

## Sudden cardiac death in the young: gender differences

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**Summary.** Sudden cardiac death (SCD) is a major cause of death also in women, despite a lower prevalence compared to men. We assessed gender differences in SCD in our case history and considered the main cardiovascular causes of SCD in women. In our experience, one third of the young SCD victims are women and – while atherosclerotic coronary artery disease and arrhythmogenic cardiomyopathy are the leading causes in male SCD victims – SCD in young women is usually associated with non-atherosclerotic causes, like spontaneous coronary dissection and mitral valve prolapse, with a similar prevalence. Myocarditis, structural inherited cardiomyopathies and *mors sine materia*, with a structurally normal heart, are involved in two thirds of female cases. Most cardiovascular causes of SCD in the young are genetically determined. Despite the incomplete penetrance and the variable expressivity observed in family lineages, the dominant autosomal mechanism of inheritance does not explain the complexity of SCD-associated gender differences, thus emphasizing the growing role of epigenetic and environmental/hormonal mechanisms.

**Key words.** Sudden cardiac death, gender differences, sex differences.

### *La morte cardiaca improvvisa nei giovani: differenze di genere*

**Riassunto.** La morte cardiaca improvvisa (MCI) rimane una delle principali cause di morte nelle donne, nonostante la prevalenza inferiore rispetto agli uomini. Abbiamo valutato le differenze di genere nella MCI nella nostra casistica e abbiamo considerato le maggiori cause cardiovascolari di MCI nelle donne. Nella nostra esperienza, un terzo delle giovani vittime di MCI è donna e, mentre l'aterosclerosi coronarica e la cardiomiopatia aritmogena rappresentano le principali cause nelle vittime maschili di MCI, la MCI nella giovane donna è solitamente associata a dissezione coronarica spontanea e prolasso della valvola mitrale. La miocardite, cardiomiopatie strutturali ereditarie e la *mors sine materia*, con cuore strutturalmente normale, sono coinvolte in due terzi dei casi femminili. La maggior parte delle cause cardiovascolari di MCI nei giovani è geneticamente determinata. Nonostante la penetranza incompleta e l'espressività variabile osservate nelle genealogie familiari, la principale modalità di trasmissione autosomica dominante non spiega la complessità delle differenze di genere associate alla MCI,

evidenziando il ruolo crescente di meccanismi epigenetici e ambientali/ormonali.

**Parole chiave.** Morte cardiaca improvvisa, differenze di genere, differenze sessuali.

### Introduction

#### *Definition, epidemiology and etiology of sudden cardiac death in the young*

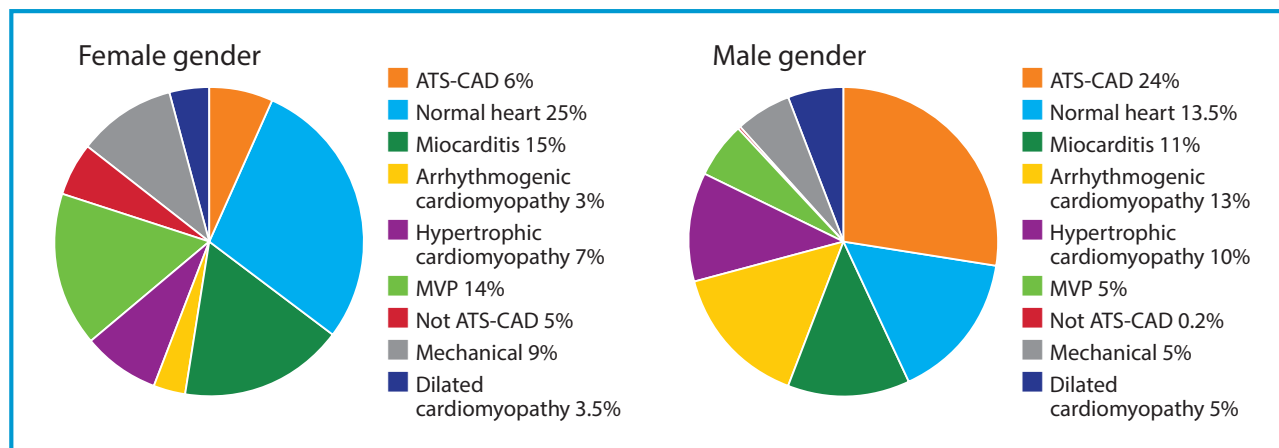
Sudden cardiac death (SCD) is defined as death due to cardiac causes occurring within one hour from the onset of symptoms in an apparently healthy subject without a prior condition that would appear abruptly fatal.<sup>1</sup> Although SCD may be associated with 'mechanical' causes, in more than 90% of cases the mechanism is arrhythmic ('electric' SCD),<sup>2</sup> leading to cardiac arrest.

In the Veneto Region the annual rate of SCD in young subjects ( $\leq 35$  years) is currently estimated at 0.5/100,000/year in women, and 1.5/100,000/year in men.<sup>3</sup> The lower SCD rate in women is probably due to different cardiovascular disease profiles and to a different prevalence of atherosclerotic coronary artery disease (ATS-CAD), which is the most frequent cause of SCD in men. However, sex differences in SCD decrease with age, possibly due to menopause and related hormonal changes in women.<sup>4</sup>

We reviewed 650 consecutive SCD cases in young people and in athletes, 69% of whom were males and 31% females, in the period 1980-2013. In our case series, major SCD causes include ATS-CAD (18%), myocarditis (12%), arrhythmogenic cardiomyopathy (AC, 10%), hypertrophic cardiomyopathy (HCM, 9%), dilated cardiomyopathy (DCM, 4%), non-ATS-CAD (7%) and mitral valve prolapse (MVP, 7.5%). In 6% of cases, SCD was mechanical and in 17% the heart was structurally normal ('unexplained SCD').

#### *Gender differences in SCD*

In our experience, myocarditis, HCM and DCM show an almost equal prevalence in males and females, while ATS-CAD and AC are the leading causes in male SCD victims (figure 1).



**Figure 1.** Etiologies for sudden cardiac death in young women and men in the Veneto region.

SCD in young women is usually associated with non-atherosclerotic disease, such as MVP, spontaneous coronary dissection, myocarditis, inherited cardiomyopathies and congenital heart diseases.<sup>3</sup> In our cohort, SCD remained unexplained in 25% of women, versus 14% of men (*mors sine materia*). In these cases, post-mortem genetic testing can help clarify a probable molecular cause of death (*molecular autopsy*).<sup>1,5</sup> Of note, a high-risk time for women with an underlying cardiovascular substrate is the peripartum period.<sup>3,5</sup>

Overall, SCD in young athletes also shows a gender preference, with a male/female ratio up to 10:1.<sup>6</sup> In our epidemiology, we observed, among athletes, a mortality rate of 2.6/100,000/year in men and 1.1/100,000/year in women.<sup>3</sup> Male gender has been recently reported to be a risk factor for sports-related SCD, due to the greater prevalence and phenotypic expression of cardiac diseases at risk of arrhythmic cardiac arrest, like cardiomyopathies and premature CAD. The higher participation rate of males in competitive sports is also an obvious explanation.<sup>7</sup>

## Coronary artery disease

### Atherosclerotic coronary artery disease

#### Definition and genetics

Atherosclerotic coronary artery disease (ATS-CAD) is an important cause of mortality mainly in men.<sup>3</sup> Although typically associated with behavioral risk factors, ATS-CAD has also been recognized in 40-60% of cases as related to a genetic background.<sup>8</sup>

Monogenic drivers of ATS-CAD include genes mainly associated with familial hypercholesterolemia and related conditions, such as APOB (encoding apolipoprotein B) and PCSK9 (encoding proprotein convertase subtilisin/kexin type 9).<sup>9</sup>

So far, genome-wide association studies have revealed that at least 164 chromosomal loci affect the risk

of ATS-CAD, involved in lipid metabolism, vascular remodeling, cell migration and adhesion, apoptosis etc. In addition, epigenetic studies identified DNA methylation alterations in ATS-CAD: methylation of critical genes and Alu and Long Interspersed Element 1 (LINE-1) repetitive elements have been found to be significantly altered in CAD. However, how the critical genes interact at the cellular level in order to cause atherosclerosis is still unclear.<sup>10</sup>

#### Gender differences in CAD

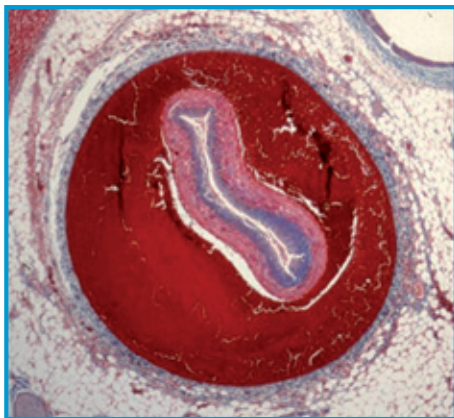
ATS-CAD is the first cause of SCD in Veneto, also in the young, where it accounts overall for 18% of deaths<sup>11</sup> at a mean age of 29.<sup>3</sup> It is mostly found in male SCD victims, being responsible of 24% of SCD cases in men, versus 6% in women.

In general, at onset, women are about 10 years older than men, with a higher expression of cardiovascular risk factors (hypertension, hypercholesterolemia, diabetes),<sup>12</sup> and are reported to develop a different form of vascular disease, the so-called 'female pattern'. Atherosclerotic plaque in women is less fibrotic and contains more lipid-laden foam cells, and acute coronary syndrome (ACS) often occurs as a result of the erosion of the atherosclerotic plaque, and less frequently from the rupture of the fibrous cap, as it is often the case in men.<sup>13</sup>

### Spontaneous coronary artery dissection

#### Definition and genetics

Spontaneous coronary artery dissection (SCAD) is a rare non-atherosclerotic cause of ACS. Main risk factors of SCAD include pregnancy, high blood pressure, extreme exercise, and previous SCAD. Its etiology appears to be multifactorial, with contributions from underlying arteriopathies, genetic factors, hormonal influences, inherited or acquired arteriopathies, or systemic inflammatory diseases, often combined with environmental stressors.<sup>14</sup>



**Figure 2.** Female, 28 years old, SCD at rest due to coronary dissection in the peripartum period.

SCAD has been associated with fibromuscular dysplasia and inherited connective tissue disorders, like Ehlers-Danlos syndrome (linked to mutations in *COL3A1*), Marfan syndrome (linked to mutation in *FBN1*), or Loeys-Dietz syndrome (linked to mutations in gene-encoding members of the transforming growth factor- $\beta$  signaling cascade, such as *TGFBR1*, *TGFBR2*, and *SMAD3*). Other mutations have been associated with arterial fragility and dissection resulting in SCAD. However the yield from routine genetic testing in patients with SCAD is low, especially among those without a family history or a suspected inherited systemic arteriopathy or connective tissue disorder.<sup>14,15</sup>

#### Gender differences in SCAD

SCAD is an important cause of SCD in young women, especially in middle-aged women, with no coronary atherosclerosis and no apparent risk factors.<sup>16</sup> Albeit rare, in Veneto SCAD is a cause of SCD in 5% of young women, versus 0.2% of men.

SCAD in women is most commonly associated with pregnancy – during which it is estimated to be responsible for 40% of myocardial infarctions<sup>17</sup> – and postpartum status within the first three months after delivery (figure 2).<sup>18,19</sup>

The higher risk of pregnancy-related SCAD is associated with the hemodynamic changes occurring during late pregnancy, in combination with a hormone-mediated arterial wall weakening.<sup>20</sup> Therefore, women with a history of SCAD should be carefully advised in regard to the risk of recurrent events whenever they are planning a pregnancy.

#### Aortic disease

##### Definition and genetics

Aneurysms and dissections are among the major diseases affecting the aorta, and are a leading cause of morbidity and mortality. In Veneto, 4% of SCD cases are

related to the laceration and external rupture of the ascending aorta with cardiac tamponade.<sup>21</sup>

Aortic dissection can be associated with several conditions, including inherited connective tissue disorders, such as Marfan syndrome, resulting from dominant autosomal mutations in the gene for microfibril component fibrillin-1 (*FBN1*), or can be component of other Mendelian disorders, such as bicuspid aortic valve (see below). In the absence of syndromic causes, about 20% of cases of thoracic aortic aneurysms leading to acute dissection (TAAD) have a genetic component. Familial TAAD is primarily linked to the genes involved in maintaining the smooth muscle contractile function (*ACTA2*, *MYH11*, *TGFBR1*, *TGFBR2*, *MYLK*). It is mainly inherited through an autosomal-dominant mechanism, with large interfamilial variability as well as variable penetrance and severity. In general, recent studies suggest the involvement of the transforming growth factor beta (TGF- $\beta$ ) pathway not only in heritable disorders of the connective tissue, but also in the pathogenesis of non syndromic aortic aneurysms and dissections.<sup>22,23</sup>

#### Gender differences in aortic disease

In Marfan syndrome, aortic dilatation does not seem to be more prevalent in males than in females, however men are reported to present a higher risk of an aortic event than female patients at any given age.<sup>24,25</sup> It is not clear why aortic dissection in Marfan syndrome occurs more frequently in men; one hypothesis involves a possible protective effect of X chromosomes in women, since women with Turner syndrome have a significantly increased risk of aortic disease, including dissection.<sup>26</sup>

Similar to such other cardiovascular diseases as CAD, aortic dissection occurs in women on average 5-10 years later. The older age and the higher proportion of women with a history of high blood pressure support the notion that hypertension – whose incidence increases with age – may play a role among the environmental factors involved in the pathogenesis of dissection. Although aortic dissections are less common in females, women experience worse outcomes, possibly due to a less typical symptomatology and a later medical contact, and greater mortality rate than men.

Finally, aortic dissection has been reported as an extremely rare complication of pregnancy, as long as the patient is not affected by any connective tissue disorder.<sup>27</sup>

#### Valve disease

##### Bicuspid aortic valve

##### Definition and genetics

Bicuspid aortic valve (BAV) is the most common congenital heart disease, with a prevalence of about 0.5-1% in the general population.<sup>28,29</sup> It is often clinically silent, but

may be complicated by aortic stenosis and regurgitation, infective endocarditis and aortic dilation and dissection.<sup>29</sup>

BAV commonly coexists with congenital heart diseases, including ventricular septal defect, isolated aortic arch obstruction and patent ductus arteriosus, and may occur as a component of different pleiotropic genetic syndromes, such as Loeys-Dietz, DiGeorge, and Marfan syndromes, as well as in patients with Turner and Williams syndrome.

Although it may occur sporadically, many cases are primarily inherited in an autosomal dominant pattern, with reduced penetrance. The male predominance and its association with Turner syndrome suggest an X-related etiology; however, the most significantly associated genes are located on autosomes. Today, *NOTCH1* (9q34-35) remains the only gene associated with both familial and sporadic BAV. Its mutations lead to signalling abnormalities that may be responsible for the development of a bileaflet aortic valve and for accelerated valvular calcium deposition.<sup>30</sup> Mutations in *TGFBR2* (3p22) and *ACTA2* (10q23.3) have been described in patients with BAV and aortic aneurysms.<sup>31,32</sup> An association between *FBN1* variants and BAV in patients with and without Marfan syndrome has been reported.<sup>33</sup> Finally, a role of *GATA4/5* and other genes in aortic valve morphogenesis and endocardial cell differentiation has been proposed.

#### Gender differences in BAV

BAV shows a strong male predominance, with a male/female ratio ranging from 3:1 to 2:1.<sup>28,29</sup> In living patients attending primary school, Basso et al found BAV in 0.5% of cases, with a higher prevalence in males than females (0.75% vs 0.24%).<sup>34</sup>

Male gender is included among the risk factors associated with progressive aortic dilatation and BAV endocarditis.<sup>35</sup> It remains unclear whether the higher frequency of endocarditis in men is related to the higher prevalence of aortic regurgitation in BAV.

A multicenter clinical study reported men with more frequent aortopathy and more frequent moderate/severe aortic regurgitation at first presentation compared with women, whereas women presented more often moderate/severe aortic stenosis.<sup>35</sup>

Men with BAV are reported to incur a higher aortic dilatation risk, a tendency for proximal ascending aorta dilation and possibly a higher risk of aortic dissection.<sup>36</sup> The authors suggest that, similarly to women with Turner syndrome, a reduced dosage of X chromosome genes may be related to the higher rate of aortopathy, with a higher dissection risk.

Finally, although men present BAV-related morbidity more frequently, it is women who carry the burden of a higher relative mortality, possibly indicating that clinical attention should focus on treatment opportunities for women.<sup>36</sup>

#### Mitral valve prolapse

##### Definition and genetics

Mitral valve prolapse (MVP) is the most common valve disease, with an estimated prevalence of 2-3% in the general population.<sup>37</sup> In Veneto is responsible for up to 8% of SCD cases.<sup>11</sup>

It is characterized by the atrial displacement of the mitral valve leaflet(s) during the ventricular systole, and is commonly considered as a benign finding. However, reported complications include mitral regurgitation, atrial fibrillation, infective endocarditis, stroke, and arrhythmic SCD.

MVP can be part of a generalized connective tissue disorder, with particular reference to Marfan syndrome or Loeys-Dietz syndrome. To date, various genetic defects have been recognized in the syndromic forms of MVP; however, the genetic analysis of non-syndromic forms – generally occurring as isolated episodes, or associated with benign extra-cardiac manifestations – remains a work in progress.

Whereas the autosomal-dominant is the common inheritance pattern, an X-related form of non-syndromic myxomatous MV disease has been described, associated with the Filamin-A gene (*FLNA*), encoding for a cytoskeleton protein involved in cardiac development.<sup>38</sup>

##### X-related *FLNA*-mitral valve disease

The X-linked *FLNA*-mitral valve disease is a genetically determined, congenital malformation of the mitral valve.<sup>39</sup> Mutations in the *FLNA* gene, encoding the actin-binding protein *FLNA*, cause a wide spectrum of connective tissue, skeletal, cardiovascular and/or gastrointestinal manifestations.

In the first report of X-linked mitral valve disease families, the penetrance of the disease was complete in men, and incomplete in women.<sup>40</sup> The disease is characterized by a severe phenotype in male patients, and a milder expression in female patients, who have another *FLNA* gene copy on their second X-chromosome, with frequent polyvalvular involvement, mitral leaflet thickening and elongation.<sup>41</sup> In general, an important characteristic of these families is the age-dependent and sex-dependent expression, the reduced penetrance and the high phenotypic variability among mutation carriers.

##### Gender differences in MVP

MVP is an important cause of arrhythmic SCD, which occurs especially in young adult women with palpitations and recorded ventricular arrhythmias (VA).<sup>11</sup>

In the SCD Registry of the Veneto Region, MVP accounts for approximately 14% of SCD cases among women, versus 5% among men. In a study involving autopsy and imaging, Basso et al reported MVP as a significant cause of SCD in young adults, and as the





**Figure 3.** Female, 27 years old, history of arrhythmias, sudden cardiac death due to mitral valve prolapse.

leading structural cause in women. Arrhythmic patients with MVP were mostly females with VA of left ventricular (LV) origin and frequent electrocardiogram (ECG) repolarization abnormalities on inferior leads. Basso et al observed fibrosis of the papillary muscles and the infero-basal LV wall, and proposed myocardial stretch by leaflet prolapse as a possible mechanism.<sup>37</sup> Finally, Marra et al reported that contrast-enhanced cardiac MRI helps identify this substrate.<sup>11,42</sup>

Female gender is thus reported as a risk factor for MVP (figure 3). Women show poorer outcomes following mitral valve surgery compared with male patients, who show a significant better post-operative long-term survival.<sup>43</sup> In addition, in a young cohort with out-of-hospital cardiac arrest seen in the Mayo Clinic, Sriram et al observed a malignant subset group of MVP patients at increased risk of SCD, characterized by bileaflet MVP, female sex, frequent complex ventricular ectopic activity, including premature ventricular contractions of the outflow tract, alternating with papillary muscle or fascicular origin.<sup>44</sup>

### Myocarditis

#### Definition and molecular pathology

Myocarditis is a leading cause of SCD in the young, accounting for up to 12% of cases in Veneto.<sup>11</sup>

Myocarditis is defined as an inflammatory myocardial disease associated with cardiac dysfunction.<sup>45</sup> Viral infections, involved in both acute and chronic myocarditis, are the most common cause of myocarditis in developed countries.

Molecular pathological techniques allow the identification of the causal microorganisms, through the detection of the viral genome in the myocardium by polymerase chain reaction (PCR) or reverse transcriptase PCR. Coxsackie virus (a RNA enterovirus) was found to be the most frequent malignant cardiotropic agent.<sup>11</sup>

#### Gender differences in myocarditis

Although some of the data on cardiac inflammation during myocarditis comes from animal models,<sup>46</sup> very few data exist on gender differences in myocarditis. Our case series shows a comparable prevalence in both sexes, being responsible of 11% of SCD cases in males and 15% in females.

### Genetically determined cardiomyopathies

#### Arrhythmic cardiomyopathy

##### Definition and genetics

AC is a genetically determined myocardial disorder, characterized by the progressive replacement of dead cardiomyocytes by fibro-fatty tissue that predisposes to the development of VA and to a high risk of SCD.<sup>47,48</sup>

It is a rare disease, with an estimated prevalence ranging from 1:2,000 to 1:5,000.<sup>49-51</sup> Clinical manifestations – including palpitations, syncope, ventricular tachycardia (VT), and/or ECG abnormalities – usually develop between the second and third decade of life, although SCD may be the only manifestation of the disease.<sup>52</sup>

AC is considered a disease of the desmosome, since in almost 50% of cases it is linked to pathogenic genetic variants in genes mostly encoding for proteins of the cardiac desmosome – plakoglobin (*PKP2*), desmoplakin (*DSP*), desmoglein-2 (*DSG2*) desmoscollin-2 (*DSC2*), and junctional-plakophilin (*JUP*)<sup>49,53,54</sup> – and presently it's the only cardiomyopathy whose diagnostic criteria include the identification of a known gene mutation.<sup>55</sup>

Usually inherited in an autosomal-dominant pattern, the disease phenotype is extremely variable, even among subjects from the same family and with the same mutation, and it is characterized by incomplete penetrance, due to the influence of modifier genes and environmental factors, such as age and gender.<sup>53</sup>

#### Gender differences in AC

In Veneto, AC accounts for 10% of SCD in the young, (3% of female victims versus 13% of male victims). AC is more prevalent in men than in women, with an approximate ratio of 3:1,<sup>56</sup> although males and females are expected to be equally affected, given the autosomal-dominant inheritance. Moreover, phenotypic expression is more common in male genetic carriers, who develop the disease earlier, and present more severe phenotypes compared to females.<sup>57</sup>

In a study including 171 AC cases, Bauce et al confirmed a higher prevalence of males, who were more likely to show an abnormal ECG and the presence of late potentials, consistent with a more severe disease expression.<sup>58</sup> In addition, men showed larger right ventricular dimensions, a lower right ventricular ejection fraction, and a more severe left ventricular (LV) involve-

ment, while female patients suffered more frequently from mild forms. Sex, however, was not associated with a high incidence of VA; on the contrary, in two independent studies on desmosomal mutation carriers, male sex was reported to predict lethal VA,<sup>59</sup> and Bhonsale et al observed earlier and more severe arrhythmic expression of the disease in males, who were more likely to be symptomatic at onset, or to present SCD as the first manifestation. However, no significant gender difference was observed in the occurrence of LV dysfunction or heart failure.<sup>60</sup> More recently, Lin et al reported male gender to predict VA recurrences after radiofrequency catheter ablation, describing different characteristics of VA and substrate properties between men and women.<sup>61</sup>

Putative reasons of gender differences in AC include sex-based difference in physical exercise and the influence of sex hormones as disease modifiers. In particular, estradiol has been advocated as a cardioprotective hormone, most likely because of its antiarrhythmic effects.<sup>62</sup>

### *Hypertrophic cardiomyopathy*

#### *Definition and genetics*

HCM is a primary myocardial disorder, defined as a concentric hypertrophy of the left ventricle, either symmetric or asymmetric, in the absence of aortic stenosis or systemic hypertension.<sup>63</sup>

It's the most common genetically determined disease affecting the heart, with an estimated prevalence in the overall population of 1:500 (0.2%).<sup>64</sup> HCM is usually transmitted as an autosomal-dominant trait, mainly caused by mutations in genes encoding the proteins of the cardiac sarcomere, the contractile unit of the cardiomyocyte. Approximately half of the mutations identified are located in 2 genes encoding the thick filament proteins, *MYH7* ( $\beta$ -myosin heavy chain) and *MYBPC3* (cardiac myosin binding protein C).<sup>65-67</sup> HCM phenocopies account for a 5-10% of the cases, mainly represented by storage diseases.<sup>68</sup>

Familial HCM is characterized by locus and allelic heterogeneity, with a high frequency of novel private mutations, and incomplete penetrance. The highly variable clinical phenotype suggests the existence of factors that may modulate the disease presentation. In fact, the phenotypic expression of HCM may vary according to the mutation involved,<sup>69,70</sup> and the contribution of environmental factors such as sex has been postulated to be central factor affecting clinical manifestations.<sup>71</sup>

#### *X-linked HCM*

The X-linked mode of inheritance is a rare genetic cause of gender differences in HCM. Only one report describes isolated HCM with a X-chromosomal inheritance pattern, linked to the *FHL1* gene (four-and-a-half LIM domain 1). A novel frameshift mutation was detected in 3

affected males, and co-segregated also with 1 case of apical HCM in heterozygous females in the family.<sup>72</sup>

#### *Gender differences in HCM*

In Veneto, HCM accounts for 9% of the SCD victims, and shows a less pronounced gender difference in its prevalence (10% of males versus 7% of females).

Accordingly, several mono- and multi-center clinical reports show a little lower disease penetrance in women, with a male predominance ranging from 2:1 to 3:2,<sup>73-76</sup> while a recent Italian study describes a male majority from adolescence to mid-life, with reversed female predominance evident only among patients >60 years of age.<sup>75</sup>

Some gender differences in the age at diagnosis have been also reported:<sup>73-75,77</sup> women are usually about 9 years older<sup>75,78</sup> and show a delayed onset of the symptoms and clinical manifestation of the disease.<sup>77</sup> Women are reported to be more symptomatic upon the initial evaluation, and to have more frequent heart failure events versus men.<sup>75,79</sup> Men, in fact, are described to be often diagnosed fortuitously, by routine medical examination, while women are more frequently diagnosed because of the onset of symptoms, such as palpitation and dyspnea.<sup>71</sup>

The causes of gender differences in HCM possibly involve a multifactorial etiology, related to genetic and endocrine differences between males and females, combined with a generally lower degree of attention to the cardiovascular risk among women.

### *Dilated cardiomyopathy*

#### *Definition and genetics*

DCM is currently defined as the presence of LV or biventricular dilatation and contractile dysfunction, in the absence of abnormal loading conditions (hypertension, valve disease) or coronary artery disease, sufficient to cause global systolic impairment.<sup>80,81</sup>

Almost half of the cases (20-35%) are genetically determined, with a familial basis.<sup>82</sup> Familial DCM is one of the most genetically heterogeneous cardiac disease, with mutations in more than 50 single genes encoding proteins whose functions are closely connected to the function and structure of the cardiomyocytes, in particular cytoskeletal, but also nuclear envelope, sarcomeric, mitochondrial, and calcium-handling proteins. Titin and lamin A/C remain the major disease-causing genes. The most common mode of inheritance for familial DCM is autosomal-dominant, while X-linked, autosomal recessive, and mitochondrial inheritance are less common. Presentation in patients is generally characterized by great clinical phenotypic heterogeneity, incomplete penetrance, mostly age-dependent – with the disease developing in childhood, adolescence, and middle age, but rarely in the elderly – and variable expressivities.

### *X-linked DCM*

Rarely, DCM can be transmitted in a X-linked fashion, caused by mutations of the Duchenne and Becker muscular dystrophy *DMD* (Dystrophin) gene on the X chromosome. *DMD* is a 2.5 Mb gene located on chromosome Xp21.2-p21.1, and is the largest known gene, encoding a 427-kDa dystrophin protein, as part of a dystrophin glycoprotein complex in the cytoskeleton that connects the contractile apparatus and extracellular matrix to stabilize the membrane.<sup>83</sup>

In Duchenne patients, dystrophin is virtually absent; whereas Becker patients have 10 to 40% of the normal amount. The clinical differences between Duchenne and Becker are due to the different types of *DMD* mutations. When the mutation causes a shift in the open reading frame or generates a premature stop codon in the gene, a non-functional protein is produced and the patient will suffer from Duchenne; on the contrary, when the reading frame is maintained (in-frame mutation), a partially functional protein is produced and the patients will show the milder clinical symptoms associated with Becker muscular dystrophy.<sup>84,85</sup>

X-linked DCM (XLDCM) is a distinct phenotype of dystrophinopathy, characterized by a preferential cardiac involvement with progressive ventricular dysfunction and chamber dilatation, without clinical skeletal myopathy, only with increase of the level of serum creatine kinase.<sup>86</sup>

The clinical severity of the cardiac involvement in XLDCM can be quite variable, ranging from an early onset and fatal cardiomyopathy to a milder form, compatible with a better prognosis. Probably because of the mutated X-chromosome inactivation, XLDCM female carriers usually have a lower chance of disease manifestation, with a later onset during the fifth decade of their life, with milder signs and symptoms, and present a slower progression of heart failure, in contrast to the early onset in hemizygous males.

### *Gender differences in DCM*

In Veneto, DCM is responsible for about 4% of SCD, and shows a similar prevalence in males and females (4% versus 3.5%).

However, clinical registries usually report women to achieve a better survival rate, in spite of a clinical presentation with a more advanced disease.<sup>87,88</sup>

In DCM, gender differences may also vary depending on the specific genetic subtype.<sup>89</sup> For instance, a study of familial DCM reported the *TNNT2* p.Ala171Ser mutation to result in different phenotypic expressions of the disease, with a more severe phenotype in males than in females.<sup>90</sup> In contrast, Herman et al reported in 312 DCM subjects a *TTN* mutation – found in approximately 25% of familial DCM cases and in 18% of sporadic cases – and cardiac outcomes were similar in subjects with and

without *TTN* mutation. However, adverse events occurred significantly earlier in male mutation carriers.<sup>91</sup>

### *Peripartum cardiomyopathy*

At present, the only sex-specific condition affecting females is the peripartum or postpartum cardiomyopathy (PPCM). PPCM is characterized by the rapid development of unexplained systolic heart failure during the final weeks of pregnancy, or up to 6 months postpartum. The clinical picture of PPCM has the appearance of a DCM, but differs from other forms of DCM in its rapid development. Women often recover their cardiac function, but long-lasting morbidity and mortality are not rare.<sup>92</sup>

The PPCM etiology includes autoimmunity, fetal microchimerism, virus infection, stress-activated cytokines and toxicity caused by an abnormal cleavage product of prolactin.<sup>81</sup> However, the notion that PPCM may have a hereditary or genetic component is supported by familial occurrence, genome-wide association studies, variable prevalence among different regions and ethnicities and, more recently, by investigations of the genes panels among PPCM women. Specifically, 15% of PPCM patients were recently found to have genetic mutations previously associated with DCM, and showed a lower recovery rate.<sup>93</sup>

## **Sudden cardiac death and normal heart: channelopathies**

### *Long QT syndrome*

#### *Definition and genetics*

Long QT syndrome (LQTS) is the most common cardiac channelopathy, estimated to occur in about 1 in 2,000 people.<sup>94,95</sup> LQTS is typically characterized by a prolongation of the QT interval on the ECG and by the occurrence of syncope or cardiac arrest, mainly precipitated by emotional or physical stress.

'Congenital' LQTS is associated with mutations in the genes encoding for ion channels and/or associated proteins. Currently, there are three major LQTS genes (*KCNQ1*, *KCNH2*, and *SCN5A*) that account for approximately 75% of the disorder, and about 10 minor LQTS-susceptibility genes that collectively account for less than 5% of LQTS cases.<sup>96</sup> Specifically, loss-of-function mutations in *KCNQ1* cause about 35% of type 1 LQTS (LQT1), loss-of-function *KCNH2* mutations contribute approximately 30% of LQTS (LQT2), and gain-of-function *SCN5A* mutations underlie roughly 10% of LQTS (LQT3). Interestingly, LQT3 patients are described to have a higher fatality of cardiac events than LQT1 and LQT2 patients.<sup>97</sup>

LQTS is typically inherited as an autosomal-dominant trait, while it is rarely inherited recessively, and is

characterized by a severe cardiac phenotype and sensorineural hearing loss.<sup>98</sup> About 5% to 10% of LQTS patients show a complex genotype with multiple mutations and typically present a more severe phenotype at a younger age.<sup>96</sup>

The penetrance of LQT1, LQT2 and LQT3 genes in genetically affected individuals ranges from 55 to 79%,<sup>99</sup> which emphasizes the importance of considering other factors beyond the primary defect, such as sex differences, hormonal status, etc.

### *Gender differences in LQTS*

Gender is a major factor in determining the course and clinical manifestation of LQTS. In children, although the QT interval duration is similar between young boys and girls, differences appear during puberty, when the QT interval in boys shortens. Overall, in congenital LQTS, women have longer QT intervals than men<sup>100</sup> and, in response to QT-prolonging drugs, women are more at risk of developing arrhythmias<sup>101</sup>. In addition, the risk for potentially lethal VA, especially in patients with LQT2, is particularly pronounced during the postpartum period.<sup>102</sup>

Thus, in spite of the equal distribution of the disease genotype among the two sexes, women are more often clinically affected by this syndrome, and the female gender is reported as an independent risk factor for cardiac events in LQTS patients. Specifically, women with LQT1 and LQT2 are reported to be at higher risk of torsades de pointes than men,<sup>103</sup> while both sexes are equally vulnerable to LQT3.<sup>97</sup>

Furthermore, the risk of cardiac events is described as higher in males until puberty, and higher in females during adulthood.<sup>97</sup> Thus, the diagnosis of LQTS may be more likely in females, with a later onset of repetitive nonfatal events.<sup>103</sup>

Interestingly, recent evidence correlates sex differences with the biophysical structure-function properties of the LQT mutations. In LQT1 women with mutations in the cytoplasmic loop region were reported as more likely to have an aborted cardiac event or SCD than women with mutations outside this region,<sup>104</sup> while women with LQT2 are reported to be more likely than men to suffer from a life-threatening cardiac event,<sup>97,105</sup> although women had no dependence on the location of the mutation.<sup>105</sup> Finally, no sex differences are reported in the cardiac events of LQT3, either pre- or post-puberty.<sup>97</sup>

### *Catecholaminergic polymorphic ventricular tachycardia*

#### *Definition and genetics*

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a genetic arrhythmogenic disorder characterized by stress-induced, bidirectional ventricular tachycardia, while the resting ECG, including the corrected QT interval, is normal.<sup>106</sup>

CPVT occurs in children and adolescents, and causes syncope and SCD at a young age, in the absence of structural heart disease. The prevalence of the disease in Europe is estimated to be 1:10,000.<sup>107,108</sup> Although rare, CPVT probably plays a significant role in the autopsy-negative, sudden unexplained death of young individuals, especially following exertion.

Two main variants of this disease have been described: an autosomal-dominant form (CPVT1) linked to mutations in the cardiac ryanodine receptor gene (*RyR2*),<sup>109,110</sup> and a recessive form (CPVT2) associated with mutations in the calsequestrin gene (*CASQ2*).<sup>111</sup> *RyR2* and *CASQ2* are both critically involved in the regulation of the cardiac excitation-contraction coupling, during which the opening of plasmalemmal  $Ca^{2+}$  channels triggers a much greater release of  $Ca^{2+}$  from the sarcoplasmic reticulum via *RyR2* channels.<sup>112</sup> *RyR2* mutations are frequent, whereas *CASQ2* mutations are rare; altogether, mutations are only found in 50 to 60% of patients. The third gene identified in association with autosomal-dominant CPVT is *CALM1*, encoding calmodulin, a protein that binds calcium and stabilizes the *RyR2* channel, and accounts for less than 1% of cases.<sup>113,114</sup> In 2012, the gene *TRDN* was identified in 2 families with CPVT, encoding triadin, a protein that links *RyR2* and calsequestrin in the sarcoplasmic reticulum.<sup>115</sup> Finally, a phenotype similar to CPVT is also linked to mutations in the gene encoding the potassium inward rectifying channel *Kir2.1* (*KCNJ2*).

### *Gender differences in CPVT*

Limited data exists to suggest any sex differences among CPVT patients. In some studies, male sex has shown a 4-fold increase in the risk of syncope,<sup>114</sup> and young men with cardiac *RyR2* mutations were shown to have a higher risk of cardiac events than young women.<sup>116,117</sup>

In CPVT families, the inheritance of *RyR2* mutations seems more frequent from mothers than fathers;<sup>117</sup> the low frequency of paternal inheritance may be due to the poorer prognosis of male patients compared to females.<sup>114</sup>

In addition, men are more likely to be positive for the *RyR2* mutation, while most of the *RyR2* mutation-negative patients are women,<sup>118</sup> suggesting a significant need for novel, sex-specific gene discovery.

### *Brugada syndrome*

#### *Definition and genetics*

Brugada syndrome (BrS) is a hereditary arrhythmic disorder associated with a right ventricular conduction delay and right bundle branch block, and SCD due to ventricular fibrillation.<sup>120</sup> Recent reports suggest that it could be responsible for 4% of all SCD and up to 12% of SCD in patients with structurally normal hearts.<sup>121</sup>



Currently, the prevalence of BrS is estimated at ~3-5 in 10,000 people, and it's highest in young men of Southeast Asian origin.<sup>119,120</sup>

Despite the identification of 18 associated genes, BrS is most often caused by mutations in *SCN5A*, leading to a loss of function in the sodium channel.<sup>122</sup>

Overall, only 30-35% of clinically diagnosed cases can be genetically diagnosed, indicating that 65-70% of BrS patients remain genetically unresolved, even if incomplete penetrance and variable expressivity may confound the diagnosis.

### *Gender differences in BrS*

The gender-related differences in the phenotypic expression of BrS have been widely reported.

The clinical phenotype is 8 to 10 times more frequent in males,<sup>121</sup> with specific clinical characteristics that are different from the women's. A greater rate of spontaneous type-1 Brugada-pattern electrogram is seen in men compared with women. Men are at greater risk to experience syncope, aborted sudden death, and documented VF than women.<sup>117,123</sup>

### *Molecular mechanisms of gender differences in SCD*

#### *Genetics and epigenetics*

The complex pathophysiology of SCD-associated cardiovascular disease and the underlying gender differences result from a complex interaction among the different genetic and epigenetic architecture of men and women, that interplays with hormonal and environmental factors to determine the final phenotype.

Genetic mechanisms include the expression of Y chromosome genes in men and the expression in women of those X chromosome genes that escaped transcriptional silencing in somatic cells. Interestingly, about 7% of the OMIM phenotypes are X-linked;<sup>124</sup> therefore, males are overall at increased risk of X-linked disorders, although penetrance and expressivity appear highly variable.

For instance, in familial X-linked cardiomyopathies, males are primarily affected, as it happens in metabolic disorders (Danon's disease and Fabry's disease) and in dystrophinopathies, such as Becker's and Duchenne's muscular dystrophies, which may be associated with cardiac involvement.<sup>71</sup>

Epigenetic mechanisms include chemical modification of DNA and histones (methylation, acetylation and deacetylation), miRNA and other long non-coding RNA activities, with consequent genes expression changes and gender-specific expression of the epigenome. These mechanisms influence gene expression independently of the DNA genetic code. Although heritable, epigenetic mechanisms are reversible, and can be modified by environmental factors. For instance, sex hormones such as

estrogen and testosterone have been shown to affect epigenetic DNA modifications.<sup>125</sup>

Differential methylation has been reported for genes associated with cardiovascular diseases involved in aging, lipid metabolism and in heart failure.<sup>126,127</sup> Methylation differences have been found, for example, in the *MMP2* gene (Matrix Metalloproteinase 2) in males suffering from ischemic stroke showing a higher hypomethylation of the promoter; the *F2RL3* gene (Coagulation Factor II Receptor-Like 3) hypomethylation has instead been correlated to mortality related to cardiovascular diseases, with a stronger association for males.

Variable imprinting of the *KCNQ1* gene has been demonstrated in mice models, and may provide a possible explanation of the existence of LQTS in the absence of a coding sequence mutation in *KCNQ1*.<sup>127</sup> Likewise, 59 epigenetic loci have been recently associated with DCM.<sup>128</sup>

#### *Pleiotropy, penetrance, expressivity*

The complexity of genotype-phenotype correlations in SCD-associated cardiovascular diseases is linked to pleiotropy, incomplete penetrance, and variable expressivity.

The 'pleiotropy', or phenotypic 'overlap', occurs when mutations in a single gene may have a different effect and result in different heritable cardiac diseases of the same multigenerational genealogy. For instance, in 2016 Veltmann et al demonstrated a high penetrance for LQTS, BrS and cardiac conduction disease in a large family harboring the *SCN5A* p.Glu1784Lys mutation.<sup>129</sup> This range of phenotypes may reflect the underlying functionality of the channel.<sup>130,131</sup>

In addition, distinct overlapping features have been identified in BrS and AC patients, and this has been the subject of an intense debate.<sup>132,133</sup>

Penetrance refers to the proportion of individuals with a given genotype who exhibit the genotype-associated phenotype. In many cardiovascular diseases, inherited in an autosomal-dominant manner, there is evidence for incomplete penetrance; in particular, in inherited cardiomyopathies a mutation carrier may never develop the disease.

In a report of over 500 desmosomal mutation carriers affected by AC, approximately only one-third met the diagnostic Task Force Criteria, with lower penetrance among women,<sup>63</sup> in line with a more recent report that observed the presence of the disease in about one third of copy number variations carriers, with an overall penetrance of 32%.<sup>134</sup>

In a 2000 report, Priori et al estimated that the overall disease penetrance across several small BrS families harboring mutations in the *SCN5A* gene was 16% (range 12.5 to 50%),<sup>135</sup> while for LQTS the mean penetrance is about 40%,<sup>136</sup> suggesting that the degree of incomplete penetrance and variable expressivity is highly variable also among cardiac channelopathies.<sup>136</sup>

Besides being a consequence of the combination of a variety of different genetic and environmental factors, reduced penetrance may be linked to the specific mutations involved.<sup>137</sup> Thus, in some conditions, normally characterized by an autosomal-dominant mode of inheritance, two incompletely penetrant (or otherwise non-penetrant) alleles may act in recessive fashion, while mimicking the normal dominant form of the disease, as it may happen, for instance, in inherited cardiomyopathies, where an increasing incidence of complex genotypes (homozygous, compound heterozygous) is reported.

Expressivity is the degree to which trait expression differs among individuals; therefore, individuals with the same genotype can show different degrees of the same phenotype. When a specific mutation causes a disease in a family, the carriers of the same mutation can display variable clinical manifestations. Like incomplete penetrance, variable expressivity is probably due to a combination of genetic, environmental and lifestyle factors. A wide inter-individual variability, in fact, cannot be exclusively explained by a single genetic variant, therefore there is increasing interest in searching genetic (coding or non-coding variants) and non-genetic (environmental and demographic variables, such as sex or age) modifiers that may modulate the relationship between genotype and phenotype. On the other hand, however, this complexity makes the development of genotype-specific prognostic or treatment recommendations difficult.

### Sex hormones

Differences in sex hormones have been hypothesized to underlie sex differences in the cardiovascular causes of SCD.

Estrogens are considered beneficial, because of their effects on atherosclerotic plaque progression, vasodilation, blood pressure, and their antioxidative and anti-inflammatory properties.<sup>138,139</sup> Women, in fact, are protected from atherosclerosis during their fertile age. After menopause, estrogen deficiency leads to an exponential increase in the cardiovascular risk, inducing several structural and functional changes in the cardiovascular system, such as endothelial dysfunction, visceral adiposity, enhanced systemic inflammation.<sup>140</sup>

At a molecular level, estrogen receptors are ligand-activated transcription factors which can activate the transcription of a number of genes whose promoter regions contain tandem estrogen response elements.<sup>141</sup> For instance, researchers demonstrated the mediation of the effects of 17 $\beta$ -estradiol on the cardiovascular system by estrogen receptors, e.g. rapid vasodilatation, reduction of vessel walls responses to injury, reduced development of atherosclerosis.<sup>142</sup>

Moreover, estrogen have been reported to prevent the development of cardiac hypertrophy and heart fail-

ure in female rodent models and in humans.<sup>143</sup> In different models of cardiomyopathies, experimental work has demonstrated the involvement of estrogens in calcium handling, in the extracellular matrix turnover, as well as in the metabolism of glucose, nitric oxide, and fatty acids, including an estrogen regulation of PPAR-gamma (peroxisome proliferator-activated receptor gamma), a transcriptional regulator of several genes involved mainly in fatty acid metabolism.<sup>144,145</sup>

In addition, a direct role of sex hormones in AC cellular pathogenic changes has been proposed using induced pluripotent stem cell-derived cardiomyocytes, where testosterone administration increased apoptosis and lipogenesis, whereas estradiol improved these phenotypes.<sup>62</sup>

Finally, to explain the longer QT intervals in women with inherited or acquired LQTS, both endogenous and exogenous (menopause hormone therapy), sex hormones are shown to affect the QT interval. In particular, endogenous testosterone and progesterone shorten the action potential, and estrogen prolongs the QT interval.<sup>146</sup>

These effects occur through alterations in myocardial L-type calcium channel current, the delayed rectifier potassium channel currents (consisting of the rapidly activating -I<sub>Kr</sub>- and slowly activating -I<sub>Ks</sub>- channel types) and the inward rectifier current -I<sub>K1</sub>-, which control phases 2 and 3 of the cardiac action potential.<sup>146</sup>

Future research is needed to better understand how sex hormones affect SCD-associated cardiovascular diseases in men and women, in order to implement better preventive strategies in both sexes.

### Key messages

- Sudden cardiac death is a major cause of death also in women, although significant differences exist between men and women.
- With respect to male gender, sudden cardiac death in females shows not only a lower prevalence, but also different risk factors and etiology.
- Major causes of sudden cardiac death in young women are represented by *mors sine materia*, with normal heart, mitral valve prolapse and spontaneous coronary dissection.
- Most cardiovascular causes of sudden cardiac death in the young are genetically determined, although epigenetic and environmental mechanisms, including sex hormones, may also contribute to sex differences in sudden cardiac death.
- A better understanding of gender differences in sudden cardiac death is necessary to prevent sudden death in young women.

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- Author contribution statement:* EA drafted the initial manuscript and revised the manuscript. SR and KP collected data and revised the manuscript. CB and GT coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All Authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.
- Conflict of interest statement:* the Authors declare no conflicts of interest.
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