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# Spontaneous coronary artery dissection: Role of prognostic markers and relationship with genetic analysis

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ABSTRACT

Spontaneous coronary artery dissection (SCAD) is increasingly recognized as an important cause of myocardial infarction (MI). Currently there is little knowledge about prognostic factors for unfavorable outcome at long term follow-up; furthermore, there is also little knowledge about the genetics of these patients.

*Aims:* This observational and retrospective study describes long-term cardiovascular outcomes of a population affected by SCAD and assesses predictors of recurrent de novo SCAD and major adverse cardiovascular events (MACE). Furthermore, a correlation between genotype and adverse events at follow-up was sought.

*Methods:* Baseline characteristics, angiographic features, use of medication and long-term cardiovascular events were systematically ascertained between 2000 and 2019.

Next generation sequencing was performed with a panel consisting of twenty genes of interest. Variants found were filtered based on their frequency and only frequencies <1% in the general population were considered as "positive".

*Results:* Seventy patients were enrolled and followed for a median time of 39.1 months. Median age was 52 years and the majority were women (86%). Use of hormone therapy (HT) (OR 3.64, $p = 0.041$) and presence of malignant ventricular arrhythmias (VAs) at onset (OR 7.03, $p = 0.0073$) were associated with a greater risk of recurrent de novo SCAD. Proximal type SCAD (OR 8.47, $p < 0.0001$) and presence of VAs at onset (OR 9.97, $p = 0.047$) were associated with a greater risk of MACE.

A potential SCAD-associated mutation was detected in 27 patients (44%); 6 patients (22%) defined as genetically "positive" developed MACE vs. 2 patients (6%) defined as "negative" ($p = 0.06$ at univariate analysis). MACE at follow-up is reached earlier in genetically positive patients (7.9 vs. 42.5 months).

*Conclusion:* use of HT and VAs at SCAD onset are prognostic factors for recurrent de novo SCAD. Proximal SCAD site and VAs at SCAD onset were prognostic factors for MACE. Analysis by molecular genetics seems to be a promising tool for the possible additional role it could play in MACE prediction.



## 1. Introduction

Spontaneous coronary artery dissection (SCAD) is increasingly recognized as an important cause of myocardial infarction (MI), particularly in young women, and often with few cardiovascular risk factors. It is now estimated that SCAD is the underlying cause of 1.7% to 4% of acute coronary syndromes (ACS) [1–3] and accounts for 0.5% of sudden cardiac deaths [4]. Furthermore, in women <60 years of age, SCAD accounts for 22% to 35% of ACS presentations [2].

SCAD is defined as a spontaneous tear in the coronary arterial wall that is not traumatic or iatrogenic. Contemporary usage of the term is confined to nonatherosclerotic causes [1,6]. This condition has been underdiagnosed for decades, but with an increased clinical index of suspicion, greater use of coronary angiography and intracoronary imaging (i.e., optical coherence tomography -OCT- and intravascular

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ultrasonography -IVUS-) and better pattern recognition on angiography, diagnosis of SCAD has improved substantially [6,7].

It has become increasingly apparent that multiple factors may predispose to arteriopathy that can weaken coronary arterial wall and increase vulnerability for dissection [8,9]. This vulnerability can be exacerbated by precipitating stressors (emotional or physical), that can trigger dissection.

Nevertheless, this condition remains insufficiently understood because there are limited prospective series to discern natural history and long-term cardiovascular outcomes of SCAD. The ideal management strategy has yet to be determined and there are no published randomized trials to guide therapy. Current management recommendations are based on expert opinions, mainly from retrospective, observational SCAD studies.

The majority of patients experience spontaneous healing during follow-up [11], but recurrent de novo SCAD has been reported following the index SCAD event in 12% to 27% of cases (depending on follow-up duration), accounting for the majority of recurrent MI in this population [6]. However, risk of recurrence and strategies to minimize it are widely unclear. Only arterial hypertension and beta-blocker usage have been correlated with disease recurrences, where the former increases them while the latter reduces them [10].

Studies are still underway to investigate the role of genetics in promoting SCAD. Genetic diseases most frequently associated with SCAD are Marfan syndrome and Ehler-Danlos syndrome type 4, despite their incidence in SCAD patients being low (about 5%) [12]. Furthermore, *COL3A1* mutation increases the risk of tissue fragility, with greater risk of dissection of medium vessels and large vessels, also promoting SCAD [13]. Other studies have provided additional elements in favor of a genetic substrate, including inheritance of the disease [14] and the association between SCAD and mutations of single genes, such as *F11R* [15,16], *PHACTR1* [17,18] and *TSR1* [19]. Other genetic diseases have been related to SCAD, although more rarely, including alpha-1 antitrypsin deficiency, polycystic kidney disease and nail-patella syndrome [13].

In light of these elements, it seems important to assess the link between SCAD patients and their genotype, also verifying the possible clinical applicability of genetic tests in this selected population. It is also crucial to shed light on possible prognostic factors not yet documented.

This observational and retrospective cohort study describes long-term cardiovascular outcomes of a population affected by SCAD and assesses predictors of recurrent de novo SCAD and major adverse cardiac events (MACE). Furthermore, an association between genotype and adverse events at follow-up is sought.

## 2. Methods

Seventy patients with nonatherosclerotic angiographic diagnosis of SCAD were enrolled between January 2000 and February 2019 at the Division of Cardiology, Department of Cardiothoracic Sciences in Azienda Sanitaria Universitaria Friuli Centrale (ASUFC) – Udine (Italy), a tertiary referral center for ACS management.

The effective angiographic diagnosis of SCAD is made only with the agreed opinion of two operators [1,4]. SCAD types were classified according to the latest published angiographic SCAD classification [10]. Briefly, type 1 has the classic appearance of contrast dye staining of arterial wall with multiple radiolucent lumen, type 2 shows long diffuse (typically >20 to 30 mm) and smooth narrowing that varies in severity (variant 2A is diffuse arterial narrowing bordered by normal segments proximal and distal to the intramural hematoma, and variant 2B is diffuse narrowing that extends to the distal tip of the artery), type 3 has focal or tubular stenosis that mimics atherosclerosis, typically requiring intracoronary imaging to prove presence of intramural hematoma or double lumen. The coronary segment affected by SCAD was defined by the Bypass Angioplasty Revascularization Investigation classification [20]. Location of dissected coronary arteries and corresponding wall motion abnormality were recorded. Enrollment in the registry was then carried out in order to perform long-term follow-up and subsequent analyses.

The following exclusion criteria were considered: angiographic evidence of atheromatous disease with atheromatous plaques ≥50% in at least one coronary district, iatrogenic dissection, chest traumatism occurring in the previous 7 days from clinical onset, aortic dissection with intracoronary retrograde extension and known autoimmune or vasculitis diseases.

Clinical history and baseline characteristics were obtained from patient interviews and hospital records. Baseline cardiovascular risk factors, medication on presentation, hospital presentation, electrocardiographic changes, long-term events and angiographic and noninvasive imaging characteristics were recorded.

All patients were interviewed on potential predisposing and precipitating stressors, gynecological history, clinical symptoms and family history. Significant emotional stress and intense physical activities (aerobic or isometric) preceding the SCAD event where queried and recorded. Emotional stress was defined as currently experiencing major stress prior to hospital admission and stress was considered related to SCAD only if the patient thought that stress could have been the trigger.

**Table 1**
Descriptive analysis of the population at the time of enrollment.

| Variable | *N* (%) |
|---|---|
| Female | 60 (86%) |
| Age (years) | 47,00/52,00/58,00 |
| Follow-up (months) | 13,90/39,10/86,62 |
| Hypertension | 20 (29%) |
| Body mass index (Kg/m$^2$) | 21,40/23,30/25,50 |
| Obesity | 11 (16%) |
| Current smoker/former smoker | 24 (35%) |
| Dyslipidemia | 16 (23%) |
| Family history of coronary artery disease | 28 (41%) |
| Diabetes mellitus | 3 (4%) |
| Migraines | 11 (16%) |
| Emotional stress | 21 (30%) |
| Active and prior HT | 11 (16%) |
| Coronary artery territory involved and biomarker | |
| Left main-left anterior descending artery | 28 (40%) |
| Circumflex artery | 28 (40%) |
| Right coronary artery | 14 (20%) |
| Proximal tract involved | 13 (19%) |
| Medium or distal tract involved | 57 (81%) |
| Type 1 angiographic SCAD | 39 (56%) |
| Type 2A angiographic SCAD | 14 (20%) |
| Type 2B angiographic SCAD | 16 (23%) |
| Type 3 angiographic SCAD | 1 (1%) |
| Troponin I peak (ng/mL) | 2,20/12,65/24,00 |
| Clinical and instrumental presentation | |
| STEMI | 32 (46%) |
| NSTEMI | 35 (51%) |
| Unstable angina | 2 (3%) |
| Ventricular arrhythmias (sustained VT or VF) | 7 (10%) |
| Ejection fraction, % | 53,00/59,00/63,00 |
| Dyskinesis | 2 (3%) |
| Akinesis | 32 (46%) |
| Hypokinesis | 21 (30%) |
| Normal left ventricular wall motion | 16 (23%) |
| Therapy at first medical contact and hospital discharge | |
| Coronary artery by-pass grafting | 2 (3%) |
| Percutaneous coronary intervention | 9 (13%) |
| Medical therapy | 59 (84%) |
| Dual antiplatelet theray (at least 3 months) | 32 (46%) |
| Beta-blocker | 57 (81%) |
| Ace-inhibitor or angiotensin II receptor blocker | 38 (55%) |
| Statin | 49 (70%) |
| Oral anticoagulant therapy | 4 (6%) |

Values are *N* (%) or first quartile/median value/third quartile, unless otherwise indicated.

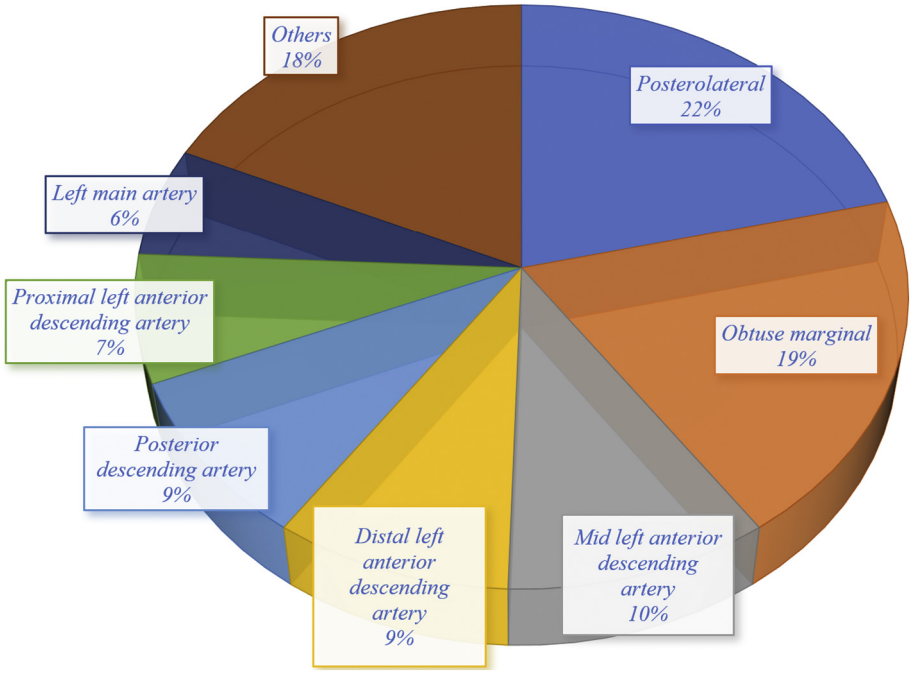


**Fig. 1.** Frequency of Coronary Artery Segment Dissection.

Presence of physical stress was defined as new or unusually intense physical activity within 1 week of hospitalization or intense isometric activity defined as lifting >20 Kg. Presence of ventricular arrhythmias (VAs) during hospitalization was defined as the presence of sustained ventricular tachycardia or ventricular fibrillation requiring cardioversion/defibrillation or antiarrhythmic agents. Active and prior hormone therapy (HT) (e.g., birth-control pills, fertility treatment, estrogen, progesterone and beta-human chorionic gonadotropin) was recorded and considered in the analysis only if used within a year of the event and/or for at least 3 consecutive years. Other potential precipitating stressors (e.g., intense retching, vomiting, straining with bowel movements, use of recreational drugs, and active pregnancy) were also recorded. Medications administered on discharge were recorded.

We compared also the study population enrolled in the period 2000–2010 versus the period 2011–2019 in terms of type of SCAD and rate of pharmacological or nonpharmacological therapeutic strategies.

### 2.1. Outcomes

Long-term cardiovascular events included the composite of cardiovascular mortality (including need for cardiac transplantation), recurrent MI (including recurrent SCAD), congestive heart failure, and revascularization, collectively termed major adverse cardiac events (MACE). Recurrent SCAD was defined as de novo recurrent spontaneous dissection with new recurrent MI symptoms and enzyme elevation, which did not involve the extension of the dissection of the original SCAD lesion.

This study complies with the Declaration of Helsinki, the locally appointed ethics committee (Comitato Etico Unico Regionale – CEUR) has approved the research protocol (Appendix A) and the informed consent has been obtained from the subjects (or from their legally authorized representative).

### 2.2. Statistical analysis

The statistical analysis software R 3.3.1 (https://cran.r-project.org/) was used. The categorical data are presented as percentages (absolute numbers), whereas the continuous data are presented as median, first and third quartile. The non-parametric Wilcoxon-Kruskal-Wallis test was used first to evaluate statistical significance for comparing groups. To account for multiplicity of testing, *p*-value were adjusted via Benjamini-Hochberg procedure.

Multivariable analysis has been performed via a Cox proportional hazard regression or logistic regression, depending on the outcome (respectively time-to-event or binary event).

### 2.3. Genetic analysis

To perform a genetic analysis, the patients' genomic DNA (gDNA) was isolated from the EDTA peripheral blood sample using the QIAsymphony® SP / AS (Qiagen) extractor. The gDNA sequencing was performed with the Ion PGM DX sequencer (ThermoFisher Scientific). A panel consisting of 20 genes involved in collagenopathies was prepared (*ACTA2*, *CBS*, *COL3A1*, *COL5A1*, *COL5A2*, *ELN*, *FBN1*, *FBN2*, *LTBP2*, *MYLK*, *NOTCH1*, *MYH11*, *SMAD3*, *SMAD4*, *SLC2A10*, *TGFB2*, *TGFB3*, *TGFBR1*, *TGFBR2*, *PLOD1*) and for this purpose the Ion AmpliSeq technology (ThermoFisher) was used.

AmpliSeq libraries were generated using the Ion AmpliSeq Library Kit Plus (ThermoFisher Scientific), according to the manufacturer's protocol. The target genes were amplified using pools of pairs of specific primers. The amplicons obtained were then subjected to enzymatic digestion and ligation with specific adapters equipped with barcodes that allowed the unambiguous identification of the different samples sequenced in multiplex.

**Table 2**
Descriptive analysis of MACE.

| | |
|---|---|
| *OUTCOMES* | |
| *Cardiovascular death (including need for heart transplantation)* | *5 (7%)* |
| *Myocardial infarction* | *6 (9%)* |
| *Recurrent* de novo *SCAD* | *6 (9%)* |
| *Revascularizations* | *3 (4%)* |
| *Heart failure requiring hospitalization* | *2 (3%)* |
| *MACE* | *12 (18%)* |
| *Time to first MACE (months)* | *1,00/12,90/50,30* |

Values are N (%) or first quartile/median value/third quartile, unless otherwise indicated.

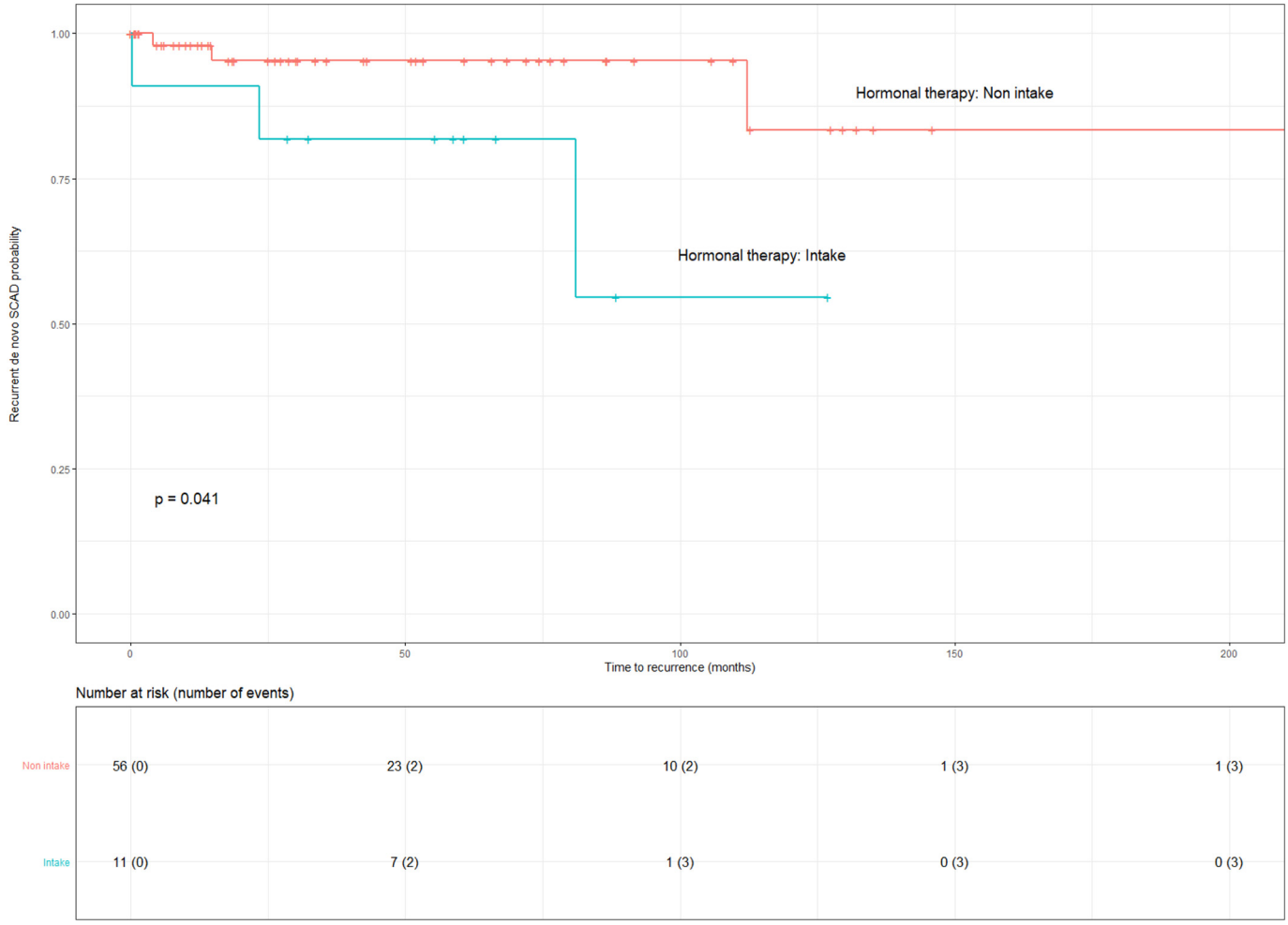


**Fig. 2.** Kaplan-Meier analysis of the probability of recurrence de novo SCAD with respect to intake/non-intake of HT.

The ligation products were purified with Agencourt AMPure XP (SPRI Technology) magnetic beads and subjected to amplification by PCR on the Ion OneTouch 2 System instrumentation, using the appropriate Ion PGM hi-Q View OT2 kit. The libraries produced were then sequenced in multiplexes of 14 samples on the Ion PGM sequencer, using the Ion PGM Hi-Q View Sequencing kit and the Ion 316 v2 BC chip.

Bioinformatics analyses were performed using the Torrent Suite software (ThermoFisher Scientific). The Variant Caller Format files generated were analyzed and reported using wANNOVAR. Variants were called when a position was covered at least 100 times and their prioritization was based on quality values, functional consequences and population frequency. Variants were filtered based on their frequency in the European-origin population described in the database (1000 Genome Project (http://www.internationalgenome.org), ESP6500SI (https://evs.gs.washington.edu), ExAC (http://exac.broadinstitute.org) and GnomAD (https://gnomad.broadinstitute.org/)) and only those with a frequency lower than 1% were considered. The potential effects of gene variants on gene functions were not taken into account because they are mostly based upon analysis of subjects suffering monogenic disease. All identified SCAD-associated mutations were confirmed by Sanger sequencing. For this purpose, Sanger sequencing reactions were analyzed by a 3500 DX DNA Sequencer (Life Technologies) analyzer using the BigDye Terminator kit v3.1 (Applied Biosystems, Foster City, CA, USA); the software Sequencing Analysis v5.4 (Life Technologies) was used for mutation detection.

## 3. Results

From January 2000 to February 2019 70 patients with nonatherosclerotic SCAD were enrolled and followed for a median time of

**Table 3**
Descriptive analysis of the population in according to presence or absence of recurrent de novo SCAD (only statistically significant variables are represented).

| | *Absence of recurrent* de novo *SCAD* | *Presence of recurrent* de novo *SCAD* | *p VALUE* * | *p VALUE* ** | *OR (95% CI)* |
|---|---|---|---|---|---|
| *Emotional stress* | *15 (26%)* | *4 (67%)* | *n.s.* | *n.s.* | *4.32 (0.58–32.2)* |
| *Hormonal therapy* | *8 (13%)* | *3 (50%)* | *n.s.* | *n.s.* | *3.64 (0.55–23.9)* |
| *Proximal tract involved* | *10 (16%)* | *3 (50%)* | *n.s.* | *p = 0,08* | *1.35 (0.17–10.6)* |
| *Ventricular arrhythmias (sustained VT or VF)* | *4 (6%)* | *3 (50%)* | *p = 0,07* | *p = 0,06* | *7.03 (0.92–53.9)* |

Values are *N* (%).
* Univariate analysis adjusted with Benjamini – Hochberg method.
** Multivariable analysis.
n.s. = non statistically significant.

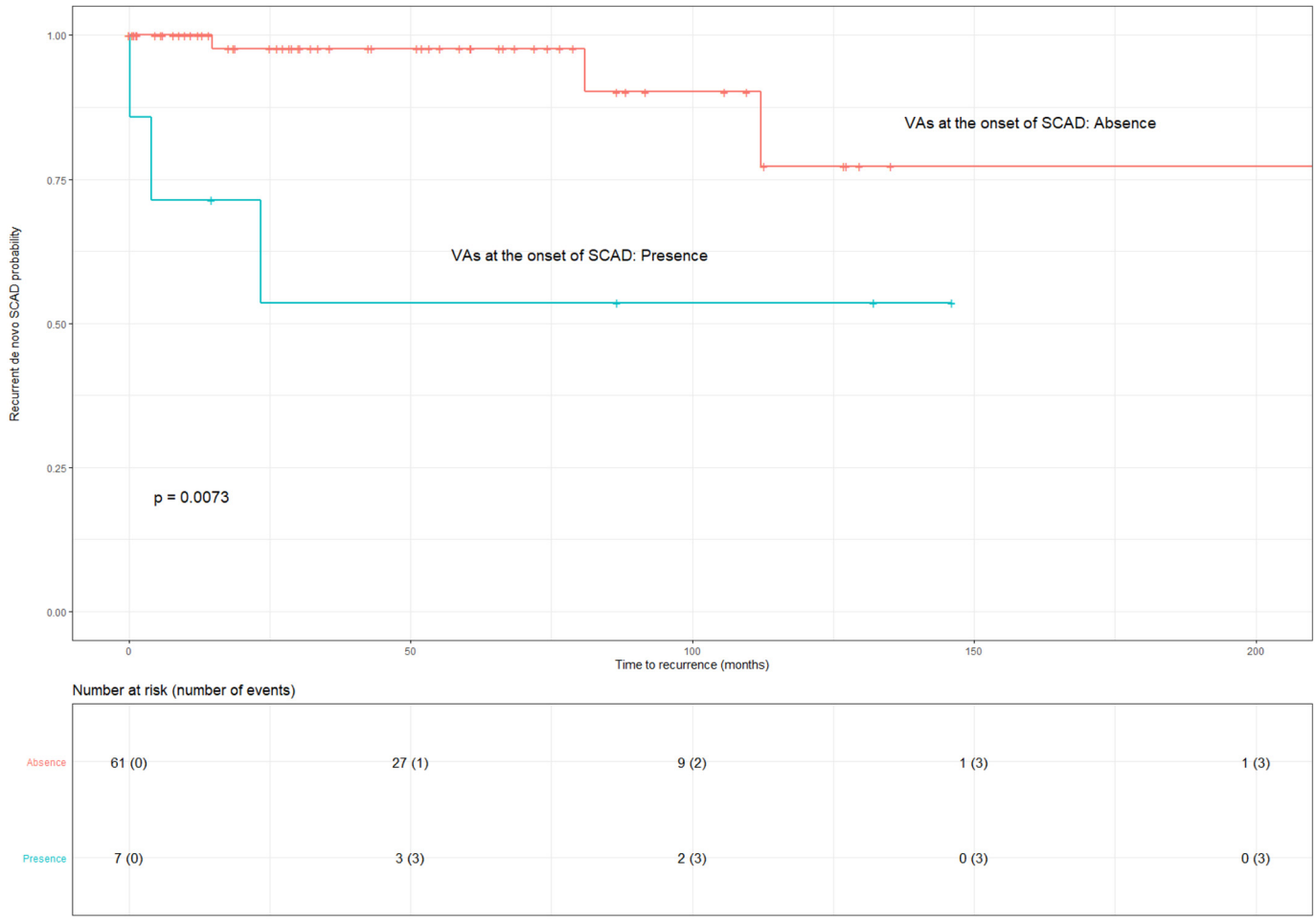


**Fig. 3.** Kaplan-Meier analysis of the probability of recurrence de novo SCAD with respect to presence/absence of VAs at the onset of SCAD.

39.1 months (interquartile range 13.9–82.6 months). Baseline characteristics are summarized in Table 1. Median age was 52 years and the majority were women (86%). Most patients had only one or no cardiovascular risk factors; however, baseline status of current smoker/former smoker and family history of coronary artery disease were present in 23% and 41% of participants, respectively. No physical stresses were documented as SCAD triggers, but 16% suffered from migraines (diagnosed by a neurologist), 30% had emotional stress as a trigger of the first event and 16% used HT or had used HT within the year of the event and/or for at least 3 consecutive years (Table 1).

Almost all patients (97%) presented with troponin-positive ACS, with approximately suffering half ST-elevation myocardial infarction (STEMI) and half non ST-elevation myocardial infarction (NSTEMI). Ventricular tachycardia or ventricular fibrillation occurred in 7 (10%) patients. The mean presenting left ventricular ejection fraction (LVEF) was 59.0% (first quartile 53%, third quartile 63%) and 77% of patients had wall motion abnormality. Two patients (45 and 56 years old, both females) showed left ventricular motion abnormalities suggestive of Tako-Tsubo pattern.

The most common coronary artery territory dissected was the left anterior descending artery (26%), followed by posterolaterals branches from the right coronary artery or circumflex artery (22%) and obtuse marginal branch (19%). The frequencies of coronary segments involved are shown in Fig. 1. Among the 70 dissected arteries at the first hospitalization, 39 had type 1 angiographic SCAD, 30 had type 2 angiographic SCAD (14 patients type 2A and 16 patients type 2B) and only one had type 3 angiographic SCAD. In 57 patients (81%) there was a medium and/or distal coronary involvement, while in the remaining 13 (19%) the SCAD was of proximal type (Table 1). In particular, in the years 2000–2010 type 1 SCAD was detected in 13 patients (68%) and type 2 in 6 patients (32%), while in the period 2011–2019 type 1 SCAD was assigned to 26 patients (51%) and type 2 to 24 patients (47%) ($p$ = ns).

Most patients (84%) were treated conservatively as their initial treatment strategy, while a smaller proportion underwent percutaneous coronary intervention (PCI) (13%) or coronary artery bypass graft (CABG) (3%).

The rate of invasive therapy differs between the period 2000–2010 vs. period 2011–2019: 8 out of 19 patients (42.1%) underwent PCI or CABG in the first period and 3 out of 51 patients (5.8%) underwent PCI in the second period ($p < 0.001$). The medications at hospital discharge and last clinical follow-up are listed in Table 1. All patients were discharged on acetylsalicylic acid therapy and a significant proportion on dual antiplatelet (46%), beta-blocker (81%) and statin therapy (70%). The proportion of dual antiplatelet therapy did not differ significantly between the period 2000–2010 and the period 2011–2019: 11 patients (58%) vs. 21 patients (41%) ($p$ = ns).

### 3.1. Genetic analysis

Among the 70 enrolled patients, the gDNA of 61 subjects has been sequenced with a panel of 20 genes associated with several collagenopathies that may be associated with SCAD. Among the 20 analyzed genes, in our cohort of patients the potential SCAD-associated mutations have been identified in *COL3A1*, *COL5A1*,

**Table 4**
Descriptive analysis of the population in according to presence or absence of MACE (only statistically significant variables are represented).

| | *MACE absent* | *MACE present* | *p VALUE* * | *p VALUE* ** | *OR (95% CI)* |
|---|---|---|---|---|---|
| *Left main- left anterior descending artery* | *20 (36%)* | *8 (67%)* | *n.s.* | *n.s.* | *n.s.* |
| *Circumflex artery* | *27 (48%)* | *1 (8%)* | *p = 0,05* | *n.s.* | *n.s.* |
| *Proximal tract involved* | *4 (7%)* | *9 (75%)* | *p = 0,0007* | *p = 0,017* | *8.47 (1.47–48.9)* |
| *Ventricular arrhythmias (sustained VT or VF)* | *3 (5%)* | *4 (33%)* | *p = 0,017* | *p = 0,0036* | *9.97 (2.12–46.9)* |
| *Ejection fraction, %* | *54,75/59,00/63,25* | *40,00/54,15/58,87* | *p = 0,0016* | *p = 0,06* | *1.28 (0.43–3.83)* |

Values are *N* (%) or first quartile/median value/third quartile, unless otherwise indicated.
* Univariate analysis adjusted with Benjamini – Hochberg method.
** Multivariable analysis.
n.s. = non statistically significant.

*COL5A2, FBN1, FBN2, MYLK, MYH11, LTBP2, NOTCH1, SMAD3, PLOD1, CBS* and *ELN*.

In detail, twenty-seven patients (44%) were found to be carrying a potential SCAD-associated mutation and they were considered as "positive" (Table 5) and one patient (2%) had a new diagnosis of Ehlers-Danlos type 4 disease. For most of the SCAD-associated mutations, a very low allelic frequency is present in population databases, suggesting a phenomenon of negative selection.

The positivity to genetics also strongly correlates with the development of proximal-type coronary dissections ($p = 0.004$ at univariate analysis, Table 6).

### 3.2. Outcomes

During follow-up twelve patients (18%) experienced MACE. In particular, five patients (7%) underwent cardiovascular death (including the need for cardiac transplantation), six patients (9%) developed recurrent de novo SCAD with an average of 2.8 recurrences/patient among those who suffered recurrences, three patients (4%) required revascularization by PCI and two patients (3%) experienced heart failure (Table 2).

Predictors of recurrent de novo SCAD are use of HT (Kaplan-Meier -KM- analyses in Fig. 2) and the presence of VAs at onset ($p = 0.06$ at multivariable anaylsis in Table 3, KM analyses in Fig. 3). Emotional

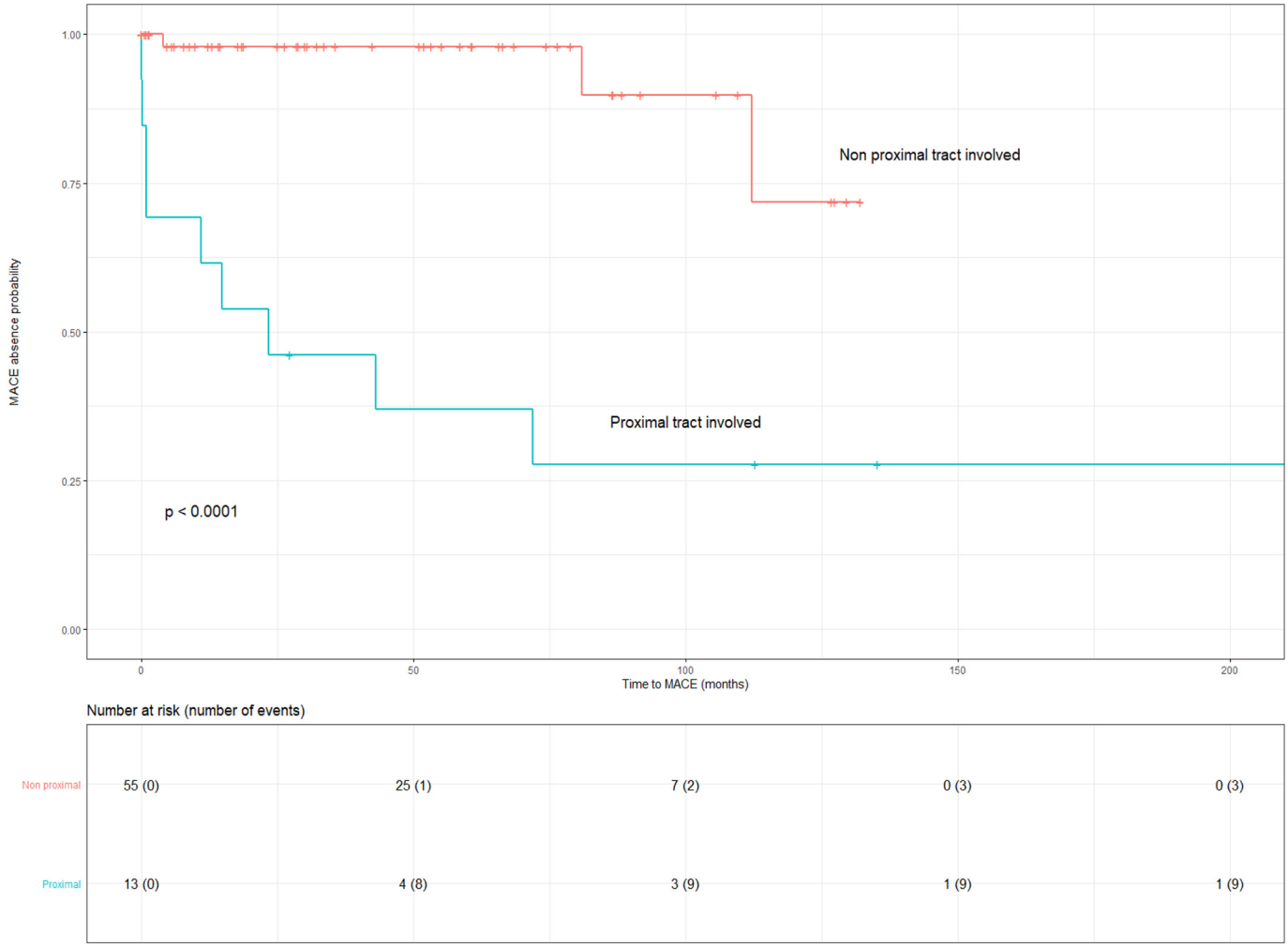


**Fig. 4.** Kaplan-Meier analysis of the probability of MACE with respect to proximal/non proximal coronary tract involving at the onset of SCAD.

stress as the trigger of the first event is more represented in patients with recurrences than in patients without recurrent disease, as well as the proximal SCAD site (Table 3); both, however, are not proven to be predictors of recurrences neither in KM analyses nor in the multivariable analysis. Gender, age at onset, cardiovascular risk factors, type of dissection, LVEF, troponin I peak and drug therapy prescribed did not change the risk of recurrences.

Predictors of MACE at follow-up are proximal coronary site of the lesion at the first event ($p = 0.017$ at multivariable anaylsis in Table 4 and statistically significant in KM analysis in Fig. 4) and presence of VAs at onset ($p = 0.0036$ at multivariable analysis in Table 4 and statistically significant in KM analysis in Fig. 5). LVEF and coronary territories involved show a trend of significance only in univariate analyses, but data are not confirmed by KM analyses nor in multivariable analysis. No other variable is related to the MACE development, including drug therapy prescribed.

Six patients (22%) defined as genetically "positive" developed MACE vs. two patients (6%) defined as "negative" ($p = 0.06$ at univariate analysis) (Table 6). Mutated patients had earlier MACE events than negative ones, although statistical significance was not reached.

## 4. Discussion

In this observational single-centre retrospective cohort study including 70 patients affected by SCAD and enrolled in a period of years 2000–2019, we comprehensively assessed long-term cardiovascular outcomes and explored clinical predictors of recurrent de novo SCAD and of MACE. In addition, the assessment of genetics through next-generation sequencing and its clinical implication during the follow-up was considered.

Importantly, our cohort is not different from cohorts of other SCAD registries in terms of age at clinical presentation (median value 52 years), female sex (86%), cardiovascular risk factors, clinical onset and LVEF [5,6,10,11,21–23]. The use of HT is in line with the literature data [5,9,10,24], while on the contrary we did not observe cases related to pregnancy. We have also observed a similar distribution of the coronary artery territories involved in the SCAD process [5,10,24,25], including the proximal vs. non proximal coronary artery tract [6,9,26]. More type 1 SCAD was reported in our study compared to other experiences [1,5,6,11] likely due to selection bias. Probably, some type 2 and type 3 SCAD may have been not recognized in the first years due to less extensive use of coronary angiography and other invasive examinations such as IVUS and OCT, in association to lesser sensitivity of operators to recognition of coronary dissection.

Mean presenting LVEF was 59.0% and 77% of patients had wall motion abnormality in the form hypokinesia, akinesia and dyskinesia.

Several investigators have reported association between SCAD and TakoTsubo syndrome (TTS) [31,32] but in our study only two patients were considered to have simultaneously SCAD and a TTS due to the typical ECG evolution, limited increases of troponin I and presence of typical wall motion abnormalities in myocardial segments not related by

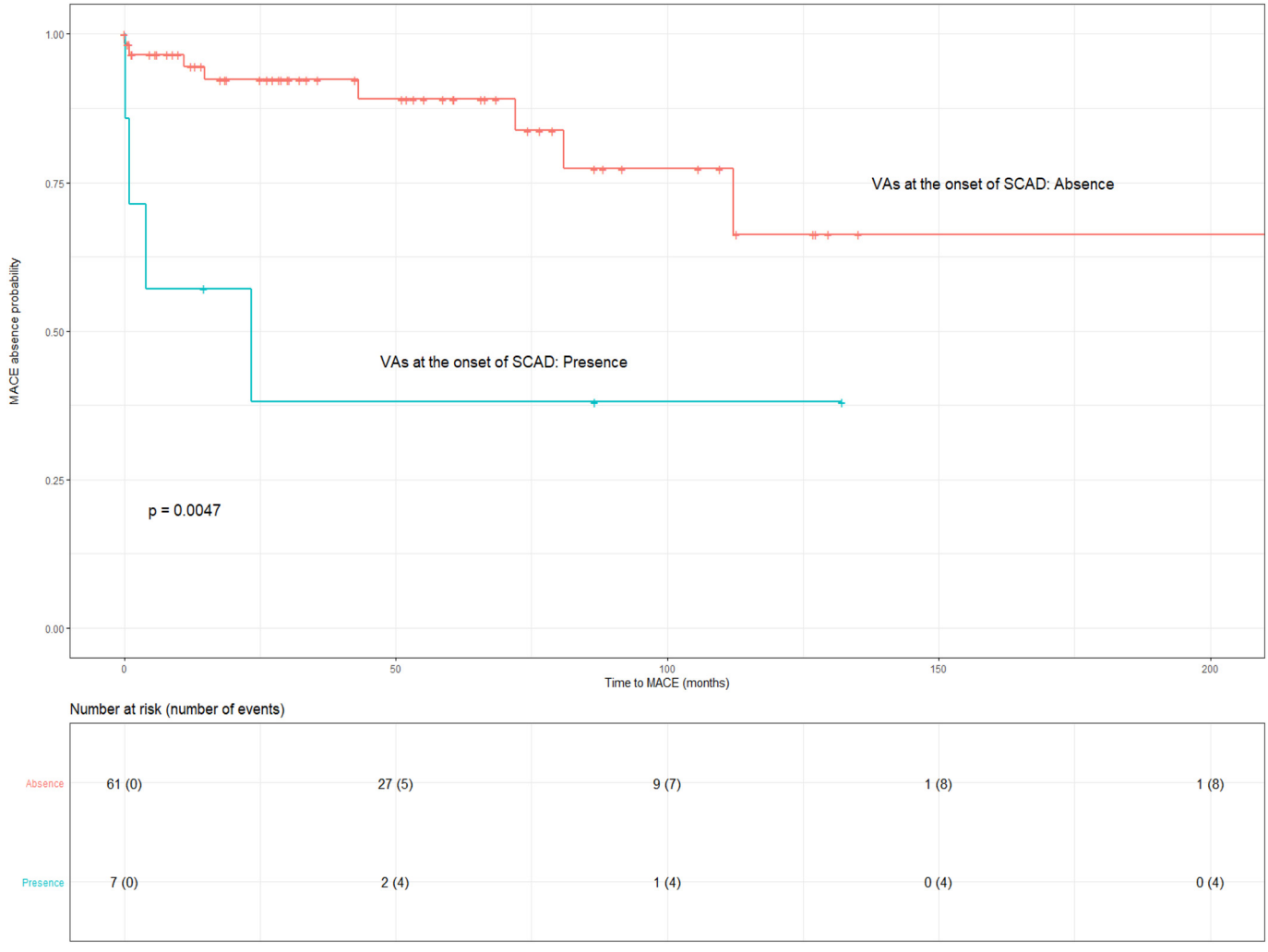


**Fig. 5.** Kaplan-Meier analysis of th probability of MACE with respect to presence/absence of VAs at the onset of SCAD.

**Table 5**
Descriptive analysis of genetic test results.

| Patient | Genetic mutation | Allelic frequence in the European population (gnomAD) | Follow-up events |
|---|---|---|---|
| 1 | ***COL3A1 c.G719A (p.G240E)*** | ***N.P.*** | ***Early recurrent* de novo *SCAD in new diagnosis of Ehlers-Danlos type 4*** |
| 2 | *COL5A1 c.C341A (p.A114D)* | *0,001* | / |
| 3 | *COL5A1 c.C3491T (p.P1164L)* | *0,0001* | / |
| 4 | *COL5A1 c.C761T (p.S254L)* | *0,0002* | / |
| 5 | *COL5A2 c.T5C (p.M2T)* | $7{,}92 \cdot 10^{-5}$ | / |
| 6 | ***COL5A2 c.G4083C (p.W1361C)*** | ***N.P.*** | ***Cardiac transplatation after 1 months from SCAD due to cardiogenic shock*** |
| 7 | *FBN1 c.C4213A (p.L1405I)* | *N.P.* | / |
| 8 | *FBN1 c.A3058G (p.T1020A)* | *0,0006* | / |
| 9 | *FBN2 c.A6946T (p.I2316F)* | *0,0004* | / |
| 10 | *FBN2 c.G1592C (p.G531A)* | *0,0015* | / |
| 11 | ***FBN2 C.c68g (p.A23G)*** | ***0,0004*** | ***Multiple recurrent* de novo *SCADs*** |
| 12 | *FBN2 c.C4141A (p.H1381N)* | *0,0057* | / |
| 13 | *MYLK c.G91A (p.V31M)* | $8{,}97 \cdot 10^{-6}$ | / |
| | *FBN2 c.G6064A (p.A2022T)* | $8{,}81 \cdot 10^{-6}$ | |
| 14 | *MYLK c.A1889G (p.N630S)* | $7{,}17 \cdot 10^{-5}$ | / |
| 15 | *MYLK c.G4142A (p.R1381Q)* | *0,0001* | / |
| | *MYLK c.G94A (p.A32T)* | $1{,}79 \cdot 10^{-5}$ | |
| 16 | *MYLK c.C3082T (p.R1028W)* | *0,0002* | / |
| | *MYLK c.G94A (p.A32T) MYH11 c.A3766C (p.K1256Q)* | $1{,}79 \cdot 10^{-5}$ *0,0003* | |
| 17 | ***LTBP2 c.T3296C (p.F1099S)*** | ***N.P.*** | ***Recurrent* de novo *SCAD after 10 years*** |
| 18 | *LTBP2 c.G5047A (p.E1683K)* | $7{,}16 \cdot 10^{-5}$ | / |
| 19 | *LTBP2 c.G11C (p.R4P)* | *N.P.* | / |
| 20 | *LTBP2 c.G5047A (p.E1683K)* | $7{,}16 \cdot 10^{-5}$ | / |
| 21 | *NOTCH1 c.G2482A (p.V828M)* | $1{,}82 \cdot 10^{-5}$ | / |
| 22 | ***NOTCH1 c.A1897T (p.T633S)*** | $\mathbf{9{,}14 \cdot 10^{-6}}$ | ***Heart failure requiring hospital admission one month after SCAD*** |
| 23 | *MYH11 c.G5869C (p.E1957Q)* | $9{,}85 \cdot 10^{-5}$ | / |
| | *SMAD3 c.A106T (p.K36)* | *N.P.* | |
| 24 | *MYH11 c.G18741A (p. R624H)* | *0,0001* | / |
| 25 | *PLOD1 c.C1534T (p.R512C)* | *0,0049* | / |
| | *PLOD1 c.G555T (p.K185N)* | *0,0055* | |
| 26 | *CBS c.G1643A (p.R548Q)* | *0,0003* | / |
| 27 | ***ELN c.G430A (p.V144M)*** | ***0,0002*** | ***Multiple recurrent* de novo *SCADs*** |

N.P.: not present in the database.

the territory of the SCAD affected coronary artery. We think that SCAD and TTS may coexist in some patients, also considering the similar epidemiologic pattern of both pathologies (middle age, female sex, emotional/physical stress), and that the SCAD could be the trigger for the development of TTS rather than a "pure" overlap.

The therapeutic strategy, preferring conservative medical therapy, was superimposable compared to the most recent studies, reserving PCI and CABG for more complicated cases [1,5,6,9,10,21,24,25]. The choice of therapeutic strategy has changed over the years moving from more interventionist attitudes in the period 2000–2010 to more conservative attitudes in the second period (2011–2019), according to the most recent indications [1,6]. Only patients with defined high-risk features, such as left main stem involvement, haemodynamic or arrhythmic instability, and persistent ischemia, were treated with PCI or CABG in two patients considered not eligible for PCI [1,6].

In our study we reported significant clinical predictors of recurrent SCAD, which has not been previously reported in other SCAD series. Strong emotional stress as a precipitating factor was reported in 67% of patients with recurrent disease. An HT history is reported in 50% of patients with recurrent disease, while a history of VAs at the onset of the disease is reported in 50% of these patients (Table 3, Fig. 2, Fig. 4). As for emotional stress, it is thought that the catecholaminergic discharge resulting from it can sharply increase wall stress. Considering this, it could be useful to observe in the future if the use of benzodiazepines or antidepressants can reduce the incidence of recurrence. Beta blockers may also be useful for this purpose [10] because, in addition to reducing parietal stress, they also mitigate the catecholaminergic discharge [27], although in our study we have not shown that they give a benefit or reduce the possibility of recurrences, nor do they reduce MACE in follow-up.

Regarding the use of HT, Tweet et al. [21] found that some patients who had recurrent de novo SCAD took HT but no other studies, as far as we know, showed a significant correlation with recurrent de novo SCAD. In our study we observed a strong association between the use of HT and the increased possibility of recurrent de novo SCAD. The increase in recurrences in these patients could be explained by vascular

**Table 6**
Descriptive analysis of genetic test results matched with clinical variables (only statistically significant variables are represented).

| | *Patients negative for gene mutations (N = 34)* | *Patients positive for gene mutations (N = 27)* | *p VALUE* * |
|---|---|---|---|
| *Proximal tract involved* | *1 (3%)* | *8 (30%)* | *p = 0,004* |
| *Medium or distal tract involved* | *33 (97%)* | *19 (70%)* | *p = 0,004* |
| *OUTCOMES* | | | |
| *Cardiovascular death (including need for heart transplantation)* | *0% (0)* | *1 (4%)* | *n.s.* |
| *Myocardial infarction* | *2 (6%)* | *4 (15%)* | *n.s.* |
| *Recurrent* de novo *SCAD* | *2 (6%)* | *4 (15%)* | *n.s.* |
| *Revascularizations* | *1 (3%)* | *2 (7%)* | *n.s.* |
| *Heart failure requiring hospitalization* | *1 (3%)* | *1 (4%)* | *n.s.* |
| *MACE* | *2 (6%)* | *6 (22%)* | *p = 0,06* |
| *Time to first MACE (months)* | *23,25/42,50/61,75* | *1,00/7,90/21,25* | *n.s.* |

Values are N (%) or first quartile/median value/third quartile, unless otherwise indicated.
* Univariate analysis adjusted with Benjamini – Hochberg method.
n.s. = non statistically significant.

changes caused by estrogen and progesterone [28]. Therefore, the lifelong suspension of HT after SCAD should be strongly considered.

No drug therapy proved to be protective against recurrent de novo SCAD, although Saw et al. [ (10)] demonstrated the positive role of beta-blockers. The explanation for this divergence is not unique and include adequate doses selection and selection bias.

Dual antiplatelet therapy did not show an improvement in the long-term outcome in these patients, neither in terms of recurrences nor in terms of mortality. Our study confirms the results obtained in the most recent literature data [10,22], and along time we reserved dual antiplatelet therapy for patients undergoing PCI or patients who, although treated conservatively, were considered to be at high ischemic risk and low bleeding risk. However, clear evidence supporting the use of dual-antiplatelet therapy in patients with SCAD, who do not undergo PCI, is lacking. Although theoretical benefits of early dual antiplatelet therapy in SCAD includes protection from additional thrombosis in the prothrombotic condition caused by intimal dissection, many physicians avoid its use in light of an increased bleeding risk and no evidence of clear benefit.

The MACE rate (18% at 39.1 months) is in line with most recent literature data [1,10,11,21,22]. Another key finding of this study is a list of significant clinical predictors of MACE, including proximal coronary tract involvement for the first SCAD and VAs at the onset of the disease. The proximal involvement of the coronary vessel by the spontaneous dissection process would imply a larger myocardial territory at risk than dissections of middle or distal coronary tracts, worsening the prognosis of these patients. In addition, the presence of ventricular arrhythmias could be due to a more prolonged occlusion of the vessel and, therefore, to a deeper myocardial ischemia, worsening patient outcomes again.

Regarding to genetic analysis, 44% of the patients tested were "positive" for mutations in the genes tested, with genes involved similar to those reported in other smaller series [12,13,29]. Some studies have observed that genetic positivity is correlated both to an early age of SCAD onset [13], not shown in our study, and to the association with migraine, fibromuscular dysplasia and dissections of the cervical arteries [17,18,30], not analyzed in our study.

The prognostic role of genetic analysis highlight how patients defined as "positive" to genetic test are also those with the greatest probability of developing MACE at follow-up, although it is only a trend of significance ($p = 0.06$). We may not have reached statistical significance, likely due to the small sample size and to selection of some genes that were not directly involved in SCAD etiopathogenesis. Furthermore, focusing only on genes that led to MACE (*COL3A1*, *COL5A2*, *FBN2*, *LTBP2*, *NOTCH1* and *ELN*) could enhance predictability of adverse events. It is also important to highlight the association between positivity to genetic tests and development of proximal SCAD (Table 6), resulting in a ten-fold likelihood of proximal type SCAD as compared to patients with negative genetic tests.

In addition, considering that the genetically positive population had no significant differences with respect to the genetically negative population in coronary area affected, prevalence of diabetes mellitus and VAs, we hypothesize that genetics may play an independent role in predicting events at follow-up.

We think that genetic testing could become a promising tool in the future to predict adverse events at follow-up in patients with SCAD, thus also improving the clinical management of such patients. However, it will be crucial in the future to carry out larger studies to confirm these results. To our opinion, genetic testing is not indicated in family members of patients with SCAD, except for diagnoses of known genetic syndromes in the proband and gene mutations considered to be pathogenic or likely pathogenic.

### 4.1. Study limitations

1) Due to the non randomized design of the study, the results could incur into the selection bias already described for observational type study.
2) It appears difficult to be certain of the homogeneity of the population included, because along the study period the clinical management, the availability of sensitive biomarker tests and the indication to coronary angiography in patients with ACS have radically changed. A larger use of advanced diagnostic methods such as IVUS and OCT, could also have led to a better definition of SCAD distribution and its prognostic role.
3) SCAD-associated genetic variants have been defined on the basis of population frequency only; bioinformatics and resulting protein conformation analyzes have not been investigated, therefore this may have affected the results.
4) No clinical and genetic data were collected on fibromuscular dysplasia, so we are unable to provide any correlation with SCAD.

## 5. Conclusions

Our study confirmed that SCAD is a rare disease with a higher prevalence in women. The prevalence of cardiovascular risk factors in patients with SCAD does not seem to differ much from that of the general population.

Use of HT and VAs at SCAD onset were prognostic factors for recurrent de novo SCAD at follow-up, indicating the importance of suspension of HT after the first event. The proximal SCAD site and VAs at SCAD onset were prognostic factors for MACE at follow-up.

Gene mutations, in particular of *COL3A1*, *COL5A2*, *FBN2*, *LTBP2*, *NOTCH1* and *ELN* genes, are associated to MACE development. Further and larger studies are needed to confirm if genetics may play a role in SCAD etiopathogenesis and prognostic stratification.

## Author statement

**Marco Antonutti:** Conceptualization, Methodology, Software, Original Draft preparation, data collection, Writing, Editing.

**Federica Baldan.**: Conceptualization, collection of genetic samples and their processing.

**Corrado Lanera**: Statistic analysis.

**Leonardo Spedicato, Davide Zanuttini, Teodoro Bisceglia, Enrico Favaretto, Stefano Poli, Chiara Tioni and Dario Sut**: data collection and organization of the register.

***Dario Gregori:*** Supervision of statistic analysis.

***Giuseppe Damante:*** Supervision of collection of genetic samples and their processing.

***Alessandro Proclemer:*** General supervision, Validation, Reviewing.

## Declaration of Competing Interest

None declared.

## Acknowledgements

None.

## Appendix A

Favorable opinion of the single regional ethics committee (CEUR) for the start of the observational study.

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**OGGETTO: Parere CEUR (seduta dd.05/06/2018 - odg 5.11) SCIOGLIMENTO RISERVE**

| | |
|---|---|
| ID studio | 2380 |
| Codice interno al centro | n.a. |
| Tipologia studio | Osservazionale con farmaco |
| Tipo studio | No Profit |
| Codice studio | NA |
| Titolo studio | Implicazioni cliniche e genetiche nella dissezione coronarica spontanea |
| EudraCT | \\ |
| Promotore | Azienda Sanitaria Universitaria Integrata di Udine – ASUI UD |
| CRO | NA |
| Centro - UO | Azienda Sanitaria Universitaria Integrata di Udine - SOC Cardiologia |
| Centro coordinatore | \\ |
| Sperimentatore | Antonutti Marco |

In riferimento allo studio indicato ed alla precedente comunicazione Prot. N. 18629 del 12.06.2018 di trasmissione del parere "*sub-condicione*", **si ritiene che la documentazione sotto indicata trasmessa a mezzo e-mail in data 23.06.2018:**

- Consenso Informato e Revoca Consenso v. 2 del 23.06.2018, clean e TC
- Informativa v. 2 del 23.06.2018, clean e TC
- Protocollo v. 2 del 23.06.2018 clean e TC

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