ORIGINAL RESEARCH

Phenotypic Analysis of Arrhythmogenic Cardiomyopathy in the Hutterite Population: Role of Electrocardiogram in Identifying High-Risk Desmocollin-2 Carriers

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***Background*—**The p.Gln554X mutation in desmocollin-2 (DSC2) is prevalent in ≈10% of the Hutterite population. While the homozygous mutation causes severe biventricular arrhythmogenic right ventricular cardiomyopathy, the phenotypic features and prognosis of heterozygotes remain incompletely understood.

***Methods and Results*—**Eleven homozygotes (mean age 32±8 years, 45% female), 28 heterozygotes (mean age 40±15 years, 50% female), and 22 mutation-negatives (mean age 43±17 years, 41% female) were examined. Diagnostic testing was performed as per the arrhythmogenic right ventricular cardiomyopathy modified Task Force Criteria. Inverted T waves in the right precordial leads on ECG were seen in all homozygotes but not in their counterparts ($P<0.001$). Homozygotes had higher median daily premature ventricular complex burden than did heterozygotes or mutation-negatives (1407 [IQR 1080 to 2936] versus 2 [IQR 0 to 6] versus 6 [IQR 0 to 214], $P=0.0002$). Ventricular tachycardia was observed in 60% of homozygotes but in none of the remaining individuals ($P<0.001$). On cardiac magnetic resonance imaging, homozygotes had significantly larger indexed end-diastolic volumes (right ventricular: 122±24 versus 83±17 versus 83±12 mL/m^2, $P<0.0001$; left ventricular: 93±18 versus 76±13 versus 80±11 mL/m^2, $P=0.0124$) and lower ejection fraction values compared with heterozygotes and mutation-negatives (right ventricular ejection fraction: 41±9% versus 59±9% versus 61±6%, $P<0.0001$; left ventricular ejection fraction: 53±8% versus 65±5% versus 64±5%, $P<0.0001$). Most affected individuals lacked right ventricular wall motion abnormalities. Thus, few met cardiac magnetic resonance imaging task force criteria.

***Conclusions*—**The ECG reliably identifies homozygous p.Gln554X carriers and may be useful as an initial step in the screening of high-risk Hutterites. The cardiac phenotype of heterozygotes appears benign, but further prospective follow-up of their arrhythmic risk is needed. **(*J Am Heart Assoc*. 2014;3:e001407 doi: 10.1161/JAHA.114.001407)**

Key Words: arrhythmogenic cardiomyopathy • arrhythmogenic right ventricular cardiomyopathy/dysplasia • ECG screening • Hutterite population • risk stratification • sudden cardiac death

Arrhythmogenic right ventricular (RV) cardiomyopathy (ARVC) is an inherited disease of cardiac muscle associated with ventricular arrhythmias and predominantly RV structural abnormalities.[1–3] The prevalence of ARVC in the general population is at least 1:5000,[1,4] and it is a leading cause of sudden cardiac death (SCD).[2,3] ARVC is caused primarily by mutations in the 5 desmosomal proteins—plakoglobin, desmoplakin, desmoglein-2, plakophillin-2, and desmocollin-2 (DSC2)—and is inherited in an autosomal dominant manner in the majority of cases.[5–12] Less frequently, a recessive inheritance pattern is observed and is associated with cardiocutaneous manifestations such as occur in Naxos disease and Carvajal syndrome.[13–15] Biventricular and left ventricular (LV) dominant forms of ARVC are being increasingly recognized[16–18]; hence it is also broadly referred to as arrhythmogenic cardiomyopathy (AC).

The c.1660C>T (p.Gln554X) mutation in DSC2 is associated with AC and is prevalent in ≈10% of Hutterites, a genetically isolated population originating in 16th-century Europe who are descendants from <100 founders.[19–22] There are 3 branches (leuts) of Hutteries: Dariusleut, Lehrerleut, and Schmiedeleut; marriages between leuts are rare.[20] It is estimated that >45 000 Hutterites reside in North America. While the

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homozygous p.Gln554X mutation is associated with a severe biventricular arrhythmogenic cardiomyopathy in the absence of cutaneous manifestations, the phenotypic features and prognosis of heterozygote carriers remain incompletely understood.[22] Guidelines for screening of carriers follow the ARVC Modified Task Force Criteria (TFC)[23] and consist of extensive and costly testing. We sought to characterize the phenotypic features of the p.Gln554X mutation carriers to optimize screening strategies and to determine their arrhythmic risk.

Methods

Patients

All members of 3 large Hutterite families (representing all 3 leuts) aged >14 years followed at the University of Calgary Inherited Arrhythmia Clinic who had been genotyped for the p.Gln554X mutation and had clinical investigations were included in the study. Clinical evaluation included 12-lead ECG, 24-hour ambulatory monitoring, exercise testing using the Bruce protocol, signal-averaged ECG (SAECG), and cardiac magnetic resonance imaging (CMR). The diagnosis of ARVC was as per 2010 TFC.[23] The presence of ventricular tachycardia (VT) was determined from all ECG data (rest recordings, ambulatory monitoring, and exercise testing) and was defined as ≥3 consecutive QRS complexes of ventricular origin >100 bpm. Epsilon waves were defined as discrete low-amplitude complexes occurring after the end of the QRS in leads V_1 through V_3.[24] All study participants provided informed consent, and the study was approved by the University of Calgary Ethics Review Board (ID-23441).

Genotyping

Genotyping of study participants was performed as described previously.[22] Study participants underwent targeted screening for the c.1660C>T (p.Gln554X) mutation in DSC2 via PCR amplification of exon 11 followed by direct Sanger sequencing.

CMR Examination

CMR was performed by using a 1.5- or 3.0-T scanner (Avanto/Skyra; Siemens) equipped with a 32-channel cardiac coil. LV systolic function determination was obtained from 6 radial long-axis and 3 short-axis steady state free precession cine images (gated, 15 to 26 seconds breath-hold, slice thickness 10 mm). RV systolic function was determined from short-axis, cine images acquired along the axis of the RV (slice thickness 6 mm). Analysis of RV structure was also performed on sagittal RV steady state free precession cine imaging. Late gadolinium enhancement imaging was performed using a standard phase sensitive inversion recovery sequence 10 to 15 minutes after 0.2 mmol/kg gadolinium chelate administration. Because detailed assessment of LV morphology is not part of the standard CMR ARVC protocol at our institution, all studies were reread by 2 expert readers (S.G.W. and L.K.), blinded to the participant genotypes.

Data Analysis

Continuous variables are expressed as mean±SD or median and IQR, if the data were not normally distributed. Categorical variables are expressed as frequencies. Three group comparisons of continuous data were performed by using ANOVA or the Kruskal–Wallis test if the data were not normally distributed. Two group comparisons of continuous data were performed by using the Student *t* test or the Wilcoxon rank-sum test if the data were not normally distributed. Fisher's exact test was used for comparison of categorical variables. Two-tailed probability values <0.05 were considered significant. The Bonferroni correction was used for subgroup comparisons when 3 group comparisons were statistically significant. Statistical analysis was performed using Stata version 13.0 (StataCorp LP).

Results

Patients

One hundred eleven individuals from 3 large Hutterite families were initially screened, with a total of 64 patients meeting inclusion criteria. Thirteen were homozygous for the p.Gln554X mutation (homozygotes), 29 were heterozygous (heterozygotes), and 22 did not carry the mutation (mutation-negatives). A total of 4 patients had an SCD event: 3 homozygotes and 1 presumed heterozygous (obligate) carrier. Among the 4 deceased individuals, 1 homozygote had clinical investigations performed and was included in the study. Our study population thus consisted of 11 homozygotes (mean age 32±8 years, 45% female), 28 heterozygotes (mean age 40±15 years, 50% female), and 22 mutation-negatives (mean age 43±17, 41% female). All homozygotes with an SCD event underwent autopsy (ages 14, 15, and 28), confirming the diagnosis of ARVC. An autopsy was not performed on the heterozygous obligate carrier (a 54-year-old man) due to family refusal, and cause of death could not be determined.

Repolarization and Depolarization Abnormalities

Repolarization abnormalities are summarized in Table 1. Inverted T waves in the right precordial leads (V_1 to V_3) were seen in all homozygotes but in none of the heterozygotes or mutation-negatives ($P<0.001$). Further, all heterozygotes and mutation-negatives lacked repolarization abnormalities, with

Table 1. Repolarization Abnormalities in the Study Population as per 2010 ARVC Modified Task Force Criteria

Category	Homozygotes (n=11)	Heterozygotes (n=28)	Mutation-Negatives (n=22)	*P* Value
Inverted T waves V_1 to V_3, n (%)	11/11 (100)	0/25 (0)	0/21 (0)	<0.001
Inverted T waves V_1 to V_2, n (%)	11/11 (100)	1/25 (4)	0/21 (0)	<0.001
Inverted T waves V_1 to V_{3+},* n (%)	8/11 (73)	0/25 (0)	0/21 (0)	<0.001
Inverted T waves V_4 to V_6, n (%)	5/11 (45)	0/25 (0)	0/21 (0)	<0.001
RBBB+inverted T waves V_1 to V_4, n (%)	5/11 (45)	0/25 (0)	0/21 (0)	<0.001
Inverted T waves V_2 to V_3, n (%)	11/11 (100)	0/25 (0)	0/21 (0)	<0.001
TFC repolarization (major), n (%)	11/11 (100)	0/25 (0)	0/21 (0)	<0.001
TFC repolarization (minor), n (%)	0/11 (0)	1/25 (4)	0/21 (0)	1.0

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; RBBB, right bundle branch block; TFC, Task Force Criteria.
*T-wave inversion in V_1 to V_3 and beyond.

the one exception being a heterozygous individual who had inverted T waves in V_1 to V_2. All homozygotes met major TFC for repolarization abnormalities compared with none of the heterozygotes or mutation-negatives (sensitivity and specificity both 100%). Only 1 heterozygote and 0 of the mutation-negatives met minor TFC. Table 2 summarizes TFC ECG depolarization abnormalities. The presence of an epsilon wave, the only major TFC in this category, was observed in 27% of homozygotes but in none of the remaining individuals (P=0.006). Delayed terminal QRS activation ≥55 ms was seen in 64% of homozygotes but in only 12% of heterozygotes and in none of the mutation-negatives (P<0.001). Although homozygotes had greater evidence of late potentials on SAECG, 5 heterozygotes and 1 noncarrier had at least 1 abnormality on SAECG. The majority (82%) of homozygotes met minor TFC for depolarization, compared with only 5 heterozygotes and 1 mutation-negative (P<0.001).

Ventricular Arrhythmias

The presence of ventricular arrhythmias in the study population is summarized in Table 3. Homozygotes had a significantly higher median premature ventricular complex (PVC) burden compared with heterozygotes or mutation-negatives (1407 [IQR 1080 to 2936] versus 2 [IQR 0 to 6] versus 6 [IQR 0 to 214] PVCs/24 h, respectively; P=0.0002). In addition, nearly all homozygotes (89%) had >500 PVCs/24 h, a minor TFC, while only 1 heterozygote and 1 mutation-negative had such a finding (P<0.001). Overall, 60% of homozygotes had documented VT, compared with none of the heterozygotes or mutation-negatives (P<0.001). If those with SCD are included in the analysis, the prevalence of VT among homozygotes increased to 69%. Among those individuals with VT, only 1 had left bundle branch block with superior axis morphology VT, which is the only major TFC in the arrhythmia category.

Table 2. Depolarization Abnormalities in the Study Population as per 2010 ARVC Modified Task Force Criteria

Category	Homozygotes (n=11)	Heterozygotes (n=28)	Mutation-Negatives (n=22)	*P* Value
ECG				
Epsilon wave, n (%)	3/11 (27)	0/25 (0)	0/21 (0)	0.006
Terminal QRS ≥55 ms, n (%)	7/11 (64)	3/25 (12)	0/21 (0)	<0.001
SAECG				
Filtered QRS duration, ms	123±16	102±10	97±13	0.0013
Terminal QRS <40 μV, ms	51±16	30±12	26±6	0.0025
RMS voltage terminal 40 ms, μV	12±5	42±23	56±41	0.016
Task Force Criteria				
Depolarization (major), n (%)	3/11 (27)	0/25 (0)	0/21 (0)	0.006
Depolarization (minor), n (%)	9/11 (82)	5/25 (20)	1/21 (5)	<0.001

Continuous variables presented as mean±SD. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; SAECG, signal-averaged ECG; RMS, root-mean-square.

Table 3. Presence of Ventricular Arrhythmias in the Study Population as per 2010 ARVC Modified Task Force Criteria

Category	Homozygotes (n=11)	Heterozygotes (n=28)	Mutation-Negatives (n=22)	*P* Value
Holter				
PVCs/24 h, median (IQR)	1407 (1080, 2936)	2 (0, 6)	6 (0, 214)	0.0002
>500 PVCs/24 h, n (%)	8/9 (89)	1/24 (4)	1/7 (14)	<0.001
Ventricular tachycardia				
Any morphology, n (%)	6/10 (60)	0/26 (0)	0/7 (0)	<0.001
LBBB, superior axis, n (%)	1/10 (10)	0/26 (0)	0/7 (0)	0.395
LBBB, inferior axis, n (%)	3/10 (30)	0/26 (0)	0/7 (0)	0.013
RBBB, n (%)	2/10 (20)	0/26 (0)	0/7 (0)	0.073
Task Force Criteria				
Major, n (%)	1/10 (10)	0/26 (0)	0/7 (0)	0.395
Minor, n (%)	9/10 (90)	1/26 (4)	1/7 (14)	<0.001

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; PVC, premature ventricular complex; LBBB, left bundle branch block; RBBB, right bundle branch block.

Overall, 90% of homozygotes met minor TFC compared with only 1 heterozygote and 1 mutation-negative ($P<0.001$).

Global/Regional Dysfunction and Structural Alterations

CMR findings are summarized in Table 4. Indexed RV end-diastolic volume was significantly greater in homozygotes, with 82% having abnormal values as per TFC compared with 25% of heterozygotes and 33% of mutation-negatives ($P=0.006$). Similarly, homozygotes had larger indexed LV end-diastolic volume values than their counterparts. RV and LV ejection fractions were significantly decreased in homozygotes compared with heterozygotes or mutation-negatives (RV ejection fraction: 41±9% versus 59±9% versus 61±6%, $P<0.0001$ and LV ejection fraction: 53±8% versus 65±5% versus 64±5%, $P<0.0001$). Furthermore, 73% of homozygotes had an abnormal RV ejection fraction as per TFC compared with none of the heterozygotes or mutation-negatives ($P<0.001$). Despite these significant differences, regional RV akinesis, dyskinesis, or dyssynchrony was observed in the minority of patients (4 homozygotes, 4 heterozygotes, and 0 of the mutation-negatives, $P=0.2$). As a result, only 3 homozygotes and 2 heterozygotes met major CMR TFC, while 0 met minor criteria. Interestingly, 73% of homozygotes had significant regional LV findings such as focal areas of myocardial thinning and the presence of dyskinetic/aneurysmal segments particularly involving the lateral wall. LV structural findings currently do not form part of the CMR TFC. Overall, heterozygotes lacked the profound RV and LV structural abnormalities observed in homozygotes. LV structural abnormalities were observed in 25% of heterozygotes with the typical CMR findings including apical thinning with microaneurysms (Figures 1 and 2). Similarly, 33% of heterozygotes had other abnormal RV findings, with the presence of microaneurysms and trabecular hypertrophy being the most common. Biventricular abnormalities were seen in 8%. A small, hypokinetic LV apical diastolic bulge was the sole structural abnormality found among mutation-negatives. Among heterozygotes, there was no apparent temporal association between the appearance of electrical abnormalities as seen on ECG, Holter, or SAECG, and the structural abnormalities observed on CMR. In many cases, abnormal CMR findings appeared to precede electrical abnormalities (Table 5). Older age or male sex was not associated with an increased frequency of structural or electrical abnormalities in heterozygotes (Table 6), although sample size likely limited our statistical power to detect a difference.

Overall TFC Score

There were no statistically significant differences between heterozygotes and mutation-negatives in all aforementioned categories (not shown). All homozygotes and heterozygotes received 1 major criterion for being mutation carriers. Seven mutation-negatives received a major criterion for being first-degree relatives of an individual with ARVC, while 15 received a minor criterion for having an affected second-degree relative. Thus, all homozygotes met TFC for definite ARVC compared with only 2 heterozygotes and 0 of the mutation-negatives (Table 7). Seven heterozygotes and 2 mutation-negatives had borderline scores for ARVC. The median TFC major score in homozygotes was 3 (IQR 2 to 3) compared with 1 (IQR 1 to 1) in heterozygotes and 0 (IQR 0 to 1) in mutation-negatives ($P<0.001$). In addition, the median TFC minor score in homozygotes was 2 (IQR 1 to 2) versus 0 (IQR 0 to 0.5) in

Table 4. CMR Findings in the Study Population

Category	Homozygotes (n=11)	Heterozygotes (n=28)	Mutation-Negatives (n=22)	*P* Value
RV EDV, mL	224±59	163±37	147±21	0.0005
RV ESV, mL	139±52	68±23	59±15	<0.0001
BSA, m^2	1.8±0.2	2.0±0.2	1.8±0.3	0.198
RV EDV/BSA, mL/m^2	122±24	83±17	83±12	<0.0001
RV ESV/BSA, mL/m^2	74±23	34±10	33±7	<0.0001
RVEF, %	41±9	59±9	61±6	<0.0001
TFC major volume,* n (%)	8/11 (73)	4/24 (17)	0/6 (0)	0.002
TFC minor volume,† n (%)	1/11 (9)	2/24 (8)	2/6 (33)	0.311
TFC major RVEF,‡ n (%)	5/11 (45)	0/24 (0)	0/6 (0)	0.001
TFC minor RVEF,§ n (%)	3/11 (27)	0/24 (0)	0/6 (0)	0.033
Any RV WMA, n (%)	4/11 (36)	4/24 (17)	0/6 (0)	0.2
RV akinesia, n (%)	3/11 (27)	1/24 (4)	0/6 (0)	0.106
RV dyskinesia, n (%)	2/11 (18)	1/24 (4)	0/6 (0)	0.221
RV dyssynchrony, n (%)	0/11 (0)	2/24 (8)	0/6 (0)	1.0
Any RV non-WMA, n (%)	6/8 (75)	8/24 (33)	2/6 (33)	0.155
Microaneurysm, n (%)	2/8 (25)	3/21 (13)	0/6 (0)	0.51
Segmental dilation, n (%)	1/6 (17)	1/24 (4)	1/6 (17)	0.253
Accordion sign, n (%)	2/5 (40)	2/24 (8)	0/6 (0)	0.135
Fibrofatty replacement, n (%)	2/6 (33)	0/24 (0)	0/6 (0)	0.045
Trabecular hypertrophy, n (%)	6/8 (75)	4/24 (17)	2/6 (33)	0.008
LV EDV, mL	169±36	147±27	142±17	0.0972
LV ESV, mL	82±28	52±12	51±10	0.0002
LV EDV/BSA, mL/m^2	93±18	76±13	80±11	0.0124
LVEF, %	53±8	65±5	64±5	<0.0001
Any LV abnormality, n (%)	8/11 (73)	6/24 (25)	1/6 (17)	0.014
LV wall thinning	7/11 (64)	3/24 (13)	0/6 (0)	0.004
LV apical thinning	3/11 (27)	4/24 (17)	0/6 (0)	0.526
LV hypokinesia/akinesia	4/11 (36)	0/24 (0)	1/6 (17)	0.005
LV aneurysm	7/11 (64)	1/24 (4)	0/6 (0)	<0.001
CMR TFC major, n (%)	3/11 (27)	2/24 (8)	0/6 (0)	0.311
CMR TFC minor, n (%)	0/11 (0)	0/24 (0)	0/6 (0)	1.0

Continuous variables expressed as mean±SD. RV indicates right ventricular; EDV, end-diastolic volume; ESV, end-systolic volume; BSA indicates body surface area; EF, ejection fraction; LV, left ventricular; TFC, Task Force Criteria; WMA, wall motion abnormality.
*Exceeds major cardiac magnetic resonance imaging (CMR) criteria cut-off for volume.
†Exceeds minor CMR criteria cut-off for volume.
‡Exceeds major CMR criteria cut-off for RVEF.
§Exceeds minor CMR criteria cut-off for RVEF.

heterozygotes and 1 (IQR 1 to 1) in mutation-negatives ($P<0.001$).

Discussion

The key findings in this study of the clinical characterization of DSC2 p.Gln554X mutation carriers in Hutterites are (1) repolarization abnormalities were seen in all homozygotes but rarely in heterozygotes and mutation-negatives; the presence of T-wave inversions in V_1 to V_3 was 100% specific and sensitive for the homozygous mutation; (2) depolarization abnormalities were not frequently seen in heterozygotes or mutation-negatives but were observed in a large proportion of homozygotes; (3) heterozygotes and mutation-negatives had

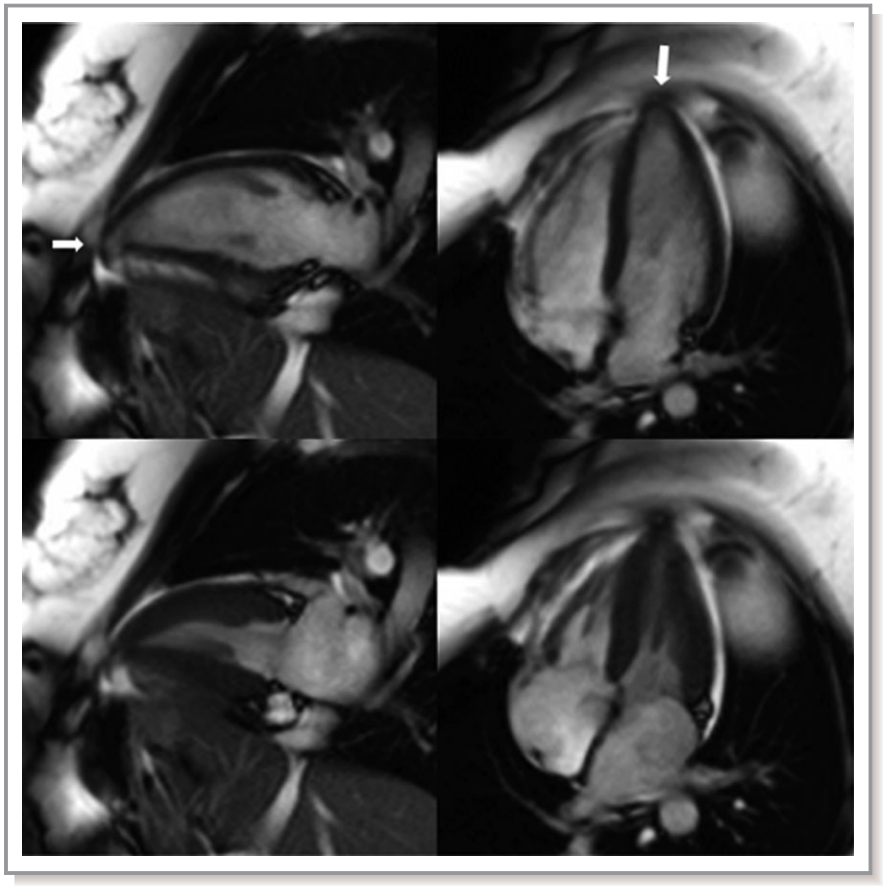

Figure 1. Cardiac magnetic resonance imaging 2-chamber and 4-chamber views at end-diastole (top panels) and end-systole (bottom panels) of a desmocollin-2 (DSC2) p.Gln554X heterozygote showing left ventricular thinning and a small aneurysm at the apex (arrows). There were no right ventricular abnormalities.

no documented VT, while it was common among homozygotes; (4) although cardiac structural abnormalities were relatively frequent, only a minority met CMR TFC; (5) CMR abnormalities in heterozygotes did not correlate with and appeared to precede electrical abnormalities; and (6) all homozygotes met definite TFC criteria for ARVC compared with only a few heterozygotes and no mutation-negatives.

AC is caused primarily by mutations in the 5 desmosomal proteins: plakoglobin, desmoplakin, desmoglein-2, plakophillin-2), and desmocollin-2.[6–11,25] To date, disease-causing AC mutations on DSC2 have been linked, for the most part, to an autosomal dominant pattern of inheritance.[5,6] This is not surprising because such mutations are likely to cause significant protein structural alterations such that a single copy of the defective gene is expected to have deleterious effects on desmosomal function. Interestingly, the DSC2 p.Gln554X mutation under study causes a severe biventricular arrhythmogenic cardiomyopathy in the homozygous state, much like in Naxos disease and Carvajal syndrome.[13–15,22] In contrast, the phenotype of heterozygote carriers remains uncertain. To the best of our knowledge, there has been only 1 study in patients with Naxos disease or Carvajal syndrome focusing on the phenotypic findings of heterozygous carriers. Antoniades et al reported on the phenotypic characterization of 46 heterozygous carriers of the 2–base pair deletion in plakoglobin (2157del2) associated with Naxos disease.[26] In this study, repolarization abnormalities were observed in 19% of heterozygotes, while regional wall motion abnormalities as identified by echocardiography and frequent PVCs were seen in 2 and 1 individual, respectively. Depolarization abnormalities, enlarged RV volumes, and VT were not observed. None of the heterozygotes fulfilled ARVC TFC using original criteria. Similarly, in our study, abnormal findings were infrequent in heterozygotes. Depolarization abnormalities were seen in 20%, while the presence of repolarization abnormalities and frequent PVCs was rare. Structural abnormalities appeared to be more prevalent in our heterozygous population with an abnormally enlarged RV volume seen in 25%, RV regional wall motion abnormalities (akinesia, dyskinesia, or dyssynchrony) observed in 17%, and other RV and LV structural abnormalities seen in 33% and 25%, respectively. One caveat is that we used CMR to identify structural alterations in our cohort, which is a more sensitive

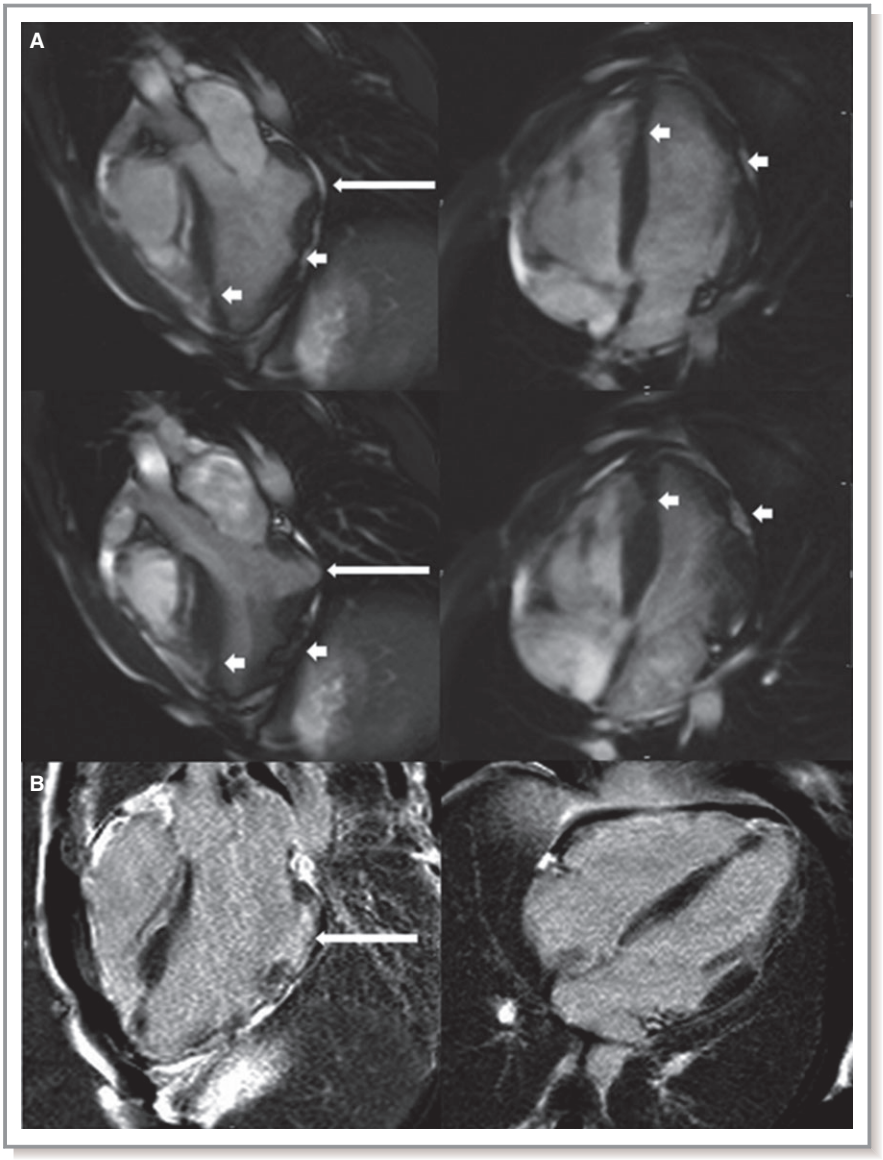

Figure 2. A, Cardiac magnetic resonance imaging 3-chamber and 4-chamber views at end-diastole (top panels) and end-systole (bottom panels) of a homozygote showing a basal inferolateral aneurysm (long arrow) with additional focal areas of thinning and akinesis in the apex, apical septum, apical anterior, and mid anterolateral walls (short arrows). There were no right ventricular abnormalities. B, Corresponding views showing small regions of late gadolinium enhancement at the edge of the basal inferolateral aneurysm (long arrow).

imaging technique than that used by Antoniades. Notwithstanding, structural abnormalities appeared out of proportion with electrical abnormalities, suggesting that CMR findings may precede electrical ones. Overall, 2 heterozygotes met definite ARVC TFC, while 7 met borderline ARVC criteria. Despite this, 0 of the heterozygotes had documented VT.

Although long-term follow-up data are lacking, the heterozygous phenotype of the p.Gln554X mutation appears to be benign. To date, we are only aware of 1 SCD event in a heterozygote: a 54-year-old smoker and hypertensive man in whom coronary artery disease is also a likely culprit. Unfortunately, the family did not permit an autopsy and the cause of death was never determined. We have previously estimated the prevalence of the DSC2 p.Gln554X mutation to be 9.4% of the Hutterite population.[22] Given that there are ≈45 000 Hutterites in North America, the potential number of gene carriers would consist of nearly 4500 individuals who would require serial clinical testing as per ARVC TFC. Our data showing a relatively benign phenotype in heterozygotes are helpful in that they allow us to counsel affected family members about their presumably low risk for SCD provided that high-risk homozygous carriers can be identified efficiently and cost-effectively. While the availability of genetic testing helps to identify homozygous carriers, Hutterites' propensity

Table 5. CMR Findings in Heterozygous Carriers Stratified by the Presence of Electrical Abnormalities

Category	No Electrical Abnormality* (n=17)	Electrical Abnormality* (n=7)	*P* Value
RV EDV, mL	165±42	156±22	0.591
RV ESV, mL	72±23	56±22	0.123
RV EDV/BSA, mL/m^2	82±18	86±15	0.654
RV ESV/BSA, mL/m^2	36±10	31±12	0.307
RVEF, %	57±7	64±11	0.067
TFC major volume,† n (%)	3/17 (18)	1/7 (14)	1.0
TFC minor volume,‡ n (%)	1/17 (6)	1/7 (14)	0.507
TFC major RVEF,§ n (%)	0/17 (0)	0/7 (0)	1.0
TFC minor RVEF,‖ n (%)	0/17 (0)	0/7 (0)	1.0
Any RV WMA, n (%)	4/17 (24)	0/7 (0)	0.283
RV akinesia, n (%)	1/17 (6)	0/7 (0)	1.0
RV dyskinesia, n (%)	1/17 (6)	0/7 (0)	1.0
RV dyssynchrony, n (%)	2/17 (12)	0/7 (0)	1.0
Any RV non-WMA, n (%)	7/17 (41)	1/7 (14)	0.352
Microaneurysm, n (%)	2/17 (12)	1/7 (14)	1.0
Segmental dilation, n (%)	1/17 (6)	0/7 (0)	1.0
Accordion sign, n (%)	2/17 (12)	0/7 (0)	1.0
Fibrofatty replacement, n (%)	0/17 (0)	0/7 (0)	1.0
Trabecular hypertrophy, n (%)	3/17 (18)	1/7 (14)	1.0
LV EDV, mL	148±29	146±24	0.844
LV ESV, mL	52±12	53±12	0.904
LV EDV/BSA, mL/m^2	74±13	80±16	0.32
LVEF, %	65±5	64±4	0.677
Any LV finding, n (%)	4/17 (24)	2/7 (29)	1.0
LV wall thinning, n (%)	3/17 (19)	0/7 (0)	0.526
LV apical thinning, n (%)	2/17 (12)	2/7 (29)	0.557
LV hypokinesia/akinesia, n (%)	0/17 (0)	0/7 (0)	1.0
LV aneurysm, n (%)	0/17 (0)	1/7 (14)	0.304
CMR TFC major, n (%)	2/17 (12)	0/7 (0)	1.0
CMR TFC minor, n (%)	0/17 (0)	0/7 (0)	1.0

Continuous variables expressed as mean±SD. RV indicates right ventricular; EDV, end-diastolic volume; BSA, body surface area; ESV, end-systolic volume; EF, ejection fraction; TFC, Task Force Criteria; WMA, wall motion abnormality; LV, left ventricular.

*Electrical abnormality was defined as presence of any major or minor Task Force Criteria in depolarization, repolarization, or arrhythmia category.

†Exceeds major cardiac magnetic resonance imaging (CMR) criteria cut-off for volume.

‡Exceeds minor CMR criteria cut-off for volume.

§Exceeds major CMRI criteria cut-off for RVEF.

‖Exceeds minor CMRI criteria cut-off for RVEF.

for living in rural areas and their variable cultural acceptance for genetic testing limit its utility as the initial step in the screening of high-risk individuals. For these reasons, noninvasive and easily accessible tests that are highly sensitive and specific for the presence of the homozygous mutation would be extremely useful. In our study, we found that the presence of T-wave inversions in V_1 to V_3 was both 100% sensitive and specific for the homozygous mutation. Further, the presence of >500 PVCs/24 h had a specificity of 89% and sensitivity of 94%. We propose that these inexpensive, noninvasive tests could be used as a first step to screen the Hutterite population to identify high-risk homozygotes. Those with abnormal testing results would then proceed to full clinical evaluation as per TFC, while those with normal testing results would undergo repeat yearly ECGs. If successful, this strategy would not only be extremely cost-effective but also increase

Table 6. Sex and Age Associations With RV Structural and Electrical Abnormalities in Heterozygotes

Category	Male (n=14)	Female (n=14)	*P* Value	Age <50 y (n=18)	Age >50 y (n=10)	*P* Value
Electrical abnormalities,* n (%)	3/14 (21)	4/13 (31)	0.678	3/17 (18)	4/10 (40)	0.365
Any structural abnormality, n (%)	6/12 (50)	3/12 (25)	0.4	4/15 (27)	5/9 (56)	0.212
Regional WMA,† n (%)	3/12 (25)	1/12 (8)	0.59	3/15 (20)	1/9 (11)	1.0
Non-WMA,‡ n (%)	5/12 (42)	3/12 (25)	0.667	3/15 (20)	5/9 (56)	0.099
RVEF, %	57±11	61±6	0.219	56±5	63±13	0.103

RVEF indicates right ventricular ejection fraction.
*Electrical abnormalities defined as presence of any major or minor Task Force Criteria in depolarization, repolarization, or arrhythmia categories.
†WMA, wall motion abnormality (any of akinesis, dyskinesis, or dyssynchrony).
‡Presence of any of RV microaneurysms, segmental RV dilation, accordion sign, fibrofatty replacement, or trabecular hypertrophy.

Hutterites' access to health evaluation, which can be limited due to their rural livelihoods. Prospective validation of such a screening approach is needed and is currently under way.

Our study also illustrates some of the limitations with the current ARVC TFC. Only a minority (27%) of homozygotes met CMR TFC under structural alterations, despite having significant biventricular findings. Further, while 60% of homozygotes had documented VT, only one individual met major TFC under the arrhythmia category by way of left-bundle branch block, superior-axis VT. These TFC shortcomings are likely secondary to the biventricular nature of the arrhythmogenic cardiomyopathy seen in p.Gln554X homozygotes and reflect the current lack of recognition of LV-dominant and biventricular forms of ARVC in the current guidelines. As the latter forms of ARVC are being increasingly recognized, it is imperative that future TFC iterations be inclusive of them.

This study has several limitations. First, our findings are based on a small number of patients and at times lacked the statistical power needed to show certain associations. Second, the retrospective nature of the study led to some missing data, which could have introduced an element of bias in the interpretation of the results. Third, since our investigation protocol for evaluating mutation-negatives typically consists of only a 12-lead ECG, complete clinical evaluation was only available for a small proportion in this subgroup. Finally, long-term follow-up data of heterozygotes are not currently available and we cannot rule out the manifestation of a latent phenotype of significance among heterozygotes. Despite these limitations, our study provides valuable insights into the phenotype and arrhythmogenic risk of DSC2 p.Gln554X heterozygotes, which will benefit from continued long-term follow-up.

Table 7. Summary of 2010 ARVC Task Force Criteria for the Study Population

ARVC Task Force Criteria	Homozygotes (n=11)	Heterozygotes (n=28)	Mutation-Negatives (n=22)	*P* Value
Repolarization major, n (%)	11/11 (100)	0/25 (0)	0/21 (0)	<0.001
Repolarization minor, n (%)	0/11 (0)	1/25 (4)	0/21 (0)	1.0
Depolarization major, n (%)	3/11 (27)	0/25 (0)	0/21 (0)	0.006
Depolarization minor, n (%)	9/11 (82)	5/25 (20)	1/21 (5)	<0.001
Arrhythmia major, n (%)	1/10 (10)	0/26 (0)	0/7 (0)	0.395
Arrhythmia minor, n (%)	9/10 (90)	1/26 (4)	1/7 (14)	<0.001
CMR major, n (%)	3/11 (27)	2/24 (8)	0/6 (0)	0.311
CMR minor, n (%)	0/10 (0)	0/24 (0)	0/6 (0)	1.0
Family history major, n (%)	11/11 (100)	28/28 (100)	7/22 (32)	<0.001
Family history minor, n (%)	0/11 (0)	0/28 (0)	15/22 (68)	<0.001
Total major score, median [IQR]	3 [2 to 3]	1 [1 to 1]	0 [0 to 1]	<0.001
Total minor score, median [IQR]	2 [1 to 2]	0 [0 to 0.5]	1 [1 to 1]	<0.001
Definite TFC, n (%)	11/11 (100)	2/28 (7)	0/22 (0)	<0.001
Borderline TFC, n (%)	0/11 (0)	7/28 (25)	2/22 (9)	0.113

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; CMR, cardiac magnetic resonance imaging; TFC, Task Force Criteria.

In conclusion, heterozygote carriers of the DSC2 p.Gln554X mutation have few abnormalities as per TFC compared with homozygotes and rarely do individuals meet definite diagnostic criteria for ARVC. Noninvasive testing by way of rest and ambulatory ECG may be useful in the screening of Hutterite colonies. Despite the noted TFC abnormalities, the prognosis of heterozygotes appears benign, but prospective, long-term data are needed.

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