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Diagnostic Yield of Genetic Testing in Young Athletes with T-wave Inversion

Running Title: *Sheikh et al.; Gene Testing Athletes with T-wave Inversion*

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Abstract

Background—T-wave inversion (TWI) is common in patients with cardiomyopathy. However, up to 25% of athletes of African/Afro-Caribbean descent (black athletes) and 5% of white athletes also have TWI of unclear clinical significance despite comprehensive clinical evaluation and long-term follow-up. The aim of this study was to determine the diagnostic yield from genetic testing, beyond clinical evaluation, when investigating athletes with TWI.

Methods—We investigated 50 consecutive asymptomatic black and 50 white athletes aged 14-35-years-old with TWI and a normal echocardiogram who were referred to a UK tertiary center for cardiomyopathy and sports cardiology. Subjects underwent exercise testing, 24-hour ECG, signal-averaged ECG, cardiac magnetic resonance imaging, and a blood-based analysis of a comprehensive 311 gene panel for cardiomyopathies including hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, left ventricular non-compaction, and ion channel disorders such as long QT syndrome and Brugada syndrome.

Results—In total, 21 athletes (21%) were diagnosed with cardiac disease on the basis of comprehensive clinical investigations. Of these, 8 (38.1%) were gene positive (MYPBC3, MYH7, GLA, and ACTC1 genes) and 13 (61.9%) were gene negative. Of the remaining 79 athletes (79%), 2 (2.5%) were gene positive (TTR and SCN5A genes) in the absence of a clinical phenotype. The prevalence of newly diagnosed cardiomyopathy was higher in white athletes compared with black athletes (30.0% *vs.* 12%, *P=0.*027). Hypertrophic cardiomyopathy accounted for 90.5% of all clinical diagnoses. All black athletes and 93.3% of white athletes with a clinical diagnosis of cardiomyopathy or a genetic mutation capable of causing cardiomyopathy exhibited lateral TWI as opposed to isolated anterior or inferior TWI; the genetic yield of diagnoses from lateral TWI was 14.0%.

Conclusions—Up to 10% of athletes with TWI revealed mutations capable of causing cardiac disease. Despite the substantial cost, the positive diagnostic yield from genetic testing was one-half of that from clinical evaluation (10% vs. 21%) and contributed to additional diagnoses in only 2.5% of athletes with TWI in the absence of a clear clinical phenotype, making it of negligible use in routine clinical practice.

Key Words: genetic testing; screening; exercise; cardiomyopathy; ethnicity; T-wave inversion

Clinical Perspective

What is new?

- The yield of testing for pathogenic disease-causing genetic mutations in athletes exhibiting T-wave inversion is low (10%), despite using a comprehensive genetic panel.
- Gene testing in selected athletes referred to a tertiary referral center contributes to additional diagnoses beyond comprehensive clinical evaluation in only 2.5% of athletes with T-wave inversion without a clear clinical phenotype.
- The overall diagnostic yield from genetic testing in athletes with T-wave inversion is one-half that of comprehensive clinical evaluation (21% vs. 10%).
- The prevalence of cardiomyopathy in white athletes with T-wave inversion is higher compared to black athletes with T-wave inversion (20% vs. 12%).

What are the clinical implications?

- Genetic testing is rarely useful in the routine investigation of athletes with T-wave inversion who have undergone comprehensive clinical evaluation.
- In contrast, comprehensive clinical evaluation can identify a clinical diagnosis in over one-fifth of athletes with T-wave inversion on initial evaluation, with a higher probability of a clinical diagnosis in white compared to black athletes (30.0% *vs.* 12%).
- This study demonstrates that lateral T-wave inversion in an athletic population referred to a specialized athletic center is associated with cardiac disease in 20% of athletes.

Introduction

A small proportion of apparently healthy adult athletes exhibit marked repolarization abnormalities in the absence of detectable structural heart disease. Of these repolarization patterns, most interest has focused on T-wave inversion (TWI),[1–9] which is a common manifestation of several inherited cardiomyopathies and some ion channel diseases implicated in sudden cardiac death (SCD).

Although rare in adult white athletes,[10–14] TWI is observed in up to a quarter of athletes of African/Afro-Caribbean descent (black athletes) and most commonly confined to the anterior leads (V1-V4).[3,5] Observational data suggest that this specific repolarization pattern represents a benign, ethnic manifestation of the athlete's heart.[3,5,15] In contrast, the clinical significance of inferior and lateral TWI is less certain. Recent studies using cardiac magnetic resonance imaging (CMR)[4] and longitudinal follow-up[1,3,6] in athletes with inferior and/or lateral TWI have reported an association with these repolarization changes and cardiomyopathy, risk of sudden cardiac arrest, or the subsequent development of cardiomyopathy over time. The majority of athletes in these studies, however, have failed to reveal any demonstrable cardiac pathology. None of these studies have incorporated genetic testing to help determine whether TWI represented an early, subtle or concealed manifestation of cardiomyopathy or ion channel disease. Genetic testing was previously expensive and impractical, but next generation sequencing[16] has now offered major advances in the molecular genetics of inherited cardiac diseases and the development of relatively inexpensive genetic panels to investigate a broad spectrum of recognized mutations in genes implicated in cardiomyopathy and ion channel disorders.

The aim of this study was to examine the additional diagnostic yield for these genetic diseases beyond standard comprehensive clinical evaluation in highly trained black and white athletes with TWI using a wide genetic panel.

Methods

Setting

The charitable organization Cardiac Risk in the Young (CRY) provides a national pre-participation screening service for several elite national sporting bodies and regional teams including Aviva Premiership Rugby, British Rowing, English cricket, English Institute of Sport, Lawn Tennis Association, Rugby Football Union and several Premiership soccer clubs. Athletes with abnormal findings undergo further investigations, of which over 80% are conducted in the Sports Cardiology Unit at St. George's Hospital. The Principle Investigator (SS) also receives referrals directly to our sports cardiology unit from other elite professional sporting bodies and other medical institutions throughout the UK. Since 2010, we have also received referrals from Qatar Orthopedic and Sports Medicine Hospital (Doha, Qatar) which has a highly active cardiovascular screening program that serves the entire Persian Gulf region. All elite athletes receive a 12-lead electrocardiogram (ECG) and two-dimensional echocardiography as part of these screening programs. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study Design and Recruitment of Athletes

Between April 2012 and April 2014 a total of 2,039 athletes (260 black and 1,779 white) were evaluated. Fifty consecutive black and 50 consecutive white athletes aged 14-35 years with TWI ≥-0.1 mV in ≥2 contiguous leads (excluding aVR, V1 and lead III in isolation) were

prospectively recruited into the study. Twenty-seven athletes were recruited through pre-participation cardiac evaluation performed by CRY in the UK and 14 through pre-participation cardiac evaluation performed by Qatar Orthopedic and Sports Medicine Hospital in Doha, Qatar. The remainder (n=59) were recruited after direct referral to St. George's Hospital for evaluation of TWI on clinical grounds from another institution or a professional sporting body in the UK. Exclusion criteria for recruitment into the study included: (i) cardiac symptoms or a family history of cardiomyopathy or ion channel disease; (ii) a previous cardiac history; (iii) a past medical history of hypertension; (iv) use of anabolic steroids or performance enhancing drugs; (v) a structurally abnormal heart or wall motion abnormalities on echocardiography.

Ethical Approval and Informed Consent

Ethical approval was obtained from the local ethics committee in accordance with the Declaration of Helsinki.[17] Written consent for enrolment of participants was obtained from individuals aged ≥16 years and from a parent or guardian for those aged <16 years. Genetic testing is not routinely performed for the evaluation of athletes in the UK and current European guidelines recommend disqualification of genotype-positive, phenotype-negative individuals from competitive sports.[13] Therefore, the results of genetic testing were not given to the athlete or sports organization, unless the athlete specifically requested the result after appropriate counselling and informed consideration of a positive test on their career.

Clinical Investigations in Athletes

All athletes were also investigated with 12-lead ECG, signal-averaged ECG, cardiopulmonary exercise stress testing, 24-hour Holter monitoring, and CMR.

12-Lead Electrocardiography

Standard 12-lead electrocardiography (ECG) was performed using a MAC 5000 or MAC 5500 digital resting ECG recorder (GE Medical Systems, Milwaukee, USA) and analyzed as previously described.[18] T-wave inversion of ≥-0.1 mV in two or more contiguous leads was considered significant, other than in leads V1, aVR and III. The distribution of TWI was classified into three groups: (i) TWI confined to the anterior leads (V1–V4); (ii) TWI involving the inferior leads (II, III, aVF), with or without anterior TWI; (iii) TWI involving the lateral leads (I, aVL, V5, V6), regardless of TWI in other leads.

Signal-Averaged ECG

Signal-averaged ECG was acquired according to accepted methodology[19] using a MAC 5000 or MAC 5500 digital resting ECG recorder.

Cardiopulmonary Exercise Stress Testing

Cardiopulmonary exercise testing was performed in an upright position with a COSMED E100w cycle ergometer (Rome, Italy) as previously described[20] using an incremental ramp protocol of 30 watts per minute in a quiet air conditioned room with an average temperature of 21°C and full resuscitation facilities. Subjects were encouraged to exercise to the point of exhaustion. Breath-by-breath gas exchange analysis was performed using a dedicated COSMED Quark CPEX metabolic cart (Rome, Italy). Blood pressure was measured pre-test and then at 3-minute intervals using an automated cuff. Signals from a 12-lead ECG were displayed continuously and recorded at 2-minute intervals using a COSMED Quark C12x electrocardiographic recorder (Rome, Italy).

24-Hour Ambulatory ECG Monitoring

Ambulatory 24-hour ECG monitoring (Lifecard CF Holters, Spacelabs Healthcare, USA) was performed specifically to detect supraventricular and/or ventricular arrhythmias.[21] Non-sustained ventricular tachycardia (NSVT) was defined as three or more consecutive ventricular beats at a rate of >120 beats per minute with a duration of <30 seconds.

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging was performed using methods previously described and analyzed with semi-automated software.[7] All volumes and masses were indexed for age and body surface area according to the DuBois and DuBois formula.[22] Late gadolinium images were acquired after intravenous gadolinium-DTPA administration.[7] The presence or absence of late gadolinium enhancement (LGE) was recorded as a binary variable.

Candidate Disease and Gene Selection for Genetic Testing

Genetic testing was performed for priority genes responsible for six inherited cardiac conditions most commonly associated with TWI, namely hypertrophic cardiomyopathy (HCM),[23] arrhythmogenic right ventricular cardiomyopathy (ARVC),[24] dilated cardiomyopathy (DCM),[25] left ventricular non-compaction (LVNC),[26] long QT syndrome (LQTS)[27,28] and Brugada syndrome (BrS).[29] To ensure that comprehensive genetic evaluation was performed, a large number of potential candidate genes were also tested after a systematic literature review. Overall, a total of 104 genes for HCM (including phenocopies), 21 genes for ARVC, 96 genes for DCM, 37 genes for LVNC, 28 genes for LQTS and 25 genes for BrS were tested (Supplemental Table 1).

Definitions of Clinical Diagnoses

The diagnosis of a cardiomyopathy (HCM, ARVC, DCM and LVNC) or an ion channel disorder (LQTS and BrS) was made in accordance with internationally recognized guidelines.[23,29–31] In particular, the diagnosis of HCM was based on LVH ≥15mm in any myocardial segment, as assessed on CMR, in the absence of another cardiac disorder or systemic condition capable of producing the same magnitude of LVH.[32,33] In cases of mild LVH, HCM was diagnosed in the context of a combination of features, including: (1) non-concentric patterns of LVH; (2) LGE on CMR; (3) an established or likely pathogenic gene mutation; (4) the presence of broader phenotypic features of the condition such as NSVT or a blunted blood pressure response to exercise; (5) in the case of apical HCM, the appearance of relative apical hypertrophy and cavity obliteration out of keeping with athletic training in combination with typical lateral deep TWI.[34,3536] Phenocopies of HCM were diagnosed on the basis of the above criteria and confirmation by a relevant pathogenic genetic test. The diagnosis of LVNC was based on increased trabeculation of the LV myocardium fulfilling recognized CMR criteria[37] and the concurrent presence of wall thinning and/or LV systolic dysfunction.

Genetic Testing in Athletes

Sample Preparation, Genetic sequencing and Analysis

Genomic DNA was extracted from 1ml of peripheral blood samples. Sequencing of all coding exons and intronic flanking regions was performed through massive parallel sequencing technology. Targeted enrichment of the genes associated with each condition was performed. Sample preparation was conducted using the SureSelect Target Enrichment Kit (Agilent, California, USA) for Illumina (California, USA) paired-end multiplexed sequencing method, following the manufacturer's instructions. Regions of interest were captured using a custom

probe library. Sequencing was performed on an Illumina HiSeq 1500 with 2x100 base read length following Illumina protocols. Low coverage regions (defined as every base with depth of coverage <15x) in those genes related with the diseases in question were re-sequenced through the Sanger method. Bioinformatic analysis was performed by means of a custom pipeline that included Novoalign (Novocraft, Selangor, Malaysia), Samtools (Genome Research Limited, Wellcome Trust Sanger Institute), Genome Analysis Toolkit (Broad Institute, Masachusettes, USA) and bcftools (Genome Research Limited, Hinxton, UK) for variant calling and genotyping, and Annovar for variant annotation.

Determination of Variant Frequency

Information regarding the frequency of identified genetic variants in different populations was analysed from: (i) Exome Variant Server, NHLBI GO Exome Sequencing Project, Seattle, WA (http://evs.gs.washington.edu/EVS/); (ii) The 1000 Genomes Project (www.1000genomes.org/); (iii) The Database of Single Nucleotide Polymorphisms (dbSNP), National Center for Biotechnology Information, US National Library of Medicine, Bethesda, MD (https://www.ncbi.nlm.nih.gov/snp/); (iv) the Human Gene Mutation Database[38] (www.hgmd.cf.ac.uk); (v) ClinVar;[39] (vi) The Exome Aggregation Consortium, Cambridge, MA and the Genome Aggregation Database (gnomAD) (http://exac.broadinstitute.org, and http://gnomad.broadinstitute.org).

Definitions and Pathogenicity of Identified Variants

Identified variants were classified as mutations if absent in control populations, rare variants if present in <1% of control populations, and polymorphisms if present in ≥1% of control populations. The pathogenicity of the identified variants was classified according to current recommendations from the American College of Medical Genetics and Genomics.[40] In summary,

variants were considered potentially pathogenic if they were: (i) absent or rare in healthy controls; (ii) previously associated with disease development; and (iii) functionally relevant variants in genes previously associated with the identified phenotype (for example in-frame or frameshift-causing insertions or deletions, variants affecting splice sites, or missense variants likely to be pathogenic as identified by software models such as SIFT,[41] PolyPhen,[42] and MutationTaster2[43]). A detailed description of the methods used to determine pathogenicity of identified variants is summarized in Supplemental Table 2.

Statistical Analysis

The Kolmogorov-Smirnov test was used to evaluate whether each continuous parameter followed a Gaussian distribution. Values are expressed as absolute numbers and percentages for categorical data and mean ± standard deviation for continuous data. Comparisons were performed using the χ^2 test or Fisher exact test for categorical variables, unpaired *t*-test for normally distributed continuous variables, and Mann-Whitney *U* test for non-normally distributed continuous variables. A two-tailed p-value of <0.05 was considered significant throughout. All analyses were performed using SPSS software, version 20.0 (IBM Analytics).

Results

Athlete Demographics

There were no differences between black and white athletes with respect to age, sex, body surface area, resting blood pressure and hours of exercise per week (Table 1). All athletes had a resting blood pressure of <140/90 mm Hg. The majority of athletes (90% or more) in both groups were men.

Electrocardiographic Characteristics

All athletes were in sinus rhythm. T-wave inversion extending into the lateral leads was the most common pattern of TWI in both black and white athletes, followed by TWI confined to the anterior leads V1-V4 (Table 2). Of note, there was a relatively high prevalence of pathological Q-waves and ST-segment depression in both groups; ST-depression was observed in at least one-fifth (Table 2). Most athletes revealed one abnormal parameter on signal-averaged ECG and 7 black (14%) and 6 white (12%) athletes revealed 2 or more abnormal parameters.

Structural Characteristics on CMR

Consistent with previous studies, white athletes had greater left ventricular (LV) and right ventricular volumes on CMR compared with black athletes, whereas black athletes had a greater mean maximal LV wall thickness (Table 2). Five black athletes (10%) and five white athletes (10%) revealed LGE.

Cardiopulmonary Exercise Testing and Holter Monitoring

Three athletes with HCM demonstrated an abnormal blood pressure response to exercise. Two athletes with HCM had a short run of NSVT. One athlete without a diagnosis of cardiomyopathy demonstrated transient asymptomatic atrioventricular re-entrant tachycardia.

Diagnoses Based on Clinical Investigations

In total, 21 athletes (21%) were diagnosed with cardiac disease on the basis of comprehensive clinical investigation. Hypertrophic cardiomyopathy was the most common diagnosis and affected 19 of the 21 athletes (90.5%). The diagnosis of HCM was based predominately on the degree and segmental nature of LVH on CMR and LGE. One athlete (4.8%) with extra-cardiac features was diagnosed with Fabry disease (later confirmed on genetic testing) and one athlete (4.8%) with LVNC. A clinical diagnosis of cardiomyopathy was more common in white athletes

compared with black athletes (30% *vs.* 12%, *P=0*.027). One black athlete (2%) revealed LGE on CMR in the absence of a clear diagnosis.

Diagnostic Yield of Genetic Testing

A genetic variant was identified in 63 athletes (63%); however, only 10 athletes revealed a disease-causing variant [pathogenic mutation (n=4) or variant of likely pathogenicity (n=6)], including 7 (14%) white athletes and 3 (6%) black athletes (Figure 1 and Table 3). The remaining 53 athletes were considered to have variants of unknown significance.

Of the 21 athletes with a clinical diagnosis, 8 individuals (33.3%; 6 white and 2 black) had a positive gene test consistent with the diagnosis (Figure 1 and Table 3). Six athletes (5 white and 1 black) were diagnosed with HCM, 1 white athlete with Fabry disease, and 1 black athlete with LVNC (Figure 1 and Supplemental Table 3).

Among the remaining 79 athletes without a clinical diagnosis, 2 (2.5%) were identified with a disease causing variant in the absence of a clinical phenotype. Specifically, 1 white athlete had a likely pathogenic rare variant in the SCN5A gene previously reported to be associated with LQTS and 1 black athlete had a pathogenic polymorphism in the TTR gene which is found in up to 4% of black individuals and associated with the development of wild-type transthyretin amyloidosis.

Electrical Changes in Athletes with a Cardiac Diagnosis

Pathological Q-waves and ST-segment depression were more common in athletes with cardiac disease compared to those without (Table 4). Almost all athletes diagnosed with cardiac disease (n=20, 95.2%) revealed TWI in the lateral leads (Figure 2). T-wave inversion limited to the anterior leads was detected in just one white athlete (4.8%) with cardiac pathology (Figure 2). The same athlete also revealed co-existing pathological Q-waves. None of the black athletes with

anterior TWI were diagnosed with a cardiomyopathy or an ion channel disorder. One black athlete with anterior TWI aged 23 harbored a pathogenic mutation in the TTR gene associated with wild-type transthyretin amyloidosis. None of the athletes with TWI confined to the inferior leads were diagnosed with cardiac disease or revealed a pathogenic genetic mutation.

Costs per Diagnosis Associated with Clinical and Genetic Testing

The cost of clinical evaluation amounted to US $1,084 per athlete using standard National Health Service Tariffs and an exchange rate of 1 British Pound to 1.28 US Dollars ($). This figure equated to a cost of $5,162 per athlete diagnosed with cardiac disease. Addition of genetic testing increased the cost of evaluation by 3-fold to $3,267 per athlete, equating to a cost of $14,204 per athlete with a clinical and/or genetic diagnosis and a cost of $32,670 per genetic diagnosis alone. The cost of making additional diagnoses beyond clinical evaluation based on genetic testing (2 genotype-positive individuals without a clear clinical phenotype) was $109,150 per athlete.

Discussion

The present study investigated whether genetic testing for mutations capable of causing cardiomyopathy and ion channel diseases has a potential role in determining the clinical significance of TWI in both black and white athletes over and above standard clinical evaluation.[18] The cohort of athletes is unique and recruitment was only possible through the assessment of a large number of athletes from several different referral sources. Although a total of 2,039 athletes were evaluated in our own sports cardiology clinic over the 2-year study period, these individuals were referred from a pool of over 5,400 athletes assessed by CRY and over 3,000 athletes assessed by Qatar Orthopaedic and Sports Medicine Unit during the same study

period. A significant proportion of athletes were also referred to us after being assessed at different institutions throughout the UK. Although it is more challenging to provide the precise denominator for this referral group, the majority of athletes in the current study (57%) revealed lateral T-wave inversion. Based on our own screening experience that only 4% of black[3,5] and 0.3% of white[2,3,5] athletes in the UK reveal lateral TWI, we estimate the total number of athletes required to derive a cohort of athletes with the TWI patterns described in this study would exceed 11,000.

Diagnostic Yield from Genetic Testing

The overall diagnostic yield from genetic testing for a pathogenic or likely pathogenic mutation in athletes with TWI was 10% compared with 21% following comprehensive clinical evaluation. Genetic testing was positive in just 8 athletes (38.1%) with a clinical diagnosis of cardiomyopathy despite a very comprehensive panel of genes being tested. Of these, 6 athletes (75.0%) were white. Genetic testing identified an additional 2 athletes (2.5%) with TWI but no clear clinical phenotype who harbored potential cardiac disease. Compared to a recent study by Kadota *et al.*[44] in which 5 out of 102 Japanese athletes (4.9%) with ECG abnormalities screened for mutations in 4 sarcomeric genes (MYH7, MYBPC3, TNNT2 and TNNI3) showed a positive result, our yield was significantly higher and likely reflects the comprehensive genetic panel used.

The yield for pathogenic mutations in our athletes clinically diagnosed with HCM was half that expected from the published literature (32% *vs.* ~60-70%). One third of the athletes diagnosed with HCM exhibited the apical variant which has been shown to have a lower than usual genetic yield.[45] The current false-negative rate of genetic testing in our cohort indicates that routine genetic testing in athletes with TWI who have undergone comprehensive clinical

evaluation will support a possible diagnosis of cardiomyopathy in a few cases at a substantial cost. These observations do not support the routine use of genetic testing for the evaluation of asymptomatic athletes with TWI in the absence of a family history of an inherited cardiac condition.

Although genetic testing identified a potentially serious SCN5A mutation implicated in LQTS in a white female athlete with TWI, she did not show any features of the disorder including a prolonged corrected QT interval. Similarly, gene testing identified a definitive transthyretin mutation in a black athlete without evidence of cardiac amyloidosis at this young age, and it is known that this variant is detected in up to 4% of the black population. These observations also highlight that routine gene testing without appropriate clinical indications may be confusing, cause unnecessary concern, and be problematic for decision making.

Association between Pattern of T-Wave Inversion and Cardiac Pathology

In both black and white athletes, TWI extending into the lateral leads was the most common pattern encountered (64% *vs.* 50%, respectively; Figure 2). Although previous studies in both black and white athletes report anterior TWI (V1-V4) to be the most common pattern observed,[3] our cohort almost certainly reflects a referral bias in favor of individuals with lateral TWI in whom suspicion of a cardiomyopathy is higher. Furthermore, given that anterior TWI is now widely recognized as a normal, ethnicity-specific training variant in black athletes, referral bias may also explain the similar prevalence of anterior TWI observed in our black and white cohorts, with less black athletes exhibiting anterior TWI being referred for evaluation.

ST-segment depression was found exclusively in athletes with lateral TWI, irrespective of ethnicity. All but one of our 21 athletes (95%) exhibiting structural disease revealed lateral TWI (Table 3 and Figure 2). Indeed, the diagnostic yield of lateral TWI for a clinical diagnosis

of cardiomyopathy in black and white athletes was 18.8% and 60%, respectively. In comparison, the yield for mutations associated with cardiomyopathy in athletes with lateral TWI was 14.0%.

Compared to white athletes, a smaller proportion of black athletes with TWI (n=6, 12%) were diagnosed with a cardiomyopathy (Table 3). Of these individuals, all exhibited TWI in the lateral leads, reinforcing the notion that this particular repolarization pattern should be viewed with caution, even in the black athletic population in whom its prevalence approaches 5%.[3] Anterior TWI in black athletes was not associated with overt cardiomyopathy, ion channel disorders or pathogenic genetic mutations suggesting that this pattern is likely benign.[46]

Overall Number of Diagnoses made and Yield from CMR

Significantly fewer black athletes were diagnosed with a cardiomyopathy compared with white athletes (12% *vs*. 30%, *P=0*.027), suggesting that TWI may be more representative of subtle forms of cardiomyopathy in white individuals. The only other study to comprehensively investigate athletes with TWI with CMR evaluated 155 athletes.[4] The authors diagnosed 37 athletes with a cardiomyopathy (predominantly HCM) on initial echocardiography. Of the remaining 118, a further 24 athletes (20.3%) were diagnosed with CMR, a figure comparable with the 19 athletes (19.0%) diagnosed in the current study, and a further 3 athletes (2.5%) on the basis of Holter monitoring and exercise testing. In this study, Holter monitoring and exercise stress testing revealed the broader phenotypic features of HCM in almost 20% of affected athletes.

Study Limitations

The current study has several limitations. Inherited cardiomyopathies exhibit an age-related penetrance, and thus it is possible that cardiac disease was under-diagnosed in our cohort. Our athlete population consisted of predominately young males, and therefore any conclusions

regarding the significance of TWI in female or master athletes cannot be extrapolated from this study. It was also not possible to perform familial evaluation of first-degree family members, which would have been a valuable source of information to help determine the genetic significance of TWI in borderline cases, even in the absence of a pathogenic mutation. Co-segregation studies were not performed, and would have been important to determine the pathogenicity of several of the identified variants. Finally, the absence of a pathogenic mutation does not exclude the possibility of underlying disease and several athletes revealed variants of undetermined clinical significance; thus, long-term clinical follow-up is warranted.

Conclusion

Up to 10% of athletes with TWI show definitive or likely pathogenic mutations for cardiomyopathy or ion channel disease. Compared with standard clinical practice, the relatively low diagnostic yield and high cost of genetic testing make it of negligible use in routine clinical practice. Although genetic testing may help identify individual athletes with TWI and a potential cardiac disorder in the absence of a clear clinical phenotype, our results suggest that it is not indicated in the routine evaluation of asymptomatic athletes with TWI in the absence of a family history of an inherited cardiac condition.

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Disclosures

Dr. Monserrat is the CEO and a stakeholder of Health in Code SL. None of the other authors have disclosures of relevance to make.

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Table 1. Demographics, ECG and CMR characteristics of black and white athletes

	Black Athletes n=50	**White Athletes n=50**	***P* value**
Age, y	22.7±7.3	25.1±7.1	0.065
Male sex, n (%)	49 (98)	45 (90)	0.092
Body surface area, m^2	1.97±0.26	2.00±0.22	0.321
Systolic Blood Pressure at rest, mm Hg	124.1±11.3	122.1±11.6	0.393
Diastolic Blood Pressure at rest, mm Hg	75.1±10.2	76.0±11.8	0.336
Amount of exercise per week, hours	13.4±4.4	13.4±5.5	0.961

Data are shown as absolute and relative (%) number of subjects for categorical variables and as mean ± SD for continuous parameters.

Table 2. ECG and CMR characteristics of black and white athletes

	Black Athletes n=50	**White Athletes n=50**	***P* value**
12-Lead ECG			
Left bundle branch block, n (%)	0 (0.0)	0 (0.0)	-
Pathological Q-waves, n (%)	2 (4.0)	3 (6.0)	0.646
T-wave Inversion, n (%)	100 (100)	100 (100)	-
Confined to V1–V4, n (%)	15 (30.0)	17 (34.0)	0.831
Extending to inferior leads, n (%)	3 (6.0)	8 (16.0)	0.200
Extending to lateral leads, n (%)	32 (64.0)	25 (50.0)	0.225
Deep T-wave Inversion, n (%)	48 (96.0)	41 (82.0)	0.025
ST-segment depression, n (%)	10 (20.0)	11 (22.0)	0.806
CMR			
Maximum left ventricular wall thickness, mm	11.9±2.1	11.3±2.4	0.232
Left ventricular end diastolic volume index, ml/m^2	85.5±15.7	98.7±17.8	<0.001
Left ventricular ejection fraction, percentage	66.7±9.7	69.0±8.7	0.251
Left ventricular wall / Left ventricular end-diastolic volume index, $mm \times m^2/mL$	0.14±0.05	0.12±0.06	0.026
Left ventricular mass index, g/m^2	85.1±18.6	84.6±20.6	0.910
Right ventricular end diastolic volume index, ml/m^2	84.9±13.5	100.3±20.9	0.001
Right ventricular ejection fraction, percentage	61.5±9.0	65.2±9.0	0.100
Late gadolinium enhancement, n (%)	5 (10.0)	5 (10.0)	-

Data are shown as absolute and relative (%) number of subjects for categorical variables and as mean ± SD for continuous parameters. CMR indicates cardiac magnetic resonance imaging.

Table 3. Clinical diagnoses made in athletes

Athlete	Age	Gene Mutation	Pathogenic	Clinical Diagnosis	T-wave Inversion	Diagnosis based on
White Athletes						
15	34	MYBPC3	Yes	Apical HCM	A, I, L	CMR and gene test
21	34	No	-	Apical HCM	A, I, L	CMR
25	35	No	-	Apical HCM	A, L	CMR
27	35	No	-	Apical HCM	A, L	CMR
31	20	No	-	HCM	I, L	CMR
35	14	No	-	HCM	I, L	CMR
47	35	No	-	HCM	A, I, L	CMR
49	19	MYBPC3	Likely	HCM	A	CMR
53	33	No	-	HCM	I, L	CMR
55	34	MYPBC3	Likely	HCM	A, I, L	CMR
60	24	MYH7	Likely	HCM	A, L	CMR
62	25	No	-	Apical HCM	A, I, L	CMR
74	34	GLA	Yes	Fabry Disease	A, I, L	CMR and gene test
77	34	MYBPC3	Yes	HCM	A, L	CMR and gene test
123	18	No	-	HCM	A, I, L	CMR
Black Athletes						
7	34	No	-	HCM	A, L	CMR
3	17	MYH7	Likely	Apical HCM	A, I, L	CMR and gene test
57	17	No	-	Apical HCM	A, I, L	CMR
86	25	No	-	HCM	A, I, L	CMR
91	15	No	-	HCM	A, L	CMR
92	13	ACTC1	Likely	LVNC	A, I, L	CMR and gene test

ACTC1 indicates Actin alpha, cardiac muscle 1; A, anterior; CMR, cardiac magnetic resonance imaging; GLA, galactosidase alpha; HCM, hypertrophic cardiomyopathy; I, inferior; L, lateral; LVNC, left ventricular non-compaction; MYBPC3, myosin binding protein C; and MYH7, myosin heavy chain 7.

Table 4. Electrical and structural characteristics of athletes diagnosed with structural cardiac disease compared to those without

Parameter	Athletes with Structural Diagnosis n=21	Athletes without Structural Diagnosis n=79	*P* Value
12-Lead ECG			
Pathological Q-waves, n (%)	4 (19.1)	1 (1.3)	0.001
Deep T-wave inversion, n (%)	21 (100)	68 (86.1)	0.070
T-wave inversion confined to anterior leads, n (%)	1 (4.8)	31 (39.2)	0.003
Lateral T-wave inversion, n (%)	20 (95.2)	37 (46.8)	<0.001
ST-segment depression, n (%)	10 (47.6)	11 (13.9)	0.001
QRS fragmentation, n (%)	2 (9.5)	16 (20.3)	0.255
Structural – CMR			
Left ventricular end diastolic volume index, ml/m^2	86.0±14.2	92.0±18.0	0.135
Right ventricular end diastolic volume index, ml/m^2	89.9±17.7	92.8±19.3	0.616
Maximum left ventricular wall thickness	14.6±2.0	10.8±1.8	<0.001
Left ventricular wall/ left ventricular end diastolic-volume index on CMR, mm × m^2/mL	0.17±0.12	0.07±0.04	0.001
Left ventricular mass index on CMR, g/m^2	89.4±23.1	83.4±18.1	0.216
Late gadolinium enhancement on CMR, n (%)	8 (38.1)	2 (2.5)	<0.001
Cardiopulmonary Exercise Testing and Holter monitoring			
Peak oxygen uptake, ml/min/kg	42.6±7.2	43.7±7.3	0.563
Predicted peak oxygen uptake, percentage	103.9±17.8	106.0±19.1	0.798
Non-sustained ventricular tachycardia, n (%)	2 (49.5)	0 (0.0)	0.051

Data are shown as absolute and relative (%) number of subjects for categorical variables and as mean ± SD for continuous parameters. CMR indicates cardiac magnetic resonance imaging.

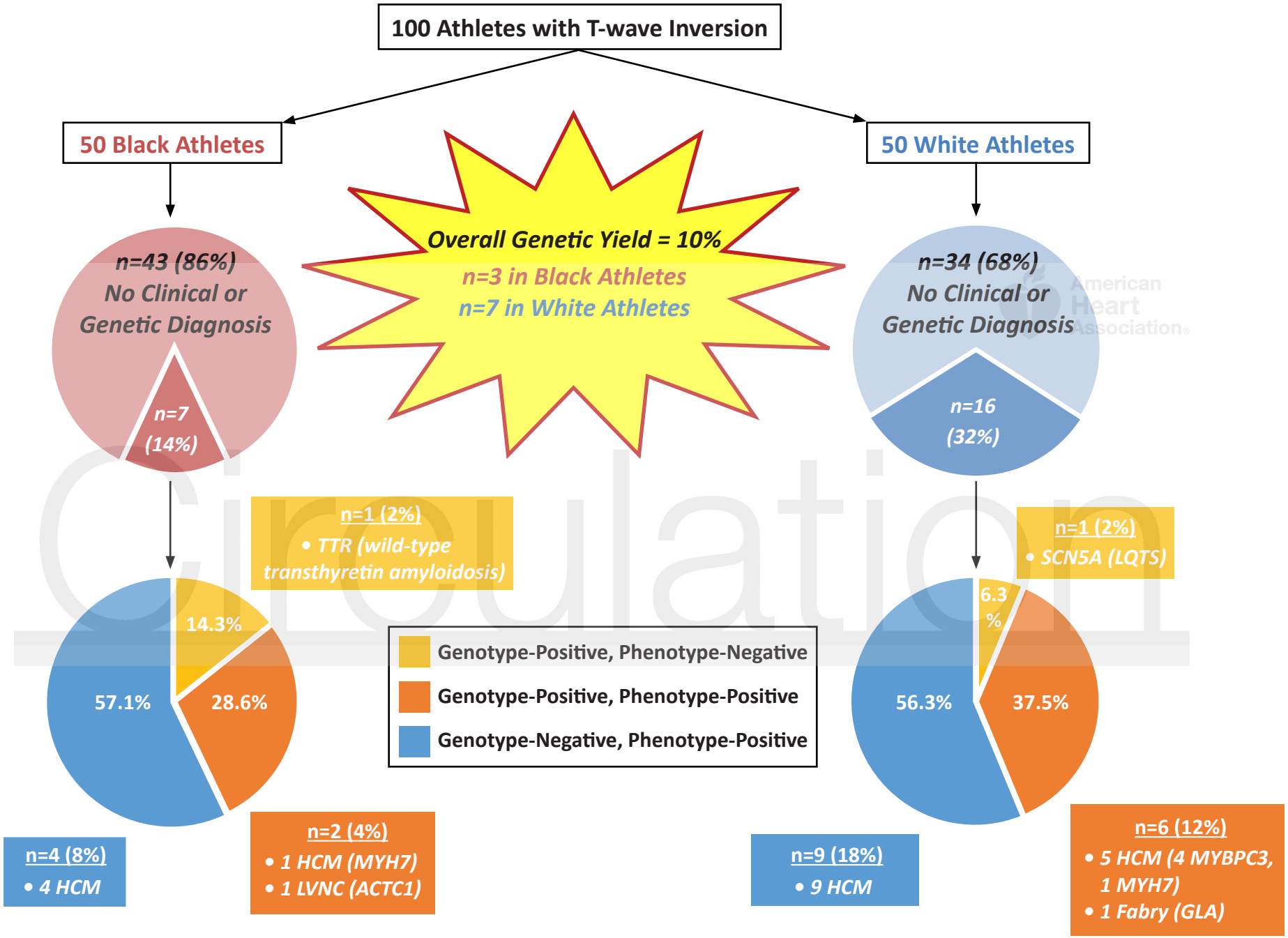
Figure Legends

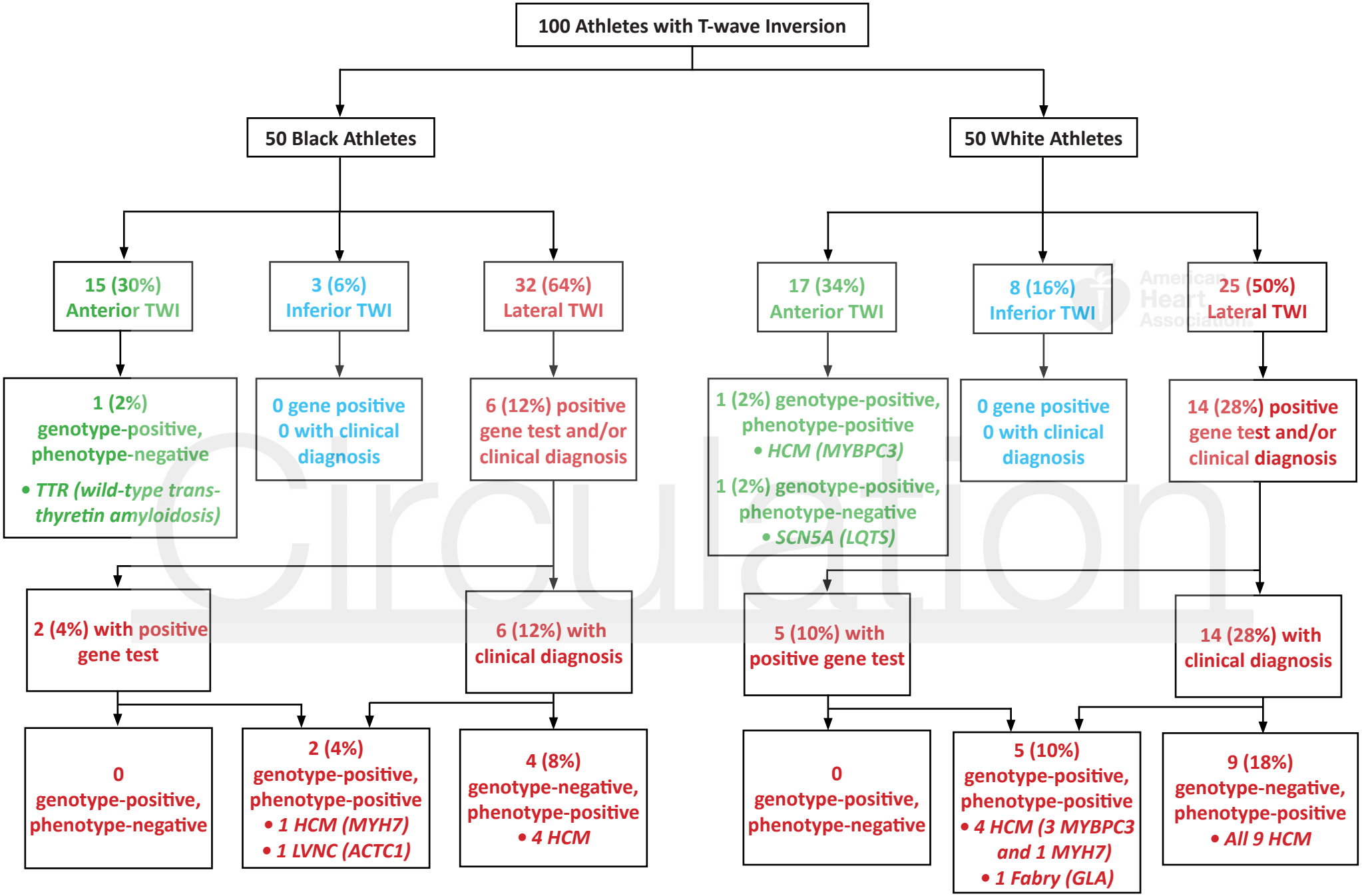
Figure 1. Breakdown of athletes with clinical and genetic diagnoses.

The diagnostic yield with comprehensive clinical investigation was 21% compared to 10% using genetic testing. Of the 21 athletes diagnosed with cardiac disease on the basis of clinical investigation, 8 (38.1%) were gene positive (MYPBC3, MYH7, GLA, and ACTC1 genes) and 13 (61.9%) were gene negative. Of the remaining 79 athletes without a clinical diagnosis, 2 (2.5%) were gene positive (TTR and SCN5A genes) in the absence of a clinical phenotype. ACTC1 indicates Actin, Alpha, Cardiac Muscle 1; GLA, galactosidase alpha; HCM, hypertrophic cardiomyopathy; n, number; LQTS, long QT syndrome; LVNC, left ventricular non-compaction; MYBPC3, myosin binding protein C; MYH7, myosin heavy chain 7; SCN5A, sodium voltage-gated channel alpha subunit 5; and TTR, transthyretin.

Figure 2. Comparison of clinical and genetic diagnoses in black and white athletes in relation to the distribution of T-wave inversion.

ACTC1 indicates Actin, Alpha, Cardiac Muscle 1; GLA, galactosidase alpha; HCM, hypertrophic cardiomyopathy; n, number; LQTS, long QT syndrome; LVNC, left ventricular non-compaction; MYBPC3, myosin binding protein C; MYH7, myosin heavy chain 7; SCN5A, sodium voltage-gated channel alpha subunit 5; TTR, transthyretin; and TWI, T-wave inversion.





Diagnostic Yield of Genetic Testing in Young Athletes with T-wave Inversion

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SUPPLEMENTAL MATERIAL

Supplemental Tables

Supplemental Table 1. Genes analyzed for variants for cardiomyopathies and ion channel disorders associated with T-wave inversion

Condition	Priority Genes Tested*	Other Candidate Genes Tested*
HCM	ACTC1, DES, FLNC, GLA, LAMP2, MYBPC3, MYH7, MYL2, MYL3, PLN, PRKAG2, PTPN11, TNNC1, TNNI3, TNNT2, TPM1, TTR	AARS2, ACAD9, ACADVL, ACTA1, ACTN2, AGK, AGL, AGPAT2, ANK2, ANKRD1, ATP5E, ATPAF2, BRAF, BSCL2, CALR3, CAV3, COA5, COA6, COQ2, COX15, COX6B1, CRYAB, CSRP3, DLD, DSP, ELAC2, FAH, FHL1, FHL2, FHOD3, FOXRED1, FXN, GAA, GFM1, GLB1, GNPTAB, GUSB, HRAS, JPH2, KRAS, LDB3, LIAS, LZTR1, MAP2K1, MAP2K2, MLYCD, MRPL3, MRPL44, MRPS22, MTO1, MYH6, MYOM1, MYOZ2, MYPN, NEXN, NF1, NRAS, OBSCN, PDHA1, PHKA1, PMM2, RAF1, SCO2, SHOC2, SLC22A5, SLC25A3, SLC25A4, SOS1, SURF1, TAZ, TCAP, TMEM70, TRIM63, TSFM, TTN, VCL, BAG3, CASQ2, IDH2, KCNJ8, KLF10, LMNA, MURC, MYLK2, OBSL1, PDLIM3
ARVC	DSC2, DSG2, DSP, FLNC, JUP, PKP2, PLN, TMEM43	CTNNA3, DES, LMNA, RYR2, TGFB3, TTN, CASQ2, CTNNB1, LDB3, PERP, PKP4, PPP1R13L, SCN5A
DCM	ACTC1, BAG3, DES,	ABCC9, ACTA1, ACTN2, ALMS1, ANKRD1, ANO5,

	DMD, DSP, FLNC, LMNA, MYBPC3, MYH7, PKP2, PLN, RBM20, TAZ, TNNC1, TNNI3, TNNT2, TPM1, TTN	CAV3, CHRM2, COL741, CRYAB, CSRP3, DNAJC19, DOLK, DSC2, DSG2, EMD, EYA4, FHL2, FHOD3, FKRP, FKTN, FOXD4, GAA, GATA4, GATA6, GATAD1, GLB1, HFE, JUP, LAMA2, LAMA4, LAMP2, LDB3, MURC, MYH6, MYL2, MYL3, MYOT, MYPN, NEBL, NEXN, PRDM16, PSEN1, PSEN2, RAF1, RYR2, SCN5A, SDHA, SGCD, SLC22A5, SPEG, SYNE1, SYNE2, TBX20, TCAP, TMEM43, TMPO, TOR1AIP1, TTR, TXNRD2, VCL, XK, BRAF, DNM1L, GATA5, GLA, IDH2, ILK, KCNJ2, KCNJ8, NKX2-5, OBSCN, OPA3, PDLIM3, PTPN11, SGCA, SGCB, TNNI3K
LVNC	ACTC1, MYBPC3, MYH7, TAZ	ACTN2, DMD, DNAJC19, DTNA, FHL1, HCN4, LDB3, LMNA, MIB1, MYH6, MYL2, NKX2-5, NNT, PLN, PRDM16, RYR2, TNNT2, TPM1, ANKRD1, BAG3, CASQ2, CSRP3, DSP, FLNC, KCNH2, KCNQ1, MLYCD, MYL3, NOTCH1, PTPN11, TNNC1, TNNI3, TTN
LQTS	CACNA1C, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, SCN5A	AKAP9, ANK2, CALM1, CALM2, CALM3, CAV3, KCND2, KCNJ5, RYR2, SCN4B, SNTA1, TRDN, FHL2, HCN4, KCNA5, KCND3, KCNE5, KCNE3, NOS1AP, PTRF, SCN1B

BrS	SCN5A, CACNA1C, CACNA2D1, CACNB2, KCNJ8, SCN1B	SCN10A, ABCC9, ANK2, FGF12, GPD1L, HCN4, KCND2, KCND3, KCNE5, KCNE3, PKP2, RANGRF, SCN2B, SCN3B, SLMAP, TRPM4, ANK3, CACNA1D, KCNH2

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; LVNC, left ventricular non-compaction.

*For full, official gene names, the reader is referred to the US National Center for Biotechnology Information (NCBI) online searchable database at https://www.ncbi.nlm.nih.gov/gene/

Supplemental Table 2. Summary of criteria used to determine variant pathogenicity

CLASSIFICATION	MAJOR CRITERIA	SUPPORTING CRITERIA
1. PATHOGENIC OR DISEASE CAUSING	1. Widely reported variant with conclusive evidence of a genotype-phenotype association and with consensus about its pathogenicity 2. Demonstrated co-segregation with a phenotype (>10 meioses) 3. Co-segregation in at least 2 families (≤10 meioses), or present in at least 5 probands with the same phenotype, and meeting at least 2 supporting criteria	1. Protein-truncating variant in a gene where loss of function is a proven pathogenic mechanism 2. Functional studies that support pathogenicity 3. *De novo* presentation in the setting of a novel disease in the family (maternity and paternity confirmed) 4. Missense variant that generates the same amino-acid change as a previously reported pathogenic variant 5. Variant with very low frequency/absent in the control population (MAF <0.001%)
2. VERY LIKELY TO BE PATHOGENIC OR DISEASE	1. Protein-truncating variant in a gene where loss of function is a proven pathogenic mechanism that explains the patient's phenotype, and that meets at least 1 supporting criterion	1. Functional studies that support pathogenicity 2. *De novo* presentation in the setting of a novel disease in the family (maternity and paternity confirmed) 3. Affecting a residue in which other pathogenic variants

CAUSING	2. Missense variant/in-frame insertion or deletion in a non-repetitive region of a gene with demonstrated genotype-phenotype association that explains the patient's disease, and that meets at least 2 supporting criteria	were previously identified. (mutational hot spot); or variant located in a relevant functional domain or region of the protein 4. Variant with very low allelic frequency/absent in the control population (MAF <0.001%) 5. Probable co-segregation in at least one family, or various index cases, but that does not meet criteria for being considered pathogenic
3. LIKELY TO BE PATHOGENIC OR DISEASE CAUSING	1. Protein-truncating variant with very low frequency or absent in the control population (MAF <0.001%) that affects a gene where loss of function is not an established pathogenic mechanism or that does not meet criteria to be considered pathogenic 2. Intronic variant outside the consensus region of the gene for which the bioinformatics predictors agree that	1. Variant with very low allelic frequency/absent in the control population (MAF <0.001%) 2. *De novo* presentation in the setting of a novel disease in the family (maternity and paternity unconfirmed) 3. Patient's phenotype or family history suggests that disease could be explained by mutations in the gene (gene with well-established phenotype-genotype

	it would affect the splicing 3. Missense variant/in-frame insertion or deletion in a non-repetitive region of a gene which does not meet criteria to be considered pathogenic/very likely to be pathogenic, but that meets at least 3 supporting criteria	association) 4. Bioinformatics predictors agree that it would be deleterious 5. Located in a mutational hot-spot, functional domain, or relevant region of the codified protein 6. Reported in at least 2 unrelated individuals that presented the same phenotype
4. UNKNOWN CLINICAL SIGNIFICANCE	1. Variants with contradictory information about their pathogenicity 2. Variants that do not meet criteria for being included in another classification category	
5. UNLIKELY TO BE PATHOGENIC OR DISEASE	1. Variant allele frequency in control populations is higher than the expected for disease or has a MAF >0.05% 2. Absence of variant co-segregation with the phenotype in at least 1 family	1. Missense variant in a gene where only variants causing protein truncation have shown association with disease 2. Functional study showing that the variant does not

CAUSING	3. Meeting at least 2 supporting criteria	affect the structure or function of the encoded protein 3. Bioinformatics predictors agree that the variant would not alter the function of the protein (including splicing variants outside the consensus region of the gene) 4. In-frame insertions/deletions in a repetitive gene region without a known function 5. Presence of the variant in homozygosis in control population
NON-PATHOGENIC (NOT DISEASE CAUSING)	1. MAF >5% in any of the control population databases 2. Previously reported in the literature with well-established evidence of consensus about its non-disease-causing classification, and with no contradictory data 3. Absence of co-segregation with the disease in at least 2 reported families	1. Variant allele frequency in control populations is higher than expected for disease or has a MAF >0.05% 2. Absence of co-segregation of the variant with the phenotype in at least 1 family 3. Functional study showing that the variant does not affect the structure or function of the encoded protein 4. Presence of the variant in healthy unaffected subjects

	4. Meeting at least 2 supporting criteria	at an age at which the disease should be fully penetrant (variant must be in homozygosis in recessively inherited diseases, or in hemizygosis in X-linked diseases)

MAF indicates minor allele frequency.

Supplemental Table 3. **Relevant genetic variants found in black and white athletes**

Athlete	Gene	Clinical Disease Associated with Identified Variant	Genotype and Population Frequency of Variant in Individuals in Control Populations	Pathogenicity	Sequence change*	Amino acid change*	Phenotype
White Athletes							
15	MYBPC3	HCM	Heterozygous; mutation (<0.0001, no homozygotes)	Pathogenic	NM_000256.3: c.1624G>C	NP_000247.2:p.Glu542Gln	Positive
74	GLA	Fabry disease	Hemizygous; mutation (not found in controls)	Pathogenic	NM_000169.2: c.902G>A	NP_000160.1:p.Arg301Gln	Positive
77	MYBPC3	HCM	Heterozygous; mutation (<0.0001, no	Pathogenic	NM_000256.3: c.3065G>C	NP_000247.2:p.Arg1022Pro	Positive

			homozygotes)				
49	MYBPC3	HCM	Heterozygous; mutation (<0.0001, no homozygotes)	Likely pathogenic	NM_000256.3: c.2552C>T	NP_000247.2:p.Ala851Val	Positive
55	MYPBC3	HCM	Heterozygous; mutation (<0.0001, no homozygotes)	Likely pathogenic	NM_000256.3: c.2198G>A	NP_000247.2: p.Arg733His	Positive
60	MYH7	HCM	Heterozygous; mutation (<0.0001, no homozygotes)	Likely pathogenic	NM_000257.3: c.3134G>T	NP_000248.2:p.Arg1045Leu	Positive
75	SCN5A	LQTS	Heterozygous; rare variant (<1%)	Likely pathogenic	NM_198056.2: c.3911C>T	NP_932173.1:p.Thr1304Met	Negative
Black Athletes							
39	TTR	Amyloid	Heterozygous; polymorphism (≥1%)	Pathogenic	NM_000371.3: c.424G>A	NP_000362.1:p.Val142Ile	Negative

3	MYH7	HCM	Heterozygous; mutation (not in controls)	Likely pathogenic	NM_000257.3: c.4259G>A	NP_000248.2:p.Arg1420Gln	Positive
92	ACTC1	HCM, DCM, LVNC	Heterozygous; mutation (<0.0001, no homozygotes)	Likely pathogenic	NM_005159.4: c.886T>C	NP_005150.1:p.Tyr296His	Positive

ACTC1 indicates Actin alpha, cardiac muscle 1; DCM dilated cardiomyopathy; GLA, galactosidase alpha; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; LVNC, left ventricular non-compaction; MYBPC3, myosin binding protein C; MYH7, myosin heavy chain 7; SCN5A, sodium voltage-gated channel alpha subunit 5; and TTR, transthyretin.

*For additional information about genomic variants, see https://www.ncbi.nlm.nih.gov/clinvar