populations) develop HCM over 15 years of follow-up, highlighting the importance of long-term surveillance.

Limitations

Although this is the largest study reporting a likely pathogenic variant in *CSRP3*, the low number of affected individuals harboring the variant precludes a more extensive evaluation of the phenotype associated with this variant. Given that haplotype studies were not performed, we cannot be certain that this is a founder mutation.

CONCLUSIONS

The p.(Cys150Tyr) variant in *CSRP3* is a cause of hypertrophic cardiomyopathy, with late onset and apparently low risk in heterozygosity. Although more evidence is needed, it is possible that homozygous subjects have a more severe phenotype.

Figure Legends

Figure 1. Scatter plot showing the relationship between age and maximal wall thickness (in millimeters) in patients harboring the p.(Cys150Tyr) variant in *CSRP3*. Broadly, males show greater degrees of hypertrophy when compared with females. It can be seen that individuals with normal degrees of LV thickness are young subjects. The homozygous patient shows an earlier age of diagnosis and the most severe hypertrophy.

Figure 2. Conservation analysis of the second LIM domain of CSRP3 in 69 orthologous species. The pink line shows the percentage of sequences with amino acids suitable for analysis, whereas the blue line shows the percentage of sequences with the reference amino acid. The red box corresponds to the Cys150 position, showing an extremely conserved residue.

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