ESC European Society of Cardiology

European Journal of Preventive Cardiology (2021) **28**, 1081–1090
doi:10.1177/2047487320934265

FULL RESEARCH REPORT

Yield and clinical significance of genetic screening in elite and amateur athletes

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Received 26 March 2020; accepted 25 May 2020; online publish-ahead-of-print 2 July 2020

Aims The purpose of this study was to assess the value of genetic testing in addition to a comprehensive clinical evaluation, as part of the diagnostic work-up of elite and/or amateur Italian athletes referred for suspicion of inherited cardiac disease, following a pre-participation screening programme.

Methods Between January 2009–December 2018, of 5892 consecutive participants, 61 athletes were investigated: 30 elite and 31 amateur athletes. Elite and amateur athletes were selected, on the basis of clinical suspicion for inherited cardiac disease, from two experienced centres for a comprehensive cardiovascular evaluation. Furthermore, the elite and amateur athletes were investigated for variants at DNA level up to 138 genes suspected to bear predisposition for possible cardiac arrest or even sudden cardiac death.

Results Of these 61 selected subjects, six (10%) had diagnosis made possible by a deeper clinical evaluation, while genetic testing allowed a definite diagnosis in eight (13%). The presence of $\geq$3 clinical markers (i.e. family history, electrocardiogram and/or echocardiographic abnormalities, exercise-induced ventricular arrhythmias) was associated with a higher probability of positive genetic diagnosis (75%), compared with the presence of two or one clinical markers (14.2%, 8.1%, respectively, p-value = 0.004).

Conclusion A combined clinical and genetic evaluation, based on the subtle evidence of clinical markers for inherited disease, was able to identify an inherited cardiac disease in about one-quarter of the examined athletes.

Keywords Athletes • genetic testing • inherited heart disease • multigene panel testing • sudden cardiac death

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Introduction

Over recent decades, sudden cardiac death (SCD) in athletes has become a highly visible event, fuelled by media reports and generating substantial concern in both physicians' and athletes' communities.[1–6] Interest in this tragic event has accelerated owing to the awareness that underlying cardiovascular diseases are often clinically identifiable, and manageable with proper prophylactic treatments.

For athletes in whom a cardiovascular disease has been identified, either at pre-participation screening[7,8] or under other circumstances, important considerations arise with respect to the appropriate advice relative to eligibility for competitive sports. The American Heart Association (AHA) – American College of Cardiology (ACC)[9] and European Society of Cardiology (ESC)[10] consensus documents have offered expert consensus recommendations for clinical practice, largely focused on competitive athletes.

At present, the majority of data have been collected on elite athletes, while knowledge on young individuals practising (or approaching for the first time) competitive sports, is relatively scant. Moreover, although the level of knowledge regarding a genetic basis of cardiovascular disease has been significantly growing in the last two decades, the role of *a priori* genetic testing in athletes is still debated.

The aim of this study was, therefore, to evaluate the role of genetic testing in addition to a comprehensive clinical evaluation, in a cohort of elite and/or amateur Italian athletes, selected by experienced centres (Osservatorio Regionale di Medicina dello Sport (ORMS), Regione Campania, and Institute of Sport Medicine and Science (CONI), Rome) for a suspicion of inherited heart disease.

Methods

Study population and study protocol

Between January 2009 and until the end of the 2018, a total of 5892 consecutive participants of which 2200 elite and 3692 amateur athletes, undergoing the Italian pre-participation screening programme,[11] were enrolled for the study. The athletes with high suspicion for underlying cardiac disease were selected for further clinical evaluation and genetic testing.

The selection and the subsequent evaluation of elite athletes were performed at CONI, while the selection of amateur athletes was performed at ORMS, and the subsequent evaluation at the Inherited and Rare Cardiovascular Diseases Clinic of the University of Campania 'Luigi Vanvitelli'. A flow-chart of the study protocol and diagnostic work-up is reported in *Figure 1* Panel (a).

Elite athletes were subjects undergoing regular training (>6 and up to 20 h/week) all year round, with a high level of achievement, competing at national or international level. Amateur athletes were subjects practicing regular exercise programmes and sport activities (three times, usually < 6 h/week, for 10 months a year) who were competing at local or regional level.

Clinical evaluation (*Figure 1* Panel (a): Step 1)

All the athletes underwent a standard clinical evaluation (pre-participation screening: including family and personal history, standard 12-lead electrocardiogram (ECG), echocardiography and exercise stress test).

Clinical markers of underlying cardiovascular condition at risk, in particular cardiomyopathies (CMPs) or primary electrical disorders (channelopathies), were investigated:

(1) Family history of cardiac arrest (CA) or SCD in ≥1 first degree relatives under 40 years of age;
(2) ECG abnormalities, such as T wave inversion (TWI) in ≥2 continuous leads, excluding aVR, V1 and lead III in isolation, ST segment depression, pathological Q waves, complete left bundle branch block (LBBB), long or short QT interval, Brugada-like repolarization;[12]
(3) Echocardiographic abnormalities, such as borderline (13–15 mm) left ventricular maximal wall thickness (MWT), left ventricular cavity enlargement (>60 mm) and/or reduction of ejection fraction (< 55%, >45%), increased ventricular trabeculation suggestive of left ventricular non-compaction (LVNC);[13]
(4) Non-sustained ventricular tachycardia (three consecutive ventricular beats at a rate of ≥120 beats per min and < 30 s in duration) during exercise stress test.

Additional clinical investigations (*Figure 1* Panel (a): Step 2A)

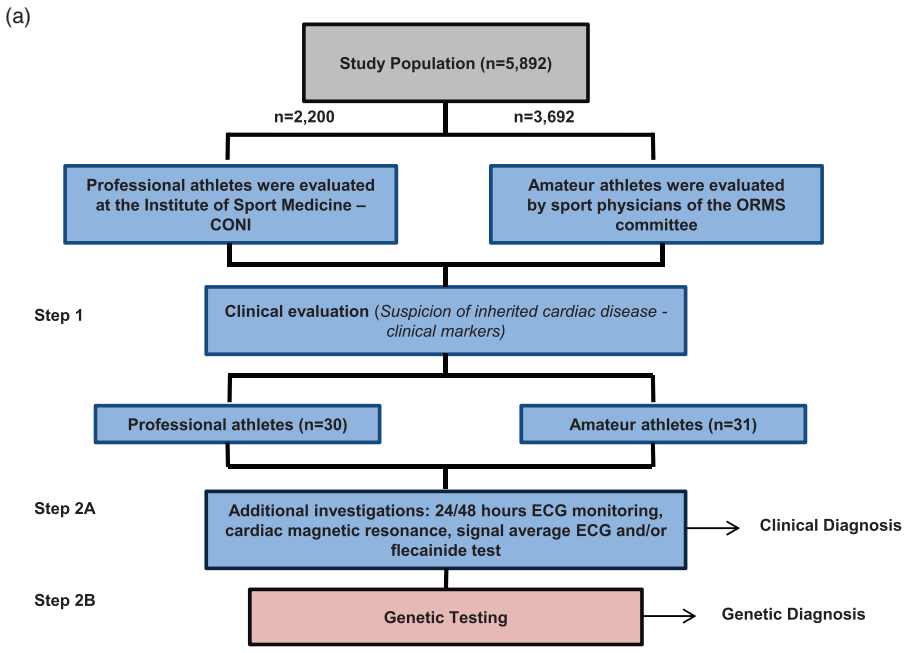
The subjects with one or more clinical markers were selected for further clinical evaluation, including, when appropriate, 24-hour ECG monitoring, cardiac magnetic resonance (CMR), signal average ECG and/or flecainide test.

Genetic testing: methods and techniques (*Figure 1* Panel (a): Step 2B)

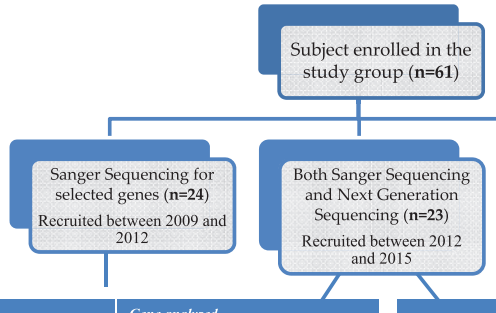
All the athletes selected performed genetic testing after obtaining informed written consent according to procedure established by the local ethics committee and according to the tenets of the Helsinki Declaration. The genetic testing was performed over an extended time period, from 2009–2018. In subjects evaluated between 2009–2012 ($n = 24$), molecular genetic testing was performed using direct Sanger sequencing (SS), guided by the clinical phenotype and according to previously described protocols.[14–19] Next generation sequencing (NGS) was available from 2012; note that many samples analyzed before 2012, were no longer available for NGS analysis. Since that year, and until 2015, all subjects ($n = 23$) underwent direct SS and, furthermore, extended molecular genetic testing using an NGS panel containing 138 genes (known to be associated to CMPs or channelopathies).[20] Between 2015–2018, subjects evaluated ($n = 14$) were analyzed using only the NGS panel of 138 genes (*Figure 1* Panel (b)). All pathogenic and likely pathogenic variants identified by NGS were also validated for their presence by SS. Identified variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines for variant interpretation concerning their pathogenicity.[21] We believe that the re-classification of variants should be carried out approximately every 6 months. All the variants presented in this study were checked when studied and then the last check was made in January 2020. In case of a positive genetic analysis for pathogenic/likely pathogenic variants, family members were invited to join the cascade programme screening. The screening in relatives was restricted to the same type of indicated variants found in probands. All the relatives carrying a putative pathogenic/likely pathogenic variant also underwent a comprehensive clinical evaluation. All relatives signed the informed consent form.

Specific diagnosis of cardiac inherited diseases

The diagnosis of a cardiomyopathy (i.e. hypertrophic cardiomyopathy (HCM), arrhythmogenic cardiomyopathy (AC), dilated cardiomyopathy



(b)



Disease	*Gene analyzed*	
Hypertrophic cardiomyopathy (HCM)	8	*MYH7, MYBPC3, TNNI3, TPM1, TNNT2, MYL2, MYL3, ACTC1*
Long-QT Syndrome (LQTS)	5	*KCNQ1, SCN5A, KCNH2, KCNE1, KCNE2*
Brugada Syndrome (BrS)/Sick Sinus Syndrome (SSS)	1	*SCN5A*
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	5	*PKP2, DSP, DSG2, DSC2, JUP*
Dilated Cardiomyopathy (DCM)	1	*LMNA/C*
Catecholaminergic polymorphic ventricular tachycardia (CPVT)	2	*RYR2, CASQ2*

Genes coding for:	Number of genes in each group, all belonging to the panel of 138 genes
1) Structural cardiac fibers and cardiomyocites proteins	49
2) Electric cardiac-voltage dependent cell structures	40
3) Cardiac proteins putative for both type of the 2 above groups of diseases	29
4) Other genes at risk for Sudden Cardiac Death (SCD) including those at risk for syndromic and metabolic diseases	20
TOT.	**138**

Figure 1 Panel (a): Flow-chart of the study protocol and diagnostic work-up: Step 1 corresponds to the clinical evaluation to search for inherited cardiac diseases and includes: (i) anamnesis, (ii) physical examination, (iii) standard 12-lead electrocardiogram (ECG), (iv) exercise stress test and (v) echocardiography. Step 2A corresponds to additional clinical investigations, as indicated; Step 2B corresponds to the genetic testing (see text for details under Methods and Results sections and Panel (b) in this Figure). Panel (b): A total of 61 athletes were analysed. Sequencing methods varied according to different year, and sequencing method available. CONI: Institute of Sport Medicine and Science; ORMS: Osservatorio Regionale di Medicina dello Sport.

(DCM) and LVNC) or of a primary electrical disorder (i.e. long QT syndrome (LQTS), Brugada syndrome (BrS), sick sinus syndrome (SSS), catecholaminergic polymorphic ventricular tachycardia (CPVT)) was made according to widely implemented, previous recommendations.[22–26]

We used the term clinical diagnosis when the athletes obtained a definitive diagnosis of inherited cardiac disease based on clinical evaluation, while the term genetic diagnosis was used when genetic testing allowed a definite diagnosis in subjects who had not obtained a clear diagnosis only

based on clinical evaluation. This dichotomy was used to stress the role of genetic tests in making a definite diagnosis also when the clinical evaluation suggests a specific phenotype but has failed to obtain a clear diagnosis.

Statistical analysis

Statistical analyses were performed using SPSS (version 25.0, SPSS Inc., Chicago, Illinois, USA). Normally distributed continuous data are presented as mean ± standard deviation (SD) and were compared by *t*-test. Categorical variables were expressed as number (and/or percentage) and analyzed by the chi-square test. Values of $p < 0.05$ (two-tailed) were considered significant.

Results

Demographic and clinical characteristics

Out of 5892 consecutive participants (median age 20 years old; male (M)/female (F) 68%/32%; 2200 elite athletes: median age 22 years old, M/F = 60%/40%; 3692 amateur athletes: median age 19 years old, M/F = 72%/28%), 61 individuals (26.1±12.8 years, median age 21 years old; M/F 92%/8%) met the criteria for further clinical evaluation and genetic testing: 30 (49%) were elite and 31 (51%) were amateur athletes. Amateur athletes were younger compared with elite (20.9±10.1 vs 33.4±13.9, *p*-value = 0.001) while no sex differences were evidenced. The inclusion of the elite athletes in the study was mainly triggered by ECG abnormalities (83%), while in amateur athletes this was based on either ECG abnormalities (55%), echocardiographic abnormalities (35%) and/or family history of sudden death in a first-degree relative (29%) (*Table 1*). ECG abnormalities were more prevalent in elite athletes compared with amateur (83% vs 55%, *p*-value = 0.016).

Diagnostic yield of clinical evaluation

Among the 30 elite and the 31 amateur athletes, a definitive (clinical or genetic) diagnosis was obtained in 14 athletes (17% or 5/30 of elite athletes and 29% or 9/31 of amateur athletes) (*Figure 2*). Based on the clinical evaluation, one elite athlete (1/5, 20% of elite athletes with

Table 1 Demographic and clinical characteristics of the total cohort.

Clinical features	Elite athletes (*n* = 30)	Amateur athletes (*n* = 31)
Age at presentation, years (±SD)	33.4 (±13.9)	20.9 (±10.1)[a]
Male sex, *n* (%)	27 (90%)	29 (93.5%)
Family history of sudden death in a first-degree relative, *n* (%)	3 (10%)	9 (29%)
ECG abormalities, n (%)	25 (83.3%)	17 (54.8%)[a]
T wave inversion, *n* (%)	23 (77%)	14 (45.2%)[a]
Anterior leads, n (%)	7 (23.4%)	8 (25.8%)
Leads V1 to V3, *n* (%)	5 (16.7%)	5 (16.1%)
Leads V1 to V4, *n* (%)	2 (6.7%)	3 (9.7%)
Lateral leads, n (%)	10 (33.3%)	5 (16.2%)
Leads V3 to V6, *n* (%)	2 (6.7%)	1 (3.2%)
Leads V4 to V6, *n* (%)	2 (6.7%)	1 (3.2%)
Leads V5 and V6, *n* (%)	6 (20%)	3 (9.7%)
Lead D1 and AVL, *n* (%)	4 (13.3%)	2 (6.4%)
Inferior leads, n (%)	13 (43.3%)	4 (12.9%)[a]
Combined inferior-lateral leads, n (%)	7 (23.3%)	3 (9.7%)
Combined inferior leads + leads V5 and V6, *n* (%)	5 (16.7%)	1 (3.2%)
Combined inferior leads + leads D1 and AVL, *n* (%)	0 (0%)	0 (0%)
Combined inferior leads + lateral leads (D1, AVL, V5, V6), *n* (%)	2 (6.7%)	2 (6.4%)
Long QT, *n* (%)	6 (20%)	1 (3.2%)[a]
Pattern Brugada-like, *n* (%)	1 (3.3%)	1 (3.2%)
Echocardiography abnormalities, *n* (%)	8 (26.7%)	11 (35.5%)
Borderline left ventricle maximal wall thickness (13–15 mm), *n* (%)	6 (20%)	3 (9.7%)
Left ventricular cavity enlargement (>60 mm) and/or reduction of ejection fraction (<55, >45%), *n* (%)	1 (3.3%)	3 (9.7%)
Increased trabeculation of the left and/or right ventricle, *n* (%)	1 (3.3%)	5 (16.1%)
Non-sustained ventricular tachycardia during exercise stress test, *n* (%)	3 (10%)	6 (19.3%)

ECG: electrocardiogram.
[a]Indicates the only values where the *p*-value ≤0.05.

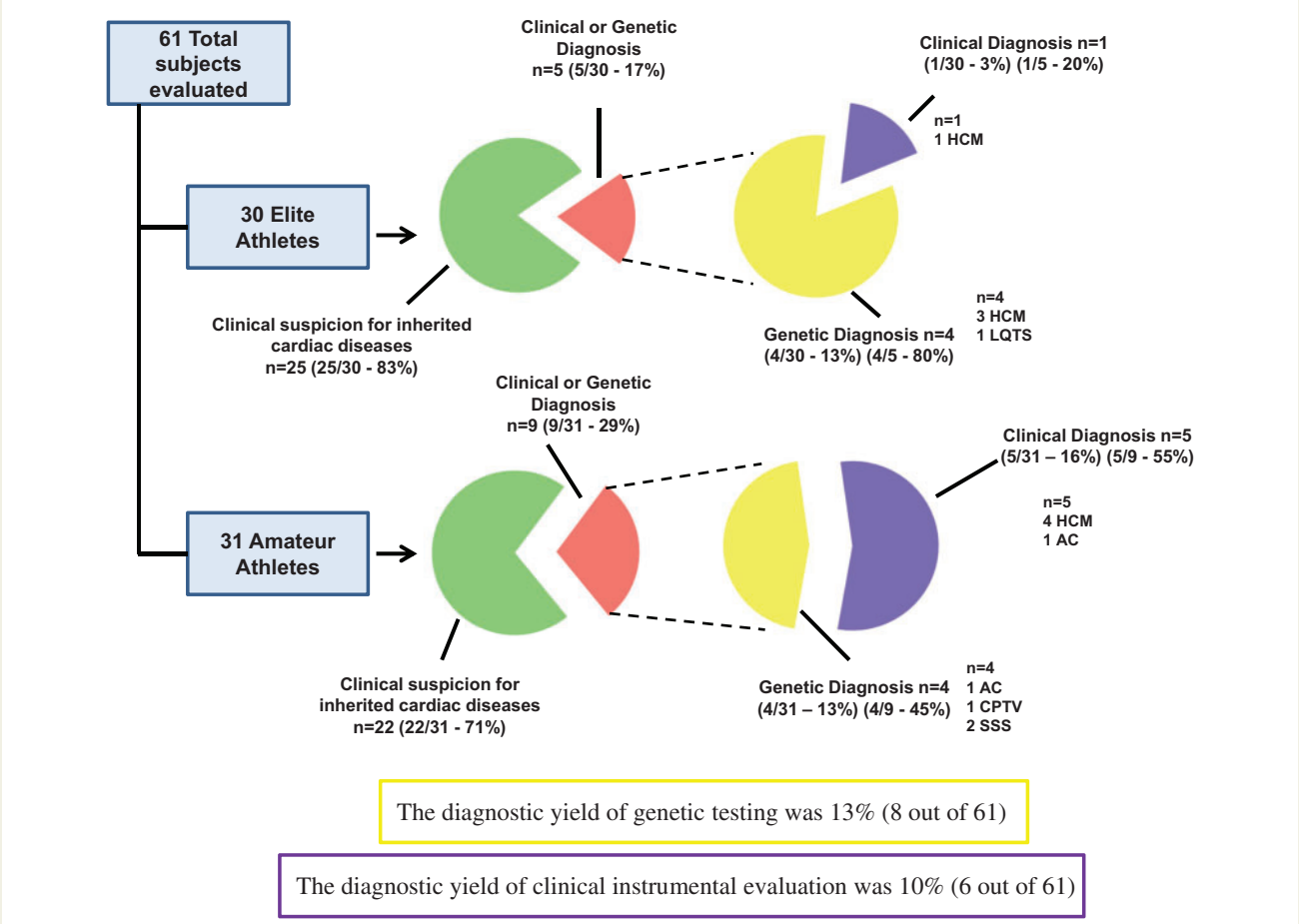


Figure 2 Diagnostic yield of clinical evaluation and of genetic testing in elite and amateur athletes. AC: arrhythmogenic cardiomyopathy; CPVT: catecholaminergic polymorphic ventricular tachycardia; HCM: hypertrophic cardiomyopathy; LQTS: long-QT syndrome; SSS: sick sinus syndrome.

definitive diagnosis) fulfilled the diagnostic criteria for HCM, and among the five amateur athletes (5/9, 55% of the amateur athletes with definitive diagnosis), four fulfilled the diagnostic criteria for HCM and one those of AC (see *Figure 2* for specifications and details).

Diagnostic yield of genetic testing

Using genetic testing, a total of 176 variants were identified (48 of them were novel variants) in 44 subjects (72%). In more detail, a total of 128 out of 176 variants were already annotated in the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), among these: five were classified as pathogenic/likely pathogenic; 116 were annotated as variants of uncertain significance (VUSs) and seven were benign or likely benign variants. The other 48 out of 176 variants were novel and not previously reported in any database (Supplemental Material *Tables S1–S4*). However, a pathogenic/likely pathogenic variant (sometimes called disease-causing mutation) was identified in only nine of these athletes (15%), while the remaining 35 were considered to have a benign or likely benign variant. All the VUSs should be continuously verified in the databases to have knowledge about the possibility of becoming definitively pathogenic or benign and, where possible, the physician should be informed.

Among the six subjects in whom cardiac disease was identified clinically (see *Figure 2* violet colour), none showed a disease-causing mutation among the genes investigated; however, one amateur athlete with clinical diagnosis of HCM and one with AC each carried a variant indicated in databases as VUS (*Table 2*).

Genetic testing allowed a definite diagnosis in eight (8/61, 13%, *Figure 2*, yellow colour). In particular, genetic diagnosis was made possible in four elite athletes (4/5, 80% of elite athletes with definitive diagnosis; three with HCM and one with LQTS), and in four amateur athletes (4/9, 45% of amateur athletes with definitive diagnosis; two with SSS, one with CPVT, one with AC) (*Figure 2*, yellow colour).

Overall, following a comprehensive clinical and genetic evaluation, 23% (14/61) of athletes had definitive diagnosis of inherited cardiac disease (17%: 5/30 in elite, and 29%: 9/31 in amateur athletes) (*Figure 2*, red colour). The diagnostic yield of genetic testing to obtain a final genetic diagnosis was 13% (8/61): 13% (4/30) in elite and 13% (4/31) in amateur athletes (*Figure 2*, yellow colour) compared with 10% of

Table 2 Clinical and genetic diagnosis of the athletes.

ID	Athlete	Age, years	Clinical markers	Gene mutated	Pathogenicity of the variant	Diagnosis	Diagnosis based on
Athletes with clinical diagnosis							
N27	Amateur	15	Family history of SD, TWI (I, L), borderline MWT	*ANK2*	VUS	HCM	CMR
N35	Amateur	30	TWI (I, L)	No	–	Apical HCM	CMR
N38	Amateur	14	Family history of SD, TWI (I, L), borderline MWT	No	–	HCM	CMR
N16	Amateur	16	TWI (I, L), borderline MWT	No	–	Apical HCM	CMR
N43	Elite	16	Borderline MWT	No	–	HCM	CMR
N18	Amateur	20	TWI (A)	*DSC2, DSG2 DSP*	Benign, benign,VUS	AC	Clinic features and CMR
Athletes with genetic diagnosis							
N31	Amateur	23	NSVT during exercise stress test	*RYR2*	Likely pathogenic	CPVT	Genetic testing
N7	Amateur	14	Bradycardia, SAB	*SCN5A*	Likely pathogenic	SSS	Genetic testing
N13	Elite	44	TWI (A), long QT	*KCNQ1*	Likely pathogenic	LQTS	Genetic testing
N15	Elite	36	TWI (L), borderline MWT, long QT	*MYBPC3*	Likely pathogenic	HCM	Genetic testing
N17	Elite	48	TWI (A), borderline MWT, NSVT during exercise stress test	*MYBPC3*	Pathogenic	HCM	Genetic testing
N49	Elite	16	TWI (I), borderline MWT	*MYH7*	Likely pathogenic	HCM	Genetic testing
N61	Amateur	9	Bradycardia, SAB	*SCN5A*	Likely pathogenic	SSS	Genetic testing
N26	Amateur	11	Family history of SD, TWI (A), increased trabeculation of the right ventricle, NSVT during exercise stress test	*PKP2, DES*	Pathogenic, likely pathogenic	AC	Genetic testing

A: anterior leads; AC: arrhythmogenic cardiomyopathy; CMR: cardiac magnetic resonance; CPVT: catecholaminergic polymorphic ventricular tachycardia; HCM: hypertrophic cardiomyopathy; I: inferior leads; L: lateral leads; LQTS: long-QT syndrome; MWT: maximal wall thickness; NSVT: non-sustained ventricular tachycardia; SAB: sino-atrial block; SD: sudden death; SSS: sick sinus syndrome; TWI: T-wave inversion; VUS: variant of uncertain significance.

Table 3 Prevalence of genetic diagnosis in athletes according to the number of clinical markers identified during the screening evaluation for the pre-participation programme.

	Athletes with 1 clinical marker ($n = 37$)	Athletes with 2 clinical markers ($n = 14$)	Athletes with 3 or more clinical markers ($n = 4$)	*p*-Value
Genetic diagnosis, *n (%)*	3 (8.1%)	2 (14.2%)	3 (75%)	0.004
In elite athletes, *n (%)*	0 (0%)	2 (14.2%)	2 (50%)	0.001
In amateur athletes, *n (%)*	3 (8.1%)	0 (0%)	1 (25%)	0.110

the clinical evaluation (6/61): 3% (1/30) in elite and 16% (5/31) in amateur athletes (*Figure 2*, violet colour).

We observed that genetic investigation confirmed the presence of cardiac disease in 75% of those that showed ≥3 clinical markers at clinical evaluation, compared with 14.2% and 8.1% of those with two and one clinical markers, respectively (*p*-value = 0.004). This difference was particularly evident in elite athletes (*Table 3*).

Screening in family members

Among the none athletes that showed a disease-causing mutation, genetic cascade screening (i.e. clinical and genetic study in relatives) was performed in three (33%).

An elite athlete (N13), showing prolonged QTc (480 ms) and TWI in the anterior lead, carried a likely pathogenic variant in *KCNQ1* gene (c.727C>T, p.Arg243Cys – rs199472713) and was diagnosed with

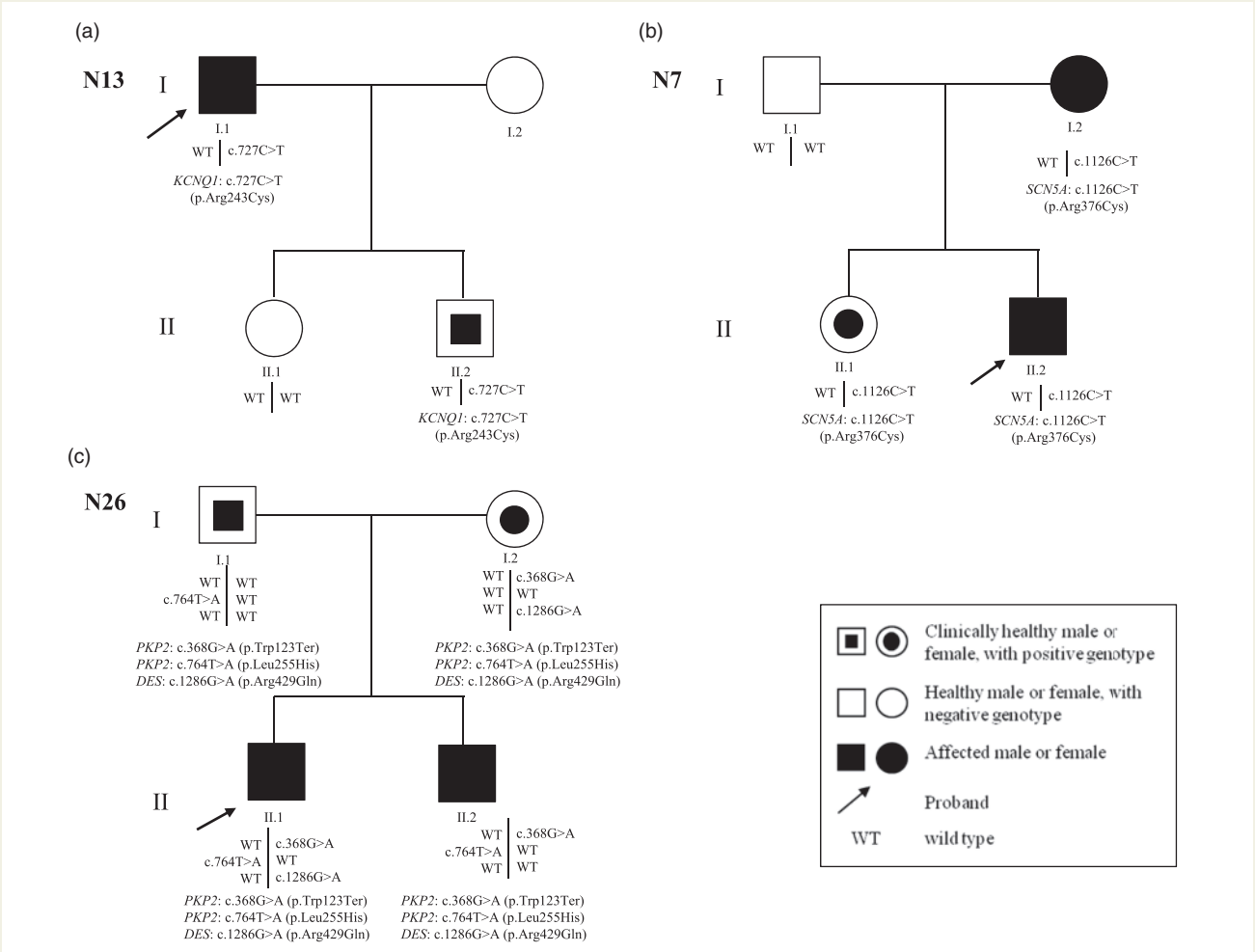


Figure 3 Families pedigree discussed in the text (see Results section on 'Screening in family members').

LQTS. Genetic testing was performed in the two sons, both practicing amateur physical activity, and one of them showed the *KCNQ1* variant. Both proband and son started beta-blockers and, after a careful discussion about the risk/benefit balance of continuing competitive sport, they decided prudently to refrain from competitive sport activity (*Figure 3(a)*).

An amateur athlete (N7), showing phases of sinus atrial block with several asymptomatic pauses at the 24-hour ECG monitoring and family history of pacemaker implantation, carried a likely pathogenic variant in *SCN5A* gene (c.1126C>T, p.Arg376Cys – rs199473100) and was diagnosed with SSS. The same variant was found in the mother (atrial fibrillation with II-degree atrioventricular block that required pacemaker implantation) and in the sister (previous syncope; numerous supraventricular ectopic beats at ECG Holter) of proband, both affected by SSS (*Figure 3(a)*).[15]

An amateur athlete (N26), showing TWI in anterior leads, increased trabeculation of the right ventricle and non-sustained ventricular tachycardia (NSVT) during exercise stress test, was a compound heterozygous, carrying a pathogenic variant (c.368G>A, p.Trp123Ter) and a VUS (c.764T>A, p.Leu255His) in the *PKP2* gene and a likely pathogenic variant in *DES* gene (c.1286G>A, p.Arg429Gln) (*Figure 3(c)*).[27] He fulfilled the 2010 revised Task Force Criteria[25] and was diagnosed with AC. He underwent implantable cardioverter defibrillator implantation and experienced two appropriate shocks during follow-up (2 and 4 years after implantation). Genetic testing was performed in the parents and in the brother. After a clinical evaluation, the brother fulfilled the 2010 revised Task Force Criteria and was diagnosed with AC, while the parents showed no clear ECG or echocardiographic abnormalities. The brother was practising soccer at competitive level but then quit competitive sport and started sotalol.

Discussion

The importance of pre-participation screening and risk stratification of athletes has been underlined in recent documents by the European Association of Preventive Cardiology (EAPC), which

indicate very carefully the type of sport and the length of time these sports could be carried out in athletes with coronary artery diseases or arterial hypertension.[28,29]

On the other hand, the natural history of CMPs and channelopathies is characterized by a concealed phase, where subclinical electrical and/or morphological abnormalities may precede, even for a long time period, overt clinical disease, making it challenging to predict the actual risk of life-threatening events, in particular those associated with exercise and sport participation.

Diagnosis of these conditions in athletes is even more difficult because of the structural, functional and electrical remodelling of the 'athlete's heart', characterized by ECG changes, increased left ventricular mass, cavity dimensions and wall thickness,[30] resulting in some cases in a 'grey zone' in which a definite diagnosis is difficult.

Thanks to the pre-participation screening programme in athletic participants and the wide implementation of the ECG,[10,12,28,29,31–34] individuals with suspicion of cardiac disease are generally identified. In a study by Calò et al.,[33] all athletes requiring sport disqualification were identified by electrocardiogram ($n = 36$). In a large cohort study concerning 12,550 athletes, Pelliccia et al.[34] identified 81 with TWI who had no apparent structural cardiac disease. After a nine-year follow up, five (6%) developed evidence for a cardiomyopathy, including a 24-year old athlete who died suddenly from clinically undetected AC. This observation supports the role of abnormal ECG and, in particular, TWI to predict the future incidence of CMPs.

A recent study performed on a cohort of 100 asymptomatic white and black athletes that showed TWI at ECG,[35] assessed the additional role of genetic testing. In this selected cohort, following a complete clinical investigation, only 21/100 athletes were definitely diagnosed with cardiac diseases. The positive yield of genetic testing was 10% and contributed to an additional diagnosis in only 2.5%. The authors comment that genetic testing should be performed in athletes only after a detailed clinical and family assessment, as negative genetic testing cannot exclude a pathological condition and the frequent occurrence of VUS is further expanding the difficulties of correct interpretation.

Diagnostic testing in the athlete populations

Our investigation aimed to define the value of gene testing, after a detailed clinical assessment, in a setting of each one of two different athlete populations. Overall, following a comprehensive clinical and genetic evaluation, 23% (*Figure 2*, red colour) of athletes had a definitive diagnosis: 17% in elite athletes and 29% in amateur athletes (*Figure 2*, red colour). The diagnostic yield of genetic testing for a disease-causing mutation was in our experience 13% (8/61): 13% (4/30) in elite and 13% (4/31) in amateur athletes (*Figure 2*, yellow colour) compared with 10% of comprehensive clinical evaluation (6/61): 3% (1/30) in elite and 16% (5/31) in amateur athletes (*Figure 2*, violet colour).

Therefore, our data support the concept that genetic testing, in the setting of evaluation of athletes suspected of carrying a genetic cardiac condition, may provide an additional diagnostic help in a subset of 13% of them (*Figure 2*, yellow colour). Among the five elite athletes with definitive diagnosis of cardiac disease, only one (1/5; 20% – *Figure 2*, violet colour) was identified by clinical evaluation, while the genetic testing was able to identify the underlying disease in the remaining four (4/5; 80% – *Figure 2*, yellow colour). On the contrary, in amateur athletes clinical diagnosis was efficient in 5/9 subjects (55%; *Figure 2*, violet colour), while a disease-causing mutation was detected in the other four (45%; *Figure 2*, yellow colour). Therefore, our experience suggests that genetic testing can be useful both in elite and amateur athletes.

None of the athletes with clinical diagnosis showed a definite pathogenic variant in the investigated genes, while only two carried a VUS. In our cohort, the diagnostic yield of genetic testing is therefore lower compared with that reported previously.[35] Differences between the two studies can be related to the population examined, the selection criteria and genetic screening (methods and number of genes investigated by NGS). Moreover, in the six athletes with clinical diagnosis, two showed the apical variant of HCM, a condition known to have a low genetic yield.[36,37]

On the other hand, in athletes showing clinical features suspicious for a cardiac disease but without a definitive clinical diagnosis, genetic testing identified an additional eight (8/61; 13%) athletes. Athletes with a higher number of clinical markers (>3) at the screening evaluation showed a higher prevalence of genetic diagnosis (75%). Therefore, our experience suggests that presence of multiple clinical markers for inherited cardiac disease may represent a reasonable indication for gene testing and conveys higher probability for identification of pathogenetic mutations.

In our cohort, three subjects ≤16 years old obtained a genetic diagnosis. Recently, Lafreniere-Roula et al.[38] showed that major cardiac events, defined as death, SCD or need for major cardiac intervention, occurred in 17/524 (0.77%) of patients with HCM < 18 years-old and seven of them experienced the event before 10 years. These latter findings further highlight the importance of an early and definitive diagnosis of an underlying inherited heart disease, especially in subjects who want to approach competitive and physically demanding sport activities.

Diagnostic testing in family members

Since most of inherited cardiac diseases (CMPs and channelopathies) show a silent clinical course that might lead to SCD in theabsence of symptoms and ventricular dysfunction,[39] an early diagnosis is required to prevent tragic events. At the present time, no data on the efficacy of clinical screening in relatives of athletes are available and genetic screening in these subjects is difficult due to cost, being time-consuming and low accuracy. We evaluated for the first time the role of cascade programme screening in relatives of athletes with an inherited cardiac disease and a disease-causing mutation. Thus, we identified six relatives with an inherited cardiac disease and, in particular, two young subjects practicing physical activity, one with LQTS and one with AC.

Clinical relevance

Out of 5892 elite and amateur consecutive athletes, 61 (1%) had relevant clinical and/or instrumental abnormalities requiring a comprehensive clinical and genetic evaluation. In our study, genetic testing helped to reach a final diagnosis in eight athletes (13%; 0.13% of the overall population). Our findings support the selective indication of genetic testing for diagnostic purposes, in both elite and amateur athletes, when a comprehensive clinical screening is suspicious (i.e. high

pre-test probability, for the presence of known clinical markers of underlying disease) but fails to identify a cardiac disease with certainty. The decision to proceed to genetic testing should be clearly outlined to the athlete during pre-test genetic counselling, after a case by case discussion, better when done by a multidisciplinary team of experts.

Positive genetic testing allows for the start of molecular cascade screening in order to verify the segregation of the mutation inside the family and potentially identify asymptomatic affected relatives.[20,40] On the other hand, the pitfalls of NGS are related to costs and to clinical interpretation, in particular to the elevated numbers of VUS identified with this method. However, when clinical features suggest an underlying disease, the presence of a VUS may deserve reinterpretation regarding its pathogenicity during follow-up according to accumulating literature knowledge.

Study limitations

Firstly, the small cohort size of the athletes that underwent a comprehensive clinical and genetic evaluation has limited our capability to offer valid explanation to specific findings (i.e. yield of genetic testing in elite vs amateur athletes). Secondly, the subsequent clinical evaluation was performed according to the clinical suspicion of the selected athletes. Thus, CMR and other investigations (i.e. flecainide test) were not performed in all of the subjects (e.g. CMR was performed in 10 elite and 12 amateur athletes). Since the long duration of the study period and the progress in genetic diagnosis of inherited cardiac disease, the genetic testing strategy was modified during the years. Similarly to previous investigation,[35] we identified an elevated number of VUSs in our genotyped cohort of athletes, which represents an important limitation (i.e. confounding factor) and challenge for a broad indication of genetic testing in the evaluation on apparently healthy individuals. VUSs were interpreted in the context of familiar cascade screening in a limited number of athletes. Comprehensive family cascade screening and a long-term follow-up are warranted to elucidate the clinical role of single and/or multiple VUSs in athletes. Finally, our study is essentially a retrospective study, which obviously contains suggestions for future studies.

Conclusion

A comprehensive clinical and genetic evaluation, based on the presence of clinical markers of inherited disease, was able to identify a cardiac disease in about a quarter of athletes investigated. Genetic testing contributed to diagnosis in 13% of athletes, while it failed to confirm the clinical diagnosis in 10% (six out of 61). Thus, the added value of genetic testing should be considered, as relevant, when clinical features suggest an underlying primary alteration, and a comprehensive cardiovascular evaluation fails to support a definitive diagnosis.

Supplemental material

Supplementary material is available at *European Journal of Preventive Cardiology* online.

Acknowledgments

This article is dedicated to the memory of Francesco M Limongelli, coordinator of the 'Osservatorio Epidemiologico della Medicina dello Sport, Campania Region', with whom we started to approach this work. He was one of the first who thought of performing this study. The conception and the design of the study was planned by ORMS and the UOC of Sports Medicine – University of Campania 'Luigi Vanvitelli' and CONI. ORMS is a regional organ of health surveillance, responsible for education of sport cardiologists and physicians, and surveillance of cardiovascular diseases which contraindicate sport eligibility, with the principal aim to build a network of sport medicine/sport cardiology referral centres in the Campania Region. CONI is a member of the International Olympic Committee, responsible for clinical evaluation and extensive support to Italian elite athletes during national and international competitions.

The authors wish to acknowledge Francesco M Limongelli, Paola Brancaccio, Antonello D'Andrea, Raffaele Canonico, Marcellino Monda and Raffaele Calabrò from the University of Campania 'Luigi Vanvitelli'; Michele Marzullo from the University of Naples 'Federico II'; Gianpaolo Palumbo from the Hospital Moscati (Avellino); Francesco Salvi from the Hospital A Cardarelli (Naples); Giuseppe Barbati, Claudio Briganti, Fioravante Capone, Franco Cerullo, Antonio Chiacchio, Ugo Dell'Unto, Gaetano Di Franco, Vincenzo Granata, Cosimo Lauro, Mario La Vecchia, Andrea Salati, Vittorio Savino, Antonietta Siciliano and Maria R Viaggiano from the ASL Campania Region, for their precious contribution.

Author contribution

GL, AP, GF and FS contributed to the conception and design of the work. MN, VD, MVE, EM, FDP, MP and PB contributed to the acquisition, analysis, or interpretation of data for the work. CM, MC, ADp, AD, EB, FDM, PWP, LDC, SR have contributed to clinical or genetic studies. GL, MN, VD, AP and FS drafted the manuscript. All the authors critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Ministry of Health Program (PRIN grant 2008: Risk stratification of sudden cardiac death in subjects practicing competitive sports: identification of clinical, instrumental, bio-humoral and genetic predictive markers. prot. 2008C5XFSS); and by grants PON03PE_00060_2 and PON03PE_00060_7 (Campania – Bioscience) from the Italian Ministry of University and Research (to FS), and CIRO and SATIN projects (to FS) from Regional (Campania Region, Italy) funds. The funding bodies had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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