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# Clinical characteristics and determinants of phenotype in TMEM43 Arrhythmogenic right ventricular cardiomyopathy type 5.

Short title: Phenotype of TMEM43 ARVC Type 5

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**ABSTRACT:**

**Background**

Arrhythmogenic right ventricular cardiomyopathy type V (ARVC-5) is the most aggressive heterozygous form of ARVC. It is predominantly caused by a fully penetrant mutation (p.S358L) in the non-desmosomal gene *TMEM43* – endemic to Newfoundland, Canada. To date, all familial cases reported worldwide share a common ancestral haplotype. It is unknown whether the p.S358L mutation by itself causes ARVC-5 or if the disease is influenced by genetic or environmental factors.

**Objective**

To examine the phenotype, clinical course and the impact of exercise on patients with p.S358L ARVC-5 without the Newfoundland genetic background.

**Methods**

We studied 62 affected individuals and 73 non-carriers from 3 *TMEM43*-p.S358L Spanish families. Impact of physical activity on phenotype was also evaluated.

**Results**

Haplotype analysis revealed that the 3 Spanish families were unrelated to ARVC-5 patients with the Newfoundland genetic background. Two families shared 10 microsatellite markers in a 4.9 cM region surrounding *TMEM43*; the third family had a distinct haplotype. Affected individuals presented a 38.7% incidence of SCD, higher in males. LV involvement was common with 40% of mutation carriers showing LVEF<50%. Compared with non-carriers, R wave in V3 was lower (3.2±2.8 vs 7.5±3.6 mV; $P<0.001$) and QRS in right precordial leads wider (104.7±24.0 vs 88.2±7.7 ms;$P=0.001$). History of vigorous exercise showed a trend towards more ventricular arrhythmias only in women ($P=0.053$).

**Conclusions**

ARVC-5 is associated with high risk of SCD and characteristic clinical and ECG features irrespective of geographical origin and genetic background. Our data suggest that, as in desmosomal ARVC, vigorous physical activity could aggravate the phenotype of *TMEM43* mutation carriers.

Word count: **256**

**Keywords:** TMEM43, arrhythmogenic right ventricular cardiomyopathy, exercise, genetics, arrhythmia.

## Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a common cause of sudden cardiac death (SCD) in young adults. It is considered primarily a disease of the desmosome, as mutations in desmosomal genes have been identified in ~50% of patients who fulfil diagnostic criteria (1).

The most aggressive heterozygous form of ARVC is ARVC type V (ARVC-5). A missense mutation on chromosome 3p25, within the non-desmosomal gene encoding transmembrane protein 43 (*TMEM43* c.1073C>T, p.S358L), was reported in 2008 as a cause of ARVC-5 in 15 families from the island of Newfoundland, Canada (2,3). These families share a common ancestral haplotype, and the affected subjects present an autosomal dominant, fully penetrant, sex-influenced and high-risk form of ARVC. Later, other mutations in the same gene have been associated with ARVC-5, but p.S358L is the predominant one (4).

The p.S358L mutation leading to ARVC-5 was also identified in families from Germany, Denmark and North America, and haplotype

analysis revealed that those families shared a common ancestor with the Newfoundland affected patients (5).

To date, the TMEM43-p.S358L missense mutation responsible for ARVC-5 has only been reported once in a patient with a non-Newfoundland origin (4). This single case was described in Toronto (Canada) and corresponds to a 43 year-old male from New Zealand with a confirmed *de novo* mutation, suggesting that a hot spot for this sequence alteration might exist at this point in *TMEM43*.

No descriptions of additional non-Newfoundland patients have appeared and data are lacking regarding patients with ARVC-5 without the Newfoundland genetic background. It is also currently unknown whether the p.S358L mutation itself causes ARVC-5 or if the disease expression is influenced by the genetic background or environmental factors.

In the present study, we sought to describe the phenotype and clinical expression of non-Newfoundland-related ARVC-5.

## Methods

**Study population**

Three apparently unrelated families with a high incidence of sudden cardiac death (SCD) across multiple generations were evaluated at four centres in Madrid, Valencia and Granada, Spain. This study was approved by the ethics committee of the participant centers and complies with the Declaration of Helsinki. All participants provided written informed consent. We investigated the phenotype and natural history of ARVC-5 disease caused by the p.S358L mutation in these families by comparing clinical events and test results in affected *versus* unaffected family members (family controls) born at *a priori* 50% risk, an ascertainment strategy unrelated to clinical presentation. Haplotypes from the three families were compared with those present in Newfoundland-related individuals from Denmark, Germany, North America and Newfoundland.

Subjects were considered affected if they were genetically confirmed, obligate carriers of the p.S358L mutation or had SCD ≤ 50 years (3). Subjects were considered unaffected if they did not carry the p.S358L

mutation. The remaining subjects were considered unknown and were not studied further.

To minimise the bias present in recognising cases with SCD and missing cases with minimal disease, we limited the analysis to subjects from sibships where the disease status (affected or unaffected) of ≥ 50% of siblings was known, as previously described (6). Clinical evaluation included a detailed medical history, physical examination, ECG and transthoracic echocardiogram.

The burden of physical activity was assessed by means of a structured telephone interview to available individuals. Participants were asked about the intensity and duration of regularly performed exercise, including leisure-related, transportation and work activities since 10 years of age. Intensity was rated according to the Multi-Ethnic Study of Atherosclerosis Typical Week Physical Activity Survey as previously described (7). Exercise duration was determined after asking the subjects between which ages and how many hours per day had they participated in each physical activity. Physical activity was considered vigorous when a high

intensity exercise (>70% of maximum oxygen uptake) was performed more than 50 hours per year (8).

**Haplotype analysis**

A total of 16 microsatellite markers spanning *TMEM43* on chromosome 3p25 were selected for haplotype analysis, which was performed in selected members from the three Spanish families and in three additional families from Newfoundland, Denmark and North America. None of the families were known to be related. The genetic distances (cM) were inferred from the deCODE genetic map.

**Statistical analysis**

Results are presented as mean (standard deviation) for continuous variables with normal distribution, as median (interquartile range) for continuous variables without normal distribution, and as number (percentage) for categorical data. For statistical analysis, Student's *t*-test and the Mann-Whitney non-parametric test were used in two-group comparisons. The chi-

square test or Fisher's exact test were used for categorical variables. A two-tailed *P*-value of <0.05 was considered to be statistically significant. All statistical analyses were performed using the SPSS package, version 20.0 (SPSS Inc., Chicago, IL, USA).

## Results

The three families comprised a total of 180 subjects (*Figure 1*). The family evaluated in Madrid (Family 1) had a total of 131 members across 7 generations. The 5-generation family from Valencia had 24 members (Family 2) and the remaining 25 subjects belonged to a 5-generation family from Granada (Family 3). Considering the three families, a total of 62 patients (59.7% males; mean age at last evaluation or death 39.1±17.6 years) were classified as affected (30 genetically-confirmed carriers, 14 were obligate carriers and 18 untested individuals who had SCD ≤50 years of age and belonged to sibships where the disease status of ≥50% of siblings were known). The remaining subjects were either non-carriers (n=73, 56.2% males; mean age at last evaluation or death 46.1±17.6 years) or their clinical status was considered as unknown (n=45, 42.2% males; mean age at last evaluation or death 50.0±41.5 years). Subjects in the unknown group were not further analysed.

The p.S358L (c.1073C>T) *TMEM43* missense mutation was initially identified using next-generation sequencing cardiomyopathy panels

(including >50 genes) or by exome sequencing in the probands from the 3 families. No other pathogenic variants were identified in desmosomal or other cardiomyopathy-related genes. The presence of the p.S358L *TMEM43* mutation in probands was confirmed by PCR amplification and Sanger sequencing. Family members who agreed to undergo genetic testing were tested for the presence of the p.S358L *TMEM43* mutation also by Sanger sequencing.

**Haplotype analysis**

Haplotype analysis revealed that index patients from Family 1 and 2 shared 10 microsatellite markers in a 4.9 cM region surrounding *TMEM43*. Comparison of the haplotypes from Family 1 and 2 with those of patients with p.S358L ARVC-5 from Newfoundland, United States and Denmark showed that only 5 common markers were shared (*Figure 2*). Thus, the p.S358L mutation leading to ARVC-5 in Family 1 and 2 was not inherited from the same ancestor as the patients in the other regions. The haplotype from Family 3 differed from the other two Spanish families and from the

Newfoundland-origin patients, and this family was considered to have a different genetic origin (*Figure 2*).

**Genotype and phenotype analysis**

Pedigree analysis and clinical follow-up of Family 1 revealed 36 affected subjects. A total of 15 subjects tested positive for the *TMEM43*-p.S358L mutation, while 11 individuals were obligate carriers and 10 had experienced SCD ≤50 years of age and belonged to sibships where the disease status of ≥50% of siblings was known. Family 2 had 12 affected subjects (5 confirmed carriers, 2 obligate carriers and 5 subjects with SCD ≤ 50 years). In Family 3, there were 14 affected subjects (10 confirmed carriers, 1 obligate carrier and 3 SCD ≤ 50 years). Clinical, ECG and echocardiographic findings of the three families are summarised in *Table 1* and pedigrees are depicted in *Figure 1*.

*TMEM43*-affected individuals showed a 31% incidence of SCD ≤50 years (n=19) and the youngest individual who suffered SCD was 22-year-old male. As in the Newfounland ARVC-5 population, SCD was significantly

more prevalent among male subjects compared to female subjects (45.9% vs 8.0%; *P*=0.002). QRS duration was also longer in male mutation carriers compared to non-carriers (115.6±27.2 vs 94.5±15.6 ms;*P*=0.02). A total of 24 carriers presented SCD (mean age 44.6±14.3 years). Heart failure was the cause of death in 2 mutation carriers (ages 47 and 70) and stroke in another 2 (ages 64 and 69).

Considering echocardiographic parameters, mean left ventricular ejection fraction (LVEF) was significantly lower in mutation carriers than in non-carriers at first evaluation (56.2±10.9% vs 62.1±7.0%;*P*<0.001), while left ventricular end diastolic volume (LVEDV) was significantly greater (106.3±40.4 vs 84.1±18.3 ml;*P*=0.03). LVEF was found to be <50% in 37.5% of the mutation carriers and almost 20% of affected patients presented clinical heart failure (*Table 1*).

Regarding right ventricular echocardiographic parameters, systolic function assessed by TAPSE was found to be lower in carriers (19.1±8.4 vs 23.5±3.5 mm;*P*=0.03) despite right ventricle (RV) size was not significantly different between carriers and non-carriers (*Table 1*).

Regarding ECG findings, mean QRS duration in right precordial leads was significantly wider in mutation carriers than in non-carriers (104.7±24.0 vs 88.2±7.7 ms;*P*=0.002), and mean R in V3 was 3.2±2.8 as compared with 7.5±3.6 mV in non-carriers (*P*<0.001) (*Table 1* and *Figure 3*).

Shown in *Figure 3* is a representative three-dimensional endomyocardial voltage map of an ARVC-5 patient who had undergone ventricular tachycardia ablation, showing extensive areas of scar in the right ventricle. Also shown are cardiac MRI from two affected patients. Additionally, *Figure 4* shows the postmortem study of the heart from an ARVC-5 patient who experienced SCD, with biventricular fatty infiltration and evidence of fibrosis in the histological analysis.

**Physical activity in patients with ARVC-5**

Among the 27 mutation carriers available for exercise telephone interviews, 13 (76.9% male) were considered to have a vigorous physical activity history since the age of 10, and the remaining 14 (35.7% male) were classified as non-vigorous exercisers. In the first group, 9 of the 13 patients

had performed daily farming and agriculture activities, including heavy carrying and lifting, for more than 10 years. One patient worked transporting heavy furniture for 7 years, another worked 15 years as a baker lifting >20 kg bags, and the remaining 2 practised sports with high dynamic demand at vigorous intensity for a mean of >50 hours per year (7).

The prevalence of ventricular tachycardia and/or ventricular fibrillation (VT/VF) was 61.5% in the vigorous physical activity group compared with 28.6% in the group with a history of less physical activity, and the difference showed a statistical trend but did not reach significance (*P*=0.08). As male ARVC-5 subjects are known to have a very poor prognosis with high incidence of arrhythmias, we undertook a sub-analysis by sex. We found no differences regarding VT/VF incidence in men who performed high intensity exercise (60% in males with both vigorous and non-vigorous exercise history; *P*=1). By contrast, vigorous exercise presented a statistical trend towards more ventricular arrhythmias in women

(66.7% vs 11.1%;*P*=0.054). However, exercise did not have a negative impact on echocardiographic and ECG findings (*Table 2*).

## Discussion

The present study provides the first clinical description of ARVC-5 in families with a non-Newfoundland-related genetic background. Our study confirms that ARVC-5 caused by the p.S358L mutation in *TMEM43* is a fully penetrant arrhythmic cardiomyopathy associated with a high risk of SCD irrespective of the patients' geographical origin and genetic background. Moreover, it confirms the worse prognosis of male mutation carriers, that left ventricle (LV) structural and functional abnormalities are frequent in ARVC-5, and that ECG signs such as lower voltages in V3 (which depicts poor R wave progression [PRWP]) and prolonged QRS in right precordial leads are hallmarks of the disease. Finally, our study shows for the first time that vigorous exercise is likely to be associated with arrhythmias in ARVC-5, particularly among female patients.

**Phenotype of ARVC-5 in non-Newfoundland patients**

The observed phenotype and clinical course of the affected Spanish individuals was in concordance with previous reports of ARVC-5 patients from Newfoundland (2,3). ECG characteristics of the affected patients were prolonged QRS duration in right precordial leads, as well as PRWP, which was identified by a lower R wave voltage in V3. Interestingly we observed that a cut-off value of R amplitude <4.5mV had a sensitivity of 80.6% and a specificity of 74.1% to predict mutation carriers in our cohort.

We also observed that the LV was enlarged and dysfunctional in affected individuals as compared with non-carriers, with almost 40% of the affected subjects with a LVEF under 50% (and ventricular arrhythmia in 89% of these). In contrast, at the right ventricle (RV) only systolic function was statistically different between mutation carriers and non-carriers. Actually, in our patients, the 2010 modified Task Force criteria (9) would have established a definite diagnosis in only 37% of affected patients.

These findings support a biventricular involvement with a LV predominance in ARVC-5 and reflect that the newly proposed term of “arrhythmogenic

cardiomyopathy" is probably more appropriate for individuals harbouring a mutation in *TMEM43* (10).

Regarding the influence of sex on the disease, we observed a more severe phenotype among male carriers (Figure 5), as previously described in the Newfoundland cohort (2,3). This finding along with the high SCD rate observed, supports the adoption of the Newfoundland protocol to prevent SCD outside its endemic area. In that protocol, tailored management based on genetic findings is recommended including the implantation of an ICD in male mutation carriers by the age of 18 years, even in the absence of any cardiac abnormality. In female carriers, ICD is recommended only in the presence of any abnormal cardiac clinical test and, in particular, when there is an excess of premature ventricular ectopics present in 24-hour Holter ECG. The therapeutic strategy adopted in Newfoundland takes into account the gender differences observed in their ARVC-5 population and that the youngest SCD case in their area was a 19-year-old male (3). Similarly, in our

cohort we observed a worst clinical course among male mutation carriers and the youngest individual who suffered SCD was a 22 years-old male.

The Newfoundland ICD protocol has proven to be highly effective in their population and is a major factor in prolonging survival among these patients (6). Indeed, as a result of its adoption in Newfoundland, the 5-year survival in males who has risen from 65% to 95%, and from 85% to 97% in females (6).

We have adopted the Newfoundland's ICD implantation protocol in our ARVC-5 Spanish families and have been using during the last 7 years. Since the adoption of the protocol, we have not had any additional cases of SCD whereas 10 VT/VF events have been aborted during this period.

Overall, our results show that the *TMEM43*-p.S358L mutation causes the aggressive ARVC-5 in non-Newfoundland-related families and in different geographical regions, suggesting that the mutation effect is not influenced by additional genetic factors. Our findings should enable clinicians worldwide encountering individuals with same genetic defect to

recognise the disease and adopt appropriate management, following the pioneering work of the Newfoundland group.

**Impact of physical activity in ARVC-5**

A history of vigorous physical activity presented a trend towards increased risk of VT/VF in our cohort. However, in the subanalysis by gender this trend was restricted to females (66.7% of VT/VF in vigorous exercise vs 11.1% in non vigororous; *P*=0.054), and males presented a high prevalence of VT/VF episodes irrespective of the exercise burden (60% in both groups). Previous studies in desmosomal ARVC have shown a clearly increased arrhythmic risk associated with endurance exercise irrespective of sex (11,12).

While our results could be influenced by the limited number of individuals evaluated, the absence of worsening by exercise in males (who are the more severely affected) together with the finding that ECG and echocardiographic parameters are comparable in exercised and unexercised male genetic carriers suggest that genotype is a very strong contributor to a severe phenotype in ARVC-5 in males and that other factors do not seem to play a primary role. Nevertheless, exercise might play a role in the disease

phenotype in female mutation carriers in whom the genotype effect on the clinical course is known to be weaker. Another factor that could be involved in these findings is hormonal. It has been described that low levels of estradiol could enhance cardiovascular events in females with ARVC (13). As regular exercise lowers estradiol levels (14), this could partly explain the mechanism of how vigorous exercise affects female ARVC5 patients.

Although, it has been previously observed that physical activity modifies cardiac structure among ARVC patients without desmosomal mutations (15), our study is the first to provide data about exercise impact on *TMEM43* mutation carriers.

A recent study from our group using an ARVC-5 transgenic mouse model has shown that TMEM43 protein is predominantly located at nuclear membrane where it interacts with emerin and β-actin. In this model, TMEM43-S358L shows partial delocalization to the cytoplasm, reduced interaction with emerin and β-actin, and activation of GSK3β (16). As ARVC-5 is a very rare disease, animal models might also be helpful in the future to elucidate potential epigenetic and environmental factors that could impact on ARVC-5 phenotype, as has been previously described for other ARVC subtypes (17,18).

**Limitations**

The number of patients included in this study is limited, even taking into account that ARVC-5 is a very rare disease. Particularly, results regarding impact of physical activity on ARVC-5 phenotype should be taken with caution as they were derived from only 27 mutation carriers. Moreover, determination of physical activity performed over a large time period by telephonic interviews using questionnaires is subject to several potential bias.

Lastly, there was a non-negligible number of subjects in which the clinical status was unknown. Although information from non-genotyped individuals with premature SCD were only considered when those subjects belonged to sibships where the disease status of ≥50% of siblings was known, and this methodology has already been used to characterize natural history in ARVC-5 in Newfoundland (3), we admit that this approach could also have caused an important selection bias.

## Conclusions

ARVC-5 is a fully penetrant arrhythmic cardiomyopathy associated with a

high risk of SCD irrespective of the patients' geographical origin and genetic background. Our data confirm that the disease is sex-influenced, with a more severe expression in male patients, and that involvement of left ventricle is common. As in other subtypes of ARVC, vigorous physical activity seems to aggravate the phenotype of *TMEM43* mutation carriers, particularly among female carriers in whom the genotype effect is weaker.

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**Figures**

**Figure 1.** Pedigrees of the three Spanish families with the p.S358L mutation in *TMEM43*. A: Family 1. Madrid, B: Family 2. Valencia, C: Family 3. Granada. Red asterisk: confirmed genetic carriers, green asterisk: confirmed non-carriers.

SCD: Sudden cardiac death

**Figure 2.** Haplotypes surrounding *TMEM43* in p.S358L mutation carriers of three Spanish families compared with those from Newfoundland, Denmark and North America. Markers from the family from Madrid are depicted in yellow and those from the family from Granada are in green. The Newfoundland markers appear framed and those shared with the other families are underlined. Families from Madrid and Valencia only share 5 markers with the Newfoundland family whereas the family from Granada shares 6 genetic markers with the Newfoundland family. In contrast, the Danish and North American families share with the Newfoundland family 10 and 8 markers, respectively. Moreover, the latter share the 4 markers surrounding the mutation with the Newfoundland family, confirming the presence of a common ancestor.

**Figure 3.** (*A*) Representative 12-lead ECG of a 55 year-old female ARVC-5 patient. Poor R wave progression with 1 mV R wave in V3 and widened QRS

(110 ms). (*B*) Three-dimensional voltage endomyocardial map of a 47 year-old ARVC-5 male with VT episodes who underwent substrate ablation. Grey areas represent low voltage tissue (scar) along the interventricular septum and inferior wall. Brown dots represent radiofrequency applications. (*C*,*D*) CMR images of a 48 year-old ARVC5 male subject with biventricular dilatation, wall motion abnormalities and dysynchronic contraction (red arrows) LVEF 35%, RVEF 46%. (*E*,*F*) CMR images of the same subject depicting severe and almost concentric intramyocardial late gadolinium enhancement (red arrows).

**Figure 4.** Post-mortem study of the heart of an ARVC-5 *TMEM43* p.S358L heterozygous carrier and victim of SCD. (*A*,*B*) Macroscopic study showing biventricular fibro-fatty infiltration, including transmural (RV), subendocardial (RV and LV) and intramyocardial (LV) localizations. (*C*,*D*) Histologic view with Masson´s trichrome staining depicting fatty infiltration (asterisks) and fibrosis (arrowheads), together with cardiomyocyte degeneration (arrows).

**Figure 5.** Survival curve in 62 ARVC-5 affected patients according to sex.

**Table 1. Clinical, electrocardiographic and echocardiographic characteristics.**

| | Affected | | | Unaffected | | | Affected vs unaffected | Affected males vs affected females |
|---|---|---|---|---|---|---|---|---|
| | **Male** | **Female** | **Total** | **Male** | **Female** | **Total** | | |
| Number of subjects (%) | 37 (59.7) | 25 (40.3) | 62 | 41 (56.2) | 32 (43.8) | 73 | | |
| Age (years), mean (SD) | 36.5 (14.9) | 43.1(20.9) | 39.1 (17.6) | 48.6 (15.0) | 43.7 (20.1) | 46.1 (17.6) | 0.07 | 0.10 |
| SCD (%) | 20/37 (54.0) | 4/25 (14.0) | 24/62 (38.7) | 0/41 (0) | 0/32 (0) | 0/73 (0) | <0.001 | 0.03 |
| SCD ≤50 years (%) | 17/37 (45.9) | 2/25 (8.0) | 19/62 (30.6) | - | - | - | - | 0.002 |
| SCD age (SD) | 41.8 (11.5) | 61.0 (15.7) | 44.6 (14.3) | - | - | - | - | 0.004 |
| Heart failure (%) | 3/21 (14.3) | 4/16 (25.0) | 7/37 (18.9) | 0/19 (0) | 0/18 (0) | 0/37 (0) | 0.01 | 0.41 |
| | **Mutation Carriers** | | | **Non-Carriers** | | | **Carriers vs non-carriers** | **Male carriers vs Female Carriers** |
| | **Male** | **Female** | **Total** | **Male** | **Female** | **Total** | | |
| 12 lead ECG | | | | | | | | |
| Subjects tested | 13 | 14 | 27 | 20 | 17 | 37 | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| QRS duration, ms (SD) | 115.6 (27.1) | 94.5 (15.5) | 104.7 (24.0) | 90.3 (9.9) | 85.8 (7.1) | 88.2 (8.9) | <0.001 | 0.02 |
| V3 R voltage, mV (SD) | 2.6 (2.3) | 3.6 (3.2) | 3.2 (2.8) | 8.6 (4.1) | 6.2 (2.4) | 7.5 (3.6) | <0.001 | 0.35 |
| Echocardiogram | | | | | | | | |
| Subjects tested | 14 | 15 | 29 | 19 | 14 | 41 | | |
| LVEF, % (SD) | 53.6 (9.0) | 58.7 (12.2) | 56.2 (10.9) | 63.3 (7.3) | 60.8 (6.6) | 62.1 (7.0) | <0.001 | 0.21 |
| LVEDD HF, % (SD) | 113.0 (18.8) | 98.7 (12.6) | 106.9 (17.5) | 97.8 (9.5) | 94.4 (10.4) | 96.6 (9.6) | 0.07 | 0.13 |
| LVEDV, ml (SD) | 125.0 (43.6) | 80.2 (12.9) | 106.3 (40.4) | 90.6 (15.64) | 70.3 (16.62) | 84.1 (18.3) | 0.03 | 0.04 |
| TAPSE, mm (SD) | 17.5 (8.9) | 22.6 (4.6) | 19.9 (7.5) | 23.5 (3.0) | 23.6 (4.1) | 23.5 (3.5) | 0.03 | 0.10 |
| RVBD 4CH, mm (SD) | 40.8 (8.3) | 32.3 (2.7) | 35.6 (7.6) | 35.9 (9.1) | 32.50 (2.7) | 34.8 (7.3) | 0.60 | 0.053 |



4CH, four-chamber view; SCD, sudden cardiac death; SD, standard deviation; RVBD, right ventricle basal diameter; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; TAPSE, tricuspid annular plane systolic excursion.

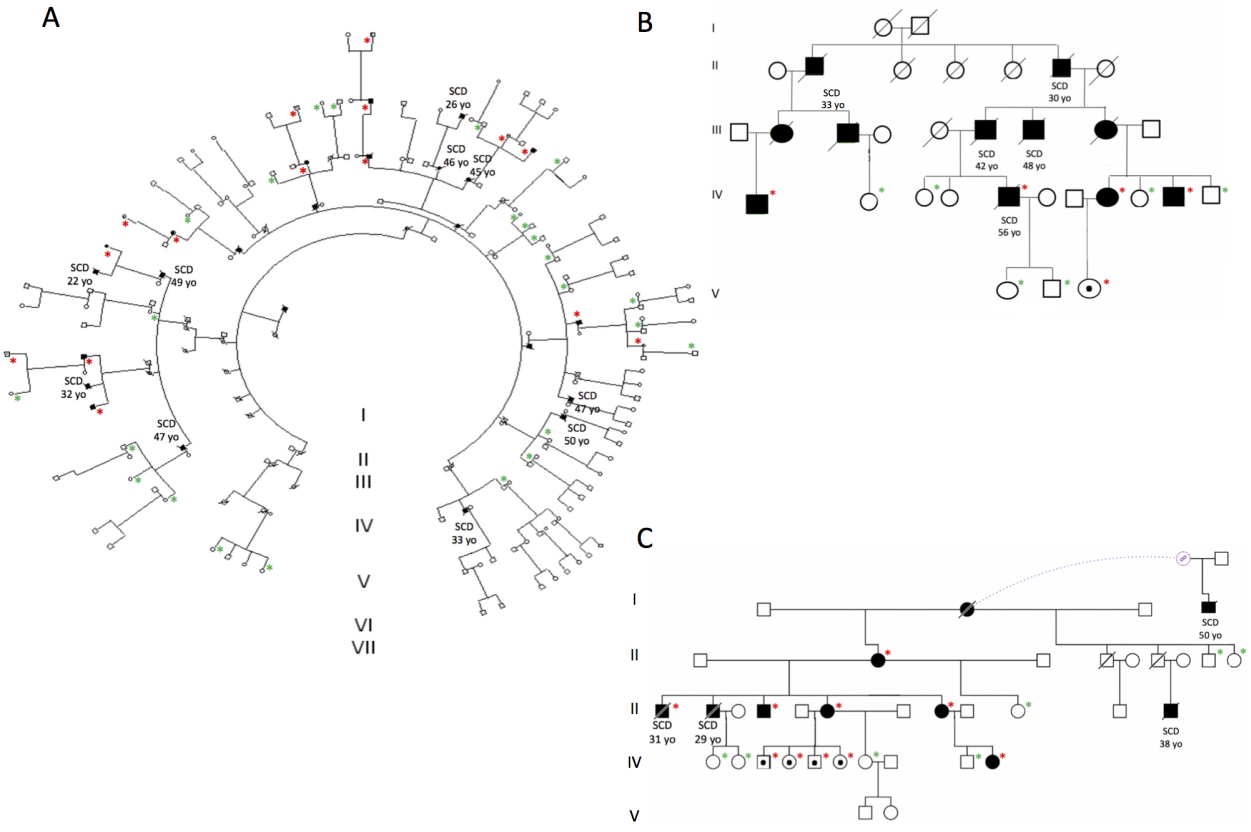
**Table 2. Impact of physical activity on clinical, electrocardiographic and echocardiographic characteristics in *TMEM43*-p.S358L mutation carriers**

| | Vigorous physical activity | | | Non-vigorous physical activity | | | Vigorous vs non-vigorous | Vigorous PA in males vs vigorous PA in females |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total | | |
| Number of subjects, *n* (%) | 10/13 (76.9) | 3/13 (23.1) | 13 | 5/14 (35.7) | 9/14 (64.3) | 14 | | |
| Age (years), mean (SD) | 38.1 (18.6) | 47.3 (9.6) | 40.2 (17.1) | 33.8 (14.7) | 38.0 (16.9) | 36.5 (15.7) | 0.56 | 0.44 |
| ICD implanted, *n* (%) | 7/10 (70.0) | 3/3 (100) | 10/13 (76.9) | 4/5 (90.0) | 3/9 (33.3) | 7/14 (50.0) | 0.24 | 0.53 |
| SVT/VF, *n* (%) | 6/10 (60.0) | 2/3 (66.7) | 8/13 (61.5) | 3/5 (60.0) | 1/9 (11.1) | 4/14 (28.6) | 0.08 | 0.85 |
| Heart failure, *n* (%) | 1/10 (10.0) | 1/3 (33.3) | 2/13 (15.4) | 1/5 (20.0) | 1/9 (11.1) | 2/14 (14.3) | 1 | 0.42 |
| 12 lead ECG | | | | | | | | |
| Subjects tested, *n* | 8 | 3 | 11 | 4 | 9 | 13 | | |
| QRS width in ms, mean (SD) | 114.0 (31.7) | 91.0 (12.7) | 107.7 (29.2) | 125.3 (7.81) | 97.1 (18.1) | 105.8 (21.5) | 0.85 | 0.27 |
| V3 R voltage in ms, | 3.1 (2.8) | 2.0 (2.0) | 2.8 (2.6) | 1.5 (1.3) | 3.1 (2.4) | 2.6 (2.2) | 0.84 | 0.55 |

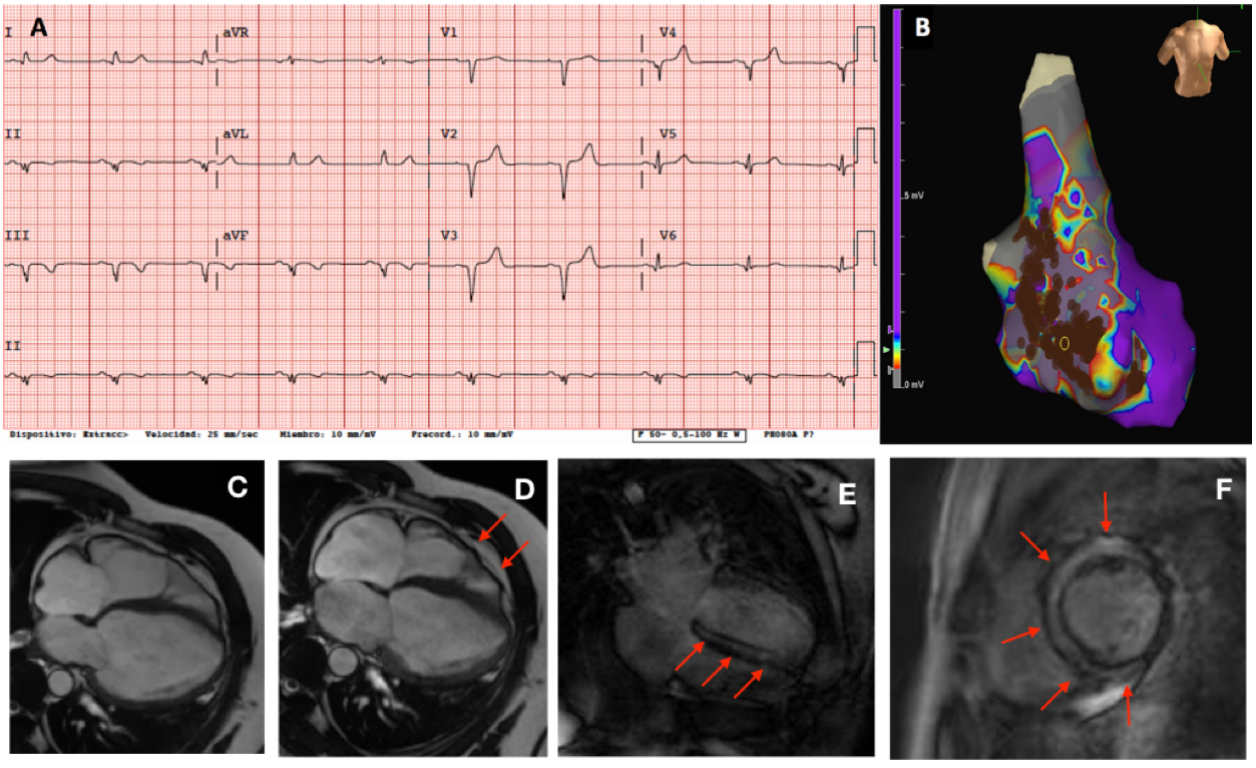
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mean (SD) | | | | | | | | |
| Echocardiogram | | | | | | | | |
| Subjects tested, n | 7 | 3 | 10 | 3 | 9 | 12 | | |
| LVEF, % (SD) | 54.4 (9.5) | 63.3 (6.4) | 56.8 (9.4) | 51.0 (9.3) | 58.4 (12.7) | 55.8 (11.8) | 0.81 | 0.17 |
| LVEDD, mm (SD) | 53.4 (11.1) | 48.0 (10.6) | 47.5 (7.6) | 52.4 (6.3) | 44.5 (7.1) | 51.8 (10.7) | 0.86 | 0.49 |
| LVEDV, ml (SD) | 119.0 (49.3) | 87.0 (10.5) | 107.0 (41.2) | 140.0 (32.5) | 70.0 (9.9) | 105.0 (44.9) | 0.94 | 0.23 |
| TAPSE, mm (SD) | 16.6 (10.4) | 17.5 (0.7) | 16.7 (9.2) | 19.5 (0.7) | 21.0 (9.6) | 20.7 (8.5) | 0.32 | 0.90 |
| RVBD 4CH, mm (SD) | 42.2 (8.5) | 30.0 (1.7) | 37.6 (9.1) | 34.0 * | 32.0 (3.2) | 32.4 (2.9) | 0.17 | 0.06 |

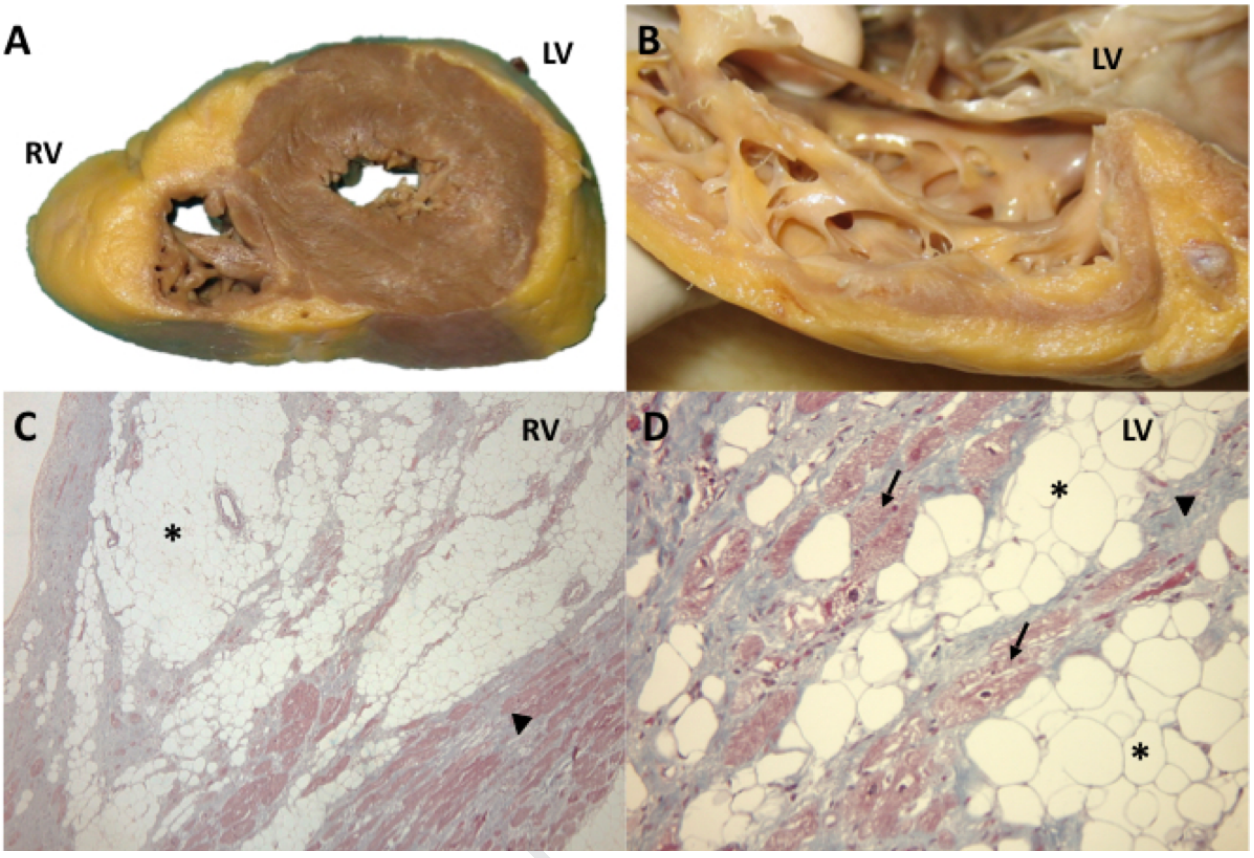
4CH, four-chamber view; PA: Physical activity, RVBD, right ventricle basal diameter; SVT, sustained ventricular tachycardia; VF, ventricular fibrillation; SD, standard deviation; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; TAPSE, tricuspid annular plane systolic excursion.

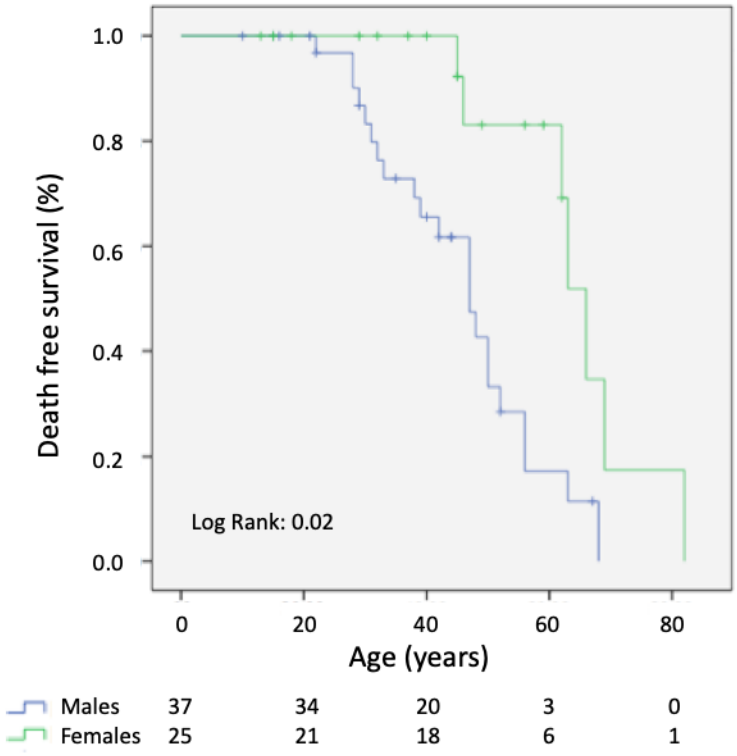
*: Only 1 subject available


A
SCD 26 yo
SCD 46 yo
SCD 45 yo
SCD 22 yo
SCD 49 yo
SCD 32 yo
SCD 47 yo
SCD 47 yo
SCD 50 yo
SCD 33 yo
I
II
III
IV
V
VI
VII
B
I
II
III
IV
V
SCD 33 yo
SCD 30 yo
SCD 42 yo
SCD 48 yo
SCD 56 yo
C
I
II
II
IV
V
SCD 50 yo
SCD 31 yo
SCD 29 yo
SCD 38 yo

| marker | Location (cM) | Madrid | | Valencia | | Granada | | Newfoundland | | Denmark | | United States | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1259 | 36.65 | **197** | 205 | 195 | 203 | 197 | 195 | 203 | 203 | 203 | 201 | 191 | 205 |
| 3610 | 37.20 | **244** | 258 | **244** | 258 | 248 | 258 | 248 | 248 | 248 | 244 | 244 | 258 |
| 2403 | 37.20 | **252** | 252 | **252** | 265 | **252** | 280 | **252** | 280 | 252 | 252 | 252 | 258 |
| 1516 | 37.20 | **353** | 357 | **353** | 331 | 331 | 335 | *347* | 357 | 361 | 365 | 331 | 351 |
| 2385 | 38.28 | **145** | 145 | **145** | 149 | 143 | 147 | 149 | 145 | 149 | 145 | 145 | 145 |
| 3602 | 38.83 | **121** | 125 | **121** | 115 | 114 | 120 | 114 | 120 | 114 | 120 | 114 | 120 |
| 1585 | | **121** | 135 | **121** | 121 | 119 | 119 | **121** | 127 | 121 | 127 | 121 | 127 |
| mut | 39.52 | + | - | + | - | + | - | + | - | + | - | + | - |
| 1554 | 41.56 | **133** | 131 | 131 | 129 | 131 | 131 | 131 | 131 | 131 | 131 | 131 | 135 |
| 3595 | 41.56 | **266** | 266 | **266** | 266 | **266** | 266 | **266** | 266 | 266 | 268 | 266 | 270 |
| 3613 | 42.10 | **189** | 181 | **189** | 201 | 203 | 181 | *195* | 199 | 199 | 189 | 191 | 185 |
| 3473 | 42.10 | **217** | 217 | 221 | 221 | 221 | 217 | 217 | 221 | 223 | 217 | 217 | 219 |
| 2338 | 42.10 | **186** | 182 | 188 | 182 | 196 | 176 | **186** | 186 | 176 | 186 | 186 | 184 |
| 4547 | 42.10 | **231** | 235 | **231** | 223 | 235 | 227 | 235 | 219 | 235 | 235 | 239 | 227 |
| 3510 | 42.10 | **285** | 283 | **285** | 279 | na | na | 283 | 281 | 283 | 281 | 283 | 281 |
| 1293 | 44.81 | **127** | 125 | 125 | 125 | 117 | 127 | *125* | 125 | 131 | 117 | 117 | 125 |






1.0
0.8
0.6
0.4
0.2
0.0
Death free survival (%)
Log Rank: 0.02
0
20
40
60
80
Age (years)
Males 37 34 20 3 0
Females 25 21 18 6 1