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Desmoplakin Missense and non-Missense Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy: Genotype-Phenotype Correlation

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ABSTRACT

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is traditionally considered as primarily affecting the right ventricle. Mutations in genes encoding desmosomal proteins account for 40-60% of cases. Genotype-phenotype correlations are scant and mostly non gene-specific. Accordingly, we assessed the genotype-phenotype correlation for desmoplakin (DSP) missense and non-missense mutations causing ARVC.

Methods and Results: We analyzed 27 ARVC patients carrying a missense or a non-missense DSP mutation, with complete clinical assessment. The two groups were compared for clinical parameters, basic demographics such as sex, age at diagnosis, age at disease onset, as well as prevalence of symptoms and arrhythmic events. Missense DSP variants were present in 10 patients and non-missense in 17. Mean age at diagnosis and at first arrhythmic event did not differ between the two groups. Also the prevalence of symptoms, either major (60% vs 59%, p=1) or all (80% vs 88%, p=0.61), did not differ. By contrast, left ventricular (LV) dysfunction was significantly more prevalent among patients with non-missense mutations (76.5% vs 10%, p=0.001), who were also much more likely to have a structural LV involvement by Cardiac Magnetic Resonance (CMR) (92% vs 22%, p=0.001).

Conclusions: For ARVC patients, both missense and non-missense DSP mutations carry a high arrhythmic risk. Non-missense mutations are specifically associated with left-dominant forms. The presence of DSP non-missense mutations should alert to the likely development of LV dysfunction. These findings highlight the clinical relevance of genetic testing even after the clinical diagnosis of ARVC and the growing clinical impact of genetics.
KEY WORDS

- Ventricular Arrhythmias
- Left Ventricular Dysfunction
- DSP truncating mutations
- Arrhythmogenic Cardiomyopathy

ABBREVIATIONS

2DE Two-dimensional echocardiography
ACA Aborted cardiac arrest
ARVC Arrhythmogenic right ventricular cardiomyopathy
CMR Cardiac magnetic resonance
DCM Dilated cardiomyopathy
DSP Desmoplakin
EF Ejection fraction
ICD Implantable cardioverter defibrillator
LGE Late gadolinium enhancement
LV Left ventricle
NSVT Non-sustained ventricular tachycardia
RV Right ventricular
SAECG Signal-averaged ECG
SCD Sudden cardiac death
VT Ventricular tachycardia

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**AUTHORSHIP AND DISCLOSURES**

All authors take the responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

The authors report no relationships that could be construed as a conflict of interest.
INTRODUCTION

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is an inherited disease characterized by risk for sudden cardiac death (SCD) and fibro-fatty replacement primarily of the right ventricle but, occasionally, also of the left ventricle (LV) (1-3). Mutations in the five major desmosomal genes account for 40-60% of cases and there is highly variable phenotypic expression and incomplete penetrance (2). The existing genotype-phenotype correlation studies (4-12) are, with one notable exception (11), of limited size, thus not allowing firm conclusions. Desmoplakin (DSP) mutations account for 2-12% of ARVC cases (11,13-15). However, the number of patients with DSP mutations is extremely small in all published reports. As an example, in the largest such study to date (11), which involved 577 genotyped ARVC patients, there were only 9 index patients with DSP mutations, definite clinical diagnosis, and follow-up data.

Three statements, reported in the literature, are of interest but are based on very small numbers. One is that DSP mutations seem to be more often associated with a predominant LV phenotype or biventricular involvement (4,8,16-21); another is that DSP mutations (4,11), especially truncations (22), are associated with a much more penetrant phenotype with SCD often as first disease manifestation; the third, and more controversial, is that ARVC missense mutations could be more severe than non-missense mutations (10,23).

The genotype-phenotype correlation, as could be expected, seems to differ according to the specific type of genetic disease and the respective underlying genetic substrate that is being affected. In channelopathies, missense mutations are usually associated with greater clinical severity, since they often exert a dominant-negative effect on wild-type channels, thereby altering their biophysical properties and functionality, whereas non-missense mutations may cause haploinsufficiency with relatively minor clinical consequences (24,25).

In cardiomyopathies such as ARVC, missense mutations seem to mostly affect protein stability, inter-domain contacts and intra-protein interactions, while preserving the overall dimensional structure, whereas non-missense mutations seem to mostly result in decreased localization of the protein in the intercalated disk (26-29).
With the goal of identifying potential phenotypic differences between carriers of missense and non-missense DSP mutations we have assembled the largest group so far of ARVC probands with a DSP mutation.

METHODS

Study Population and Clinical Assessment

The study population included 27 probands fulfilling the diagnostic criteria for ARVC and carrying a pathogenic or possibly pathogenic variant in the DSP gene. We limited the study to probands in order to avoid the confounding consequence of the incomplete penetrance which would follow the inclusion of family members. All patients were diagnosed according to the revised Task Force Criteria (2) at the Heart Hospital, University College London Hospitals NHS Trust (UCLH), London, UK and at the Center for Cardiac Arrhythmias of Genetic Origin, IRCCS Istituto Auxologico Italiano, Milan, Italy.

The assessment included clinical history, pedigree evaluation, 12-lead and signal-averaged ECG (SAECG), two-dimensional echocardiography (2DE) with detailed right ventricular (RV) views, maximal exercise stress test, 24 hour ambulatory ECG monitoring and CMR with late gadolinium enhancement (LGE) using a dedicated protocol (30). Symptoms included syncope of likely arrhythmic origin, non-sustained and sustained ventricular arrhythmias recorded during 24 hour ambulatory ECG monitoring and/or exercise stress test, aborted cardiac arrest (ACA) and implantable cardioverter defibrillator (ICD) appropriate interventions. Non-sustained ventricular tachycardia (NSVT) was defined as 3 or more consecutive ventricular beats with an RR interval of <600 ms (>120 bpm) and lasting <30 seconds, while if lasting ≥30 seconds was defined as sustained ventricular tachycardia (VT); ICD appropriate interventions were defined as any ventricular event treated by the device with anti-tachycardia pacing and/or shock. Symptoms were subdivided in major (syncope/VT/VF/ACA/ICD appropriate shocks) and minor (NSVT). Patients with atypical chest pain, palpitations and/or syncope suggestive of vasovagal origin were considered asymptomatic.

Twelve-lead electrocardiograms were recorded at baseline at a paper speed of 25 mm/s. Parameters evaluated from basal electrocardiogram were: PR, QRS and QT intervals, QRS voltages,
flattened/inverted T waves, epsilon waves, QTc (Bazett). The presence of flattened/inverted T waves and low QRS voltages was separately evaluated as to their occurrence in anterior leads (V1-V3), lateral leads (I, aVL, V5-V6), inferior leads (II, III, aVF). Flattened/inverted T waves and low QRS voltages were considered diffuse when present in ≥2 lead groups. All electrocardiographic parameters were manually measured by a single experienced cardiologist (SC) blinded to the patients’ genetic status.

Cardiac structural and functional abnormalities were defined by echocardiography in all and by CMR in 20 patients, according to the Task Force Criteria (2). Left ventricular dysfunction was defined as ejection fraction (EF) ≤ 55% (31).

All patients had ECG, SAECG, echocardiogram performed at baseline and during yearly follow-up. Exercise testing and 24hr Holter recordings were performed at baseline in all patients and repeated according to the clinical status of each patient. For the purpose of this study, age at the onset of arrhythmic symptoms was considered separately from the age at the time of diagnosis and from the age at the onset of LV dysfunction.

All patients provided written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ethics committees.

**Genetic Analysis**

Genetic testing for the main ARVC genes (PKP2, DSP, DSC2, DSG2, JUP, TMEM43) was performed through Next-Generation Sequencing (TruSeq Custom Amplicon, MiSeq platform, Illumina and SureSelect Target Enrichment, Agilent Technologies, HiSeq platform, Illumina) as previously described (32,33). All significant variants identified were then confirmed with Sanger sequencing. The patients were not tested for the newly discovered CDH2 gene (34).

Genetic variants in the DSP gene were evaluated according to their frequency in the general population (Exome Aggregation Consortium, Exome Variant Server, 1,000 Genomes Project) (35-37), presence or absence in human genetic variants databases (38-41), literature description, localization and conservation.

For the purpose of the study, and considering ARVC disease prevalence, common (minor allele frequency >0.01%) and well-established, according to literature, benign variants were excluded.
from further analyses. Only rare genetic variants (minor allele frequency <0.01%) expected to affect the protein sequence were considered. These were then stringently classified as pathogenic or possibly pathogenic. Pathogenic were considered either i) non-missense variants that are predicted to generate radical modifications of the protein sequence (i.e. frameshift insertions or deletions, splice site substitutions) and absent in the general population databases or ii) missense variants already reported more than once in literature as clearly associated with ARVC and absent in the general population databases. Possibly pathogenic were considered either i) missense variants already reported only once in literature and/or in genetic variants databases in association with ARVC and absent in the general population databases, or ii) missense variants identified in heterozygous state in the general population no more than 2 times with no other information available and affecting highly conserved residues.

Among the 27 probands with a DSP mutation, 6 were also carrying a second pathogenic or possibly pathogenic desmosomal variant.

Statistical Analyses

Analyses were performed using the statistical software SPSS Statistics, version 21.0 (IBM Co, Armonk, NY). Continuous variables were expressed as mean and standard deviation (SD) or as median and interquartile range (IQR, 25th-75th percentile) whenever the distribution was skewed. Group comparisons in continuous variables were performed with the Student t test or with the Mann-Whitney U test, as appropriate. Categorical variables were presented as absolute (n) and relative frequencies (%) and compared among genetic groups with the Fisher exact test or χ² test, as appropriate. In taking into account the potentially confounding influence on phenotype of the double variants, a sensitivity analysis was performed with all the comparisons repeated upon exclusion of the 6 patients. A 2-sided p value <0.05 was considered statistically significant.
RESULTS

Study population

The study population included 27 clinically confirmed ARVC probands (see suppl. Table 1), in whom genetic analysis identified 25 extremely rare (minor allele frequency<0.01%) DSP gene variants (see suppl. Table 2). Twenty-one patients carried a pathogenic variant (either missense or non-missense), while 6 carried a possibly pathogenic variant. All the possibly pathogenic variants were missense. Non-missense variants were found in 17 patients and missense variants in 10 patients. Most variants clustered in the head of the DSP protein (Figure 1). There were no significant differences in basal characteristics of patients according to the type of DSP variant (Table 1). In total, females were slightly overrepresented (16, 59%) and all probands but one (Afro-Caribbean) were Caucasians (26, 96%). No skin or hair abnormalities were observed in any of the DSP mutation carrier patients. The patients were referred to us because of symptoms (16, 59%) or after SCD of a family member (11, 41%). During the clinical workout, a history for SCD and/or a previous diagnosis of ARVC among first or second-degree family members was ascertained in 12 probands referred for symptoms. Therefore, in 23/27 (85%) patients a positive family history was detected and it was similarly present in the two genetic groups (70% vs 94%, p=0.13). Also, mean age at diagnosis was comparable between missense and non-missense mutation carriers (41.5±10 vs 44±11 years, p=0.52).

Symptoms

Table 1 shows that the prevalence of symptomatic patients was similar among missense and non-missense carriers (80% vs 88%, p=0.61) and this was true also for either major (6/10, 60% vs 10/17, 59%) or minor arrhythmic events (2/10, 20% vs 5/17, 29%, p=0.68). The LV ejection fraction at the time of arrhythmic events was preserved in both groups while being slightly, but significantly, higher in missense carrier patients (63±6 % vs 55±7 %, p=0.015). Age at appearance of arrhythmias did not differ between missense and non-missense mutation carriers, even in the subgroup of 16 referred for symptoms.

Electrocardiogram, Signal-Averaged ECG and Holter Recording

The majority of patients in both missense and non-missense carrier groups presented with diffusely inverted/flattened T waves. Similarly, SAECG was positive in the majority of patients in
both groups. No statistically significant differences were observed in the parameters considered (see suppl. Table 3). No differences were observed between the two groups on ECG 24-hour Holter recordings parameters (see suppl. Table 3).

**Echocardiogram**

Table 2 shows that there were no differences in the RV dimensions at presentation on echocardiogram. Although not statistically significant, a trend towards a lower LV ejection fraction was observed in the non-missense mutation carriers (50±12 vs 60±13%, p=0.07). Fourteen (52%) patients developed LV dysfunction. The occurrence of LV dysfunction was clearly more prevalent among patients with non-missense mutations (76.5% vs 10%, p=0.001). The only patient with a missense variant who developed LV dysfunction did it at age 72. No patient in either group has progressed to functional class NYHA III-IV or undergone heart transplantation during a median follow up time of 80 months (IQR 30-118).

**Cardiac Magnetic Resonance**

A standardized ARVC CMR imaging protocol was performed at a mean age of 44± 12 years in 22 /27 (81%) patients for the detection of both myocardial fibrosis by LGE and fatty infiltration. In one patient the CMR was performed in another Center and data are not available, while in the 4 remaining subjects, a previously implanted ICD precluded the examination. A significantly higher percentage of positive findings at either CMR parameter evaluation was found among non-missense mutation carriers compared to missense mutation carriers (12/13, 92% vs 2/9, 22%, respectively, p=0.001) (Table 3). This difference was primarily due to the presence of LGE that indicated a significantly more frequent LV involvement in 11/13 (85%) non-missense carrier patients compared to 1/9 (11%) missense mutation carriers. In particular, in the non-missense subset this overrepresented left ventricular involvement had a predominant isolated LV pattern (6/11, 55%), which was not present in any of the missense patients. Conversely, the detection of ventricular fat was not significantly different between the two genetic groups.
Cascade genetic screening, performed so far in 8 families, has led to the identification of 28 genotype-positive family members. The clinical evaluation was completed in 25 of 28 and 14 of them (56%), all with a non-missense variant, showed signs of left ventricular involvement.

**Sensitivity analysis**

In addition to their primary DSP gene mutation, 6 patients carried another desmosomal pathogenic or possibly pathogenic variant (4 PKP2, 1 DSC2 and 1 DSP) (see suppl. Table 4). After their exclusion from the entire study population (4 of 10 DSP missense mutation carriers and 2 of 17 DSP non-missense mutation carriers), all results, including the CMR finding, were confirmed (see suppl. Table 5 for details). With respect to the arrhythmic events, all these 6 patients had an arrhythmic phenotype as one would expect in double-mutation carriers.

**DISCUSSION**

The present study, despite the relatively small absolute numbers, represents so far the largest collection of ARVC probands carrying a DSP mutation and the first to examine clinical differences between carriers of missense vs non-missense DSP variants. This allows us to make some credible statements about genotype-phenotype correlations in carriers of DSP mutations. There are several main findings. LV dysfunction and LV structural involvement are significantly more common in carriers of non-missense mutations, in agreement with previous suggestions (5,6,17,19,22). There is no difference between carriers of missense and non-missense mutations as arrhythmic events are concerned, at variance with previous reports (10,22,23). Similarly, and in agreement with a recent publication (11), nothing in our data supports previous claims that missense mutations could be more clinically severe than non-missense mutations (10,23).

**DSP Mutations and Left Ventricular Dysfunction**

ARVC is a disease whose original definition dates back to the early 80s (42) and has been traditionally considered as an inherited disorder primarily affecting the right ventricle. Although substantial LV involvement had been reported in several patients already in 1997 (1,43), only more recently it has been established that, besides the classical right-sided disease pattern, the disease may
present with a phenotypic expression broader than previously anticipated and encompassing biventricular or even predominantly LV forms (6).

Significant LV involvement in the setting of DSP mutations has been observed since the first attempts to correlate genotype and phenotype in ARVC, with 2 out of 4 carriers of distinct DSP mutations exhibiting LV abnormalities (4). Although in ARVC the LV is often affected after the RV has developed a significant dysfunction, in a number of cases the LV is predominantly affected already early on in the disease course, exhibiting features of dilation, systolic impairment, with no or modest RV involvement (8). This is referred to as the left-dominant form of ARVC.

Recently, DSP mutations have been often associated with predominant LV or biventricular involvement (4,6,8,11,16-22). It has been suggested that, among all DSP mutations, those which are non-missense are often associated with left dominant or biventricular forms (6,17). Even in the initial clinical descriptions of DSP mutation carriers (4), only the carriers of non-missense mutations had an early LV involvement (17). This observation has been further replicated, with small numbers, in other studies reporting on single patients (17,19) or on a few more than a dozen missense and non-missense DSP mutation carriers within a population of genotyped ARVC probands (6).

We present the largest group of patients to date with a clear ARVC phenotype associated with DSP mutations, carrying 26 variants, 17 non-missense and 9 missense. Non-missense carriers had more structural involvement compared to missense carriers. This was well shown by the echocardiogram and tissue characterization performed with CMR. Particularly, LV systolic function at presentation was impaired in the vast majority of the non-missense carriers, while it was preserved in all missense carriers. LV involvement was more prominent in non-missense carriers both at echocardiogram and CMR. On the echocardiogram, LV systolic function was preserved in all but one missense carriers; on CMR, none of the missense carriers had isolated LV involvement, while this was predominant in the non-missense carriers patients. Our results confirm previous numerically limited observations and demonstrate that LV dysfunction and LV involvement are significantly more common in carriers of non-missense DSP mutations, thus highlighting DSP truncating mutations as a key emerging genetic substrate of LV disease. This concept is reinforced by the observation of left
ventricular involvement in more than half of the family members evaluated so far, all of whom had non-missense variants.

This concept is also supported by studies on the Carvajal syndrome (44) and on dilated cardiomyopathy (DCM) (45). The Carvajal syndrome, caused by homozygous DSP non-missense mutations, is a syndromic form of ARVC with an extra-cardiac cutaneous phenotype. Although initially the cardiac phenotype in the Carvajal syndrome was presented as more similar to a DCM phenotype (46), later on it was shown that the pathology of the heart as well as the ECG features were more in line with a biventricular form of ARVC with substantial LV involvement (4,47). In another study on DCM patients, desmosomal gene mutations were found in 5% of patients (45) and among all variants identified, those with the strongest evidence of pathogenicity were 2 novel non-missense DSP variants. Although it is known that end-stage ARVC and DCM can often be indistinguishable (8), that study highlighted the fact that desmosomal gene mutations, and DSP truncations in particular, may be the common denominator behind a compromised LV. Therefore, in presence of a DSP truncating mutation, a closer follow-up of LV function should be considered.

**DSP Mutations and Arrhythmic Events**

Desmoplakin mutations (4,11,17), and especially truncations (22), have been associated to more penetrant phenotypes, with SCD often being the sentinel event. In our series, the majority of DSP mutation carrier patients (59%) indeed experienced a major cardiac event. However, at variance with previous suggestions (22), there were no significant differences in either all or major arrhythmic events, nor in the patients’ age at these events, among carriers of missense and non-missense mutations. In the largest published series of patients carrying the same DSP truncating mutation (22), which however involved only 3 probands with 15 family members, a highly penetrant and malignant clinical phenotype was described, with concomitant LV non-compaction in 2 families. In that study, similarly to many of our cases, the LV was predominantly involved. Although our data support a prominent role of DSP truncating mutations in LV disease and LV or biventricular ARVC forms, they do not confirm previous suggestions that this specific type of mutations increases arrhythmic risk. On the other hand, since also in our series the majority (59%) of DSP mutation carriers experienced a life-threatening arrhythmic event, our data reinforce the earlier observations that DSP mutations,
irrespective of their type, may contribute to more penetrant phenotypes with increased arrhythmic propensity (4,11).

**DSP Missense vs Non-Missense Mutations**

We have explored the issue of ARVC clinical severity according to the type of DSP mutations. In contrast to previous reports (10,23) we found no association between the type of mutations and clinical severity, whether an earlier age at diagnosis, an earlier age of disease onset, or greater prevalence of major arrhythmic events. The only clear association found is LV dysfunction in the presence of non-missense DSP mutations.

The relatively small numbers of our study are balanced by three points of strength. First, our patient population has a clear ARVC phenotype according to the revised Task Force criteria (2). Second, our population includes a comparable number of carriers of missense and non-missense mutations and of patients with and without symptoms and major events, thus allowing us to examine separately the role of age at disease diagnosis and at disease onset. Third, we have mostly considered variants with high levels of evidence for pathogenicity, thereby avoiding to assign prognostic value to variants of unknown significance which could interfere with a correct interpretation.

Desmoplakin is a large protein and an obligatory component of the desmosome since it links desmosomal protein partners, mainly plakophilin and plakoglobin, to the intermediate filaments of the cytoskeleton, forming the basis of cell-to-cell adhesion. It has 3 unique domains, each with particular secondary structure and function (26). Although the rule of thumb that non-missense truncating mutations act through haploinsufficiency and missense mutations through dominant-negative effects generally applies, functional studies have shown that there are notable exceptions (26-29). Until elaborate functional studies will provide insights on how each particular mutation exerts its effect in the setting of a cross-talk with a dynamic cellular environment and the rest of genetic background, our data support the notion that there are no differences in terms of arrhythmic risk between missense and non-missense DSP mutations in ARVC.
CONCLUSION

One of the main objectives of genotype-phenotype correlations, besides gathering information on the underlying mechanisms of action of different mutations, is to assign prognostic implications to particular genetic substrates to implement risk stratification strategies and individualized patient management. Our findings support the concept, already validated for channelopathies such as the long QT syndrome (24,48), that even within the same disease genotype-phenotype correlations should be gene-specific. We show that ARVC-associated *DSP* mutations correlate with a high arrhythmic risk and that non-missense mutations are specifically associated with left-dominant forms. The presence of *DSP* truncating mutations should alert to the likely development of LV dysfunction. These findings highlight the potential impact on clinical management of genetic testing even after the clinical diagnosis of ARVC.
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35. Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [December, 2015 accessed].


FIGURE 1 LEGEND

Schematic representation of the main structural and functional domains of the desmoplakin protein showing the relative position of mutations reported in this study. Missense mutations are depicted in orange, non-missense mutations are depicted in blue, while mutations predicted to affect splicing are reported in italics. A, B, C plakin repeat domains; GSR glycine/serine/arginine-rich domain.
Table 1. Demographic and clinical characteristics of the 27 ARVC probands according to the genetic status.

<table>
<thead>
<tr>
<th></th>
<th>Missense (n=10)</th>
<th>Non-missense (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>6 (60)</td>
<td>10 (59)</td>
<td>1</td>
</tr>
<tr>
<td>Caucasian</td>
<td>10 (100)</td>
<td>16 (94)</td>
<td>1</td>
</tr>
<tr>
<td>Family History *</td>
<td>7 (70)</td>
<td>16 (94)</td>
<td>0.13</td>
</tr>
<tr>
<td>Age at diagnosis, yrs</td>
<td>41.5±10</td>
<td>44±11</td>
<td>0.52</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>8 (80)</td>
<td>15 (88)</td>
<td>0.61</td>
</tr>
<tr>
<td>Major arrhythmic events **</td>
<td>6 (60)</td>
<td>10 (59)</td>
<td>1</td>
</tr>
<tr>
<td>Minor arrhythmic events ***</td>
<td>2 (20)</td>
<td>5 (29)</td>
<td>0.68</td>
</tr>
<tr>
<td>EF at arrhythmia, (%)</td>
<td>63±6</td>
<td>55±7</td>
<td>0.015</td>
</tr>
<tr>
<td>Age at first arrhythmic event, yrs</td>
<td>37±9</td>
<td>43±11</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Data are absolute n and relative frequencies (%) or mean ± SD. *Family history for ARVC and/or SCD in first or second – degree family members. ** Syncope, VT, VF, CA, ICD appropriate shock. *** NSVT

EF, Ejection fraction.
Table 2. Echocardiographic parameters of the 27 ARVC probands according to the genetic status.

<table>
<thead>
<tr>
<th></th>
<th>Missense (n=10)</th>
<th>Non-missense (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAX RVOT, mm</td>
<td>3.5±0.4</td>
<td>3.5±0.7</td>
<td>0.99</td>
</tr>
<tr>
<td>SAX RVOT, mm</td>
<td>3.4±0.5</td>
<td>3.4±0.9</td>
<td>0.84</td>
</tr>
<tr>
<td>RVIT, mm</td>
<td>3.4±0.6</td>
<td>3.4±0.9</td>
<td>0.88</td>
</tr>
<tr>
<td>Impaired RVEF</td>
<td>2 (20)</td>
<td>2 (12)</td>
<td>0.61</td>
</tr>
<tr>
<td>RVWMA</td>
<td>1 (10)</td>
<td>3 (18)</td>
<td>1</td>
</tr>
<tr>
<td>LVEDd, mm</td>
<td>5.0±0.6</td>
<td>5.2±0.6</td>
<td>0.25</td>
</tr>
<tr>
<td>LVESd, mm</td>
<td>3.4±0.6</td>
<td>3.8±0.7</td>
<td>0.11</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>60±13</td>
<td>50±12</td>
<td>0.07</td>
</tr>
<tr>
<td>LV dysfunction</td>
<td>1 (10)</td>
<td>13 (76.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at LV dysfunction, yrs</td>
<td>72</td>
<td>45±12</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Data are absolute n and relative frequencies (%) or mean ± SD. LAX RVOT, right ventricular outflow tract diameter in long axis view; SAX RVOT, right ventricular outflow tract diameter in short axis view; RVIT, right ventricular inflow tract; RVEF, right ventricular ejection fraction; RVWMA, right ventricular wall motion abnormalities; LVEF, left ventricular ejection fraction; LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter.
Table 3. Cardiac Magnetic Resonance tissue characterization according to the genetic status

<table>
<thead>
<tr>
<th></th>
<th>Missense (n=9)</th>
<th>Non-missense (n=13)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive CMR, n (%)</strong></td>
<td>2 (22)</td>
<td>12 (92)*</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>LGE parameter, n (%)</strong></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>negative</td>
<td>8 (89)</td>
<td>2 (15)</td>
<td></td>
</tr>
<tr>
<td>isolated LV</td>
<td>0 (0)</td>
<td>6 (46)</td>
<td></td>
</tr>
<tr>
<td>biventricular</td>
<td>1 (11)</td>
<td>5 (39)</td>
<td></td>
</tr>
<tr>
<td><strong>FAT parameter, n (%)</strong></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>negative</td>
<td>7 (78)</td>
<td>6 (46)</td>
<td></td>
</tr>
<tr>
<td>isolated RV</td>
<td>1 (11)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>isolated LV</td>
<td>0 (0)</td>
<td>2 (15)</td>
<td></td>
</tr>
<tr>
<td>biventricular</td>
<td>1 (11)</td>
<td>5 (39)</td>
<td></td>
</tr>
</tbody>
</table>

Data are absolute n and relative frequencies (%).

*One CMR positive patient had a biventricular involvement only by fat parameter.