An inherited sudden cardiac arrest syndrome may be based on primary myocardial and autonomic nervous system abnormalities

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An inherited sudden cardiac arrest syndrome may be based on primary myocardial and autonomic nervous system abnormalities

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Abstract

Background: A recently discovered sudden cardiac arrest (SCA) syndrome is linked to a risk haplotype which harbours the dipeptidyl-peptidase 6 (DPP6) gene as plausible culprit.

Objective: Because DPP6 impacts both cardiomyocyte and neuronal function, we hypothesized that ventricular fibrillation (VF) in risk haplotype carriers arises from functional changes in both heart and autonomic nervous system.

Methods: We studied six risk haplotype carriers with prior VF (symptomatic), eight carriers without VF (asymptomatic), and seven non-carriers (controls). We analysed supine and standing heart rate variability (HRV), baroreflex sensitivity (BRS), pre-VF heart rate changes, and myocardial $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) scintigraphy.

Results: Carriers had longer interbeat intervals than controls (1.03±0.11 vs. 0.81±0.07 seconds, p<0.001), lower low-frequency (LF) and higher high-frequency (HF) activity, and lower LF/HF ratio (0.68±0.50 vs. 2.11±1.10, p=0.013) in supine position. Upon standing up, carriers had significantly larger decrease in interbeat interval and increase in LF than controls (standing to supine ratios: 0.78±0.07 vs. 0.90±0.07 [p=0.002] and 1.94±1.03 vs. 1.17±0.34 [p=0.022], respectively), and nonsignificantly larger decrease in HF (0.62±0.36 vs. 0.97±0.42 [p=0.065]) and increase in LF/HF ratio (5.55±6.79 vs. 1.62±1.24 [p=0.054]). Sixteen of seventeen VF episodes occurred at rest; heart rate immediately before VF was 110±25 beats/min. Symptomatic carriers had less heterogeneous $^{123}$I-mIBG distribution in the left ventricle than asymptomatic carriers (SPECT-score ≥3 in seven asymptomatic and one symptomatic carrier, p=0.008).

Conclusions: It can be speculated that these data are consistent with more labile autonomic tone in carriers, suggesting that the primary abnormalities may reside both in the heart and autonomic nervous system.
Keywords

sudden cardiac arrest; autonomic nervous system; dipeptidyl-peptidase 6; $^{123}$I-mIBG; heart rate variability; baroreflex sensitivity
48 Introduction

49 Sudden cardiac arrest (SCA) is mostly caused by cardiac arrhythmias (ventricular fibrillation, VF) that may result from inherited cardiac ion channel dysfunction or cardiomyopathies (1).

50 Ion channel properties may be disrupted by mutations in the encoding genes (inherited SCA syndromes) (2) and/or imbalances in neural control by the autonomic nervous system (ANS) (3). For instance, in Long QT-syndrome (LQTS), ventricular fibrillation (VF) results from dysfunction of mutant cardiac ion channels (mostly voltage-gated K⁺ channels) and their adverse responses to sympathetic stimulation (4). Accordingly, the cornerstone of therapy in LQTS are β-adrenoceptor blocking drugs and, in selected cases, cardiac sympathetic denervation (5). Yet, in LQTS, as in most other inherited SCA syndromes, the primary derangement resides in the heart, rather than the ANS.

59 In another inherited SCA syndrome (6), a risk haplotype in chromosome 7q36 harbouring the dipeptidyl peptidase 6 (DPP6) gene was identified as underlying genetic variant. DPP6 is a potential β-subunit of the cardiac transient outward K⁺ current (Ito) encoded by Kv4.x-subunits. DPP6 modulates trafficking, kinetics, and pharmacology of Kv4.x-encoded channels (7,8). Carriers of the risk haplotype have 20-fold increased DPP6 mRNA levels in the myocardium (9). DPP6 overexpression may cause gain-of-function of Kv4, resulting in arrhythmia (10). These findings suggest a role for DPP6 in the occurrence of VF. Yet, although prior studies of this SCA syndrome have focused on the heart, DPP6 is not exclusively expressed in cardiac tissue, but also plays an important role in various brain regions, e.g., thalamus, hypothalamus, hippocampus. In neuronal tissue, DPP6 is a putative β-subunit for neuronal A-type currents, encoded by Kv4.x-subunits, which increases the excitability of dendritic cells (7). Moreover, DPP6 plays a role in hippocampal synaptic development and function (11), a region involved in emotion and stress response, and coupled to ANS function.
Because increased VF risk may be associated both with increased sympathetic tone (e.g., in LQTS) and increased parasympathetic tone (e.g., in Brugada syndrome, another inherited SCA syndrome) (12), abnormal ANS function may be a primary mechanism for VF occurrence in the DPP6-related SCA syndrome.

We hypothesized that risk haplotype carriers have imbalances in ANS function, and that these imbalances are associated with VF risk. There are many ways to study ANS function in relation to arrhythmogenic disorders (13). We chose from this broad palette to analyse heart rate variability (HRV), baroreflex sensitivity (BRS), and heart rate changes immediately preceding VF (to study control of cardiac function by the ANS) (13) and $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-mIBG) myocardial scintigraphy (to map norepinephrine release and reuptake within the heart). We performed these studies in symptomatic and asymptomatic risk haplotype carriers and in healthy family members who did not carry the risk haplotype. With these studies, we aimed to obtain further insights into the mechanisms by which the ANS modulates VF risk. Moreover, we sought to obtain novel tools to predict VF risk in carriers of the risk haplotype.
Methods

Design and setting

Three groups from one extended family, recruited from the Cardiogenetics department of Amsterdam UMC, were studied: (1) carriers of the risk haplotype with prior VF (symptomatic, n=6); (2) age/sex-matched carriers of the risk haplotype without prior VF (asymptomatic, n=8); (3) age/sex-matched family members who did not carry the risk haplotype and had no cardiac history (controls, n=7). The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional Medical Ethics Committee. All subjects were aged >18 years and signed informed consent.

Analysis of HRV, BRS, and pre-VF heart rates

HRV measurement, performed in all subjects directly after the early planar $^{123}$I-mIBG image was acquired, was conducted as described previously (14). Heart rate and blood pressure were recorded for 20 minutes (10 minutes in supine position, followed by 10 minutes in standing position). These data were measured with the Nexfin Cardiovascular Monitor, a completely non-invasive blood pressure and cardiac output (CO) monitor based on finger arterial pulse contour analysis. During all measurement, patients held their hand at heart level. Data files were converted to beat-to-beat data-files.

The mean interbeat interval (IBI) between the pulse-waves and the standard deviation of all IBIs (SD-IBI) were measured. In power spectrum analysis, conducted using Fast Fourier transform in MATLAB®, we analysed very low frequency (VLF; 0.003-0.04 Hz), low frequency (LF; 0.04-0.15 Hz), and high frequency (HF; 0.15-0.4 Hz) bands. The LF band reflects a combination of sympathetic and parasympathetic activity, and the HF band an estimate of parasympathetic control. The LF/HF ratio is commonly used as a measure of sympathovagal
balance. LF and HF were expressed as normalized units by division by the total variance (including the power in the VLF-band). We also analysed the gain from systolic blood pressure changes to heart period changes in the LF-band (if coherence >0.5), a validated measure of BRS (ms/mmHg) (15,16). Standing-to-supine ratios were calculated to quantify the increase in sympathetic drive upon standing.

To gain insight into the ‘autonomic state’ preceding VF, implantable cardioverter defibrillator (ICD) recordings in symptomatic patients were analysed for heart rate before the ICD shocks delivered in the absence of β-adrenoceptor blockers or antiarrhythmic drugs.

123I-mIBG scintigraphy

123I-mIBG scintigraphy was performed in all study subjects to assess cardiac sympathetic activity. All subjects received 185 MBq (5 mCi; ±10%) of 123I-mIBG (AdreView™, GE Healthcare) intravenously after a 30 minutes rest period in supine position. At 15 minutes (early) and 4 hours (late) post-injection, 10 minutes planar anterior thorax images were acquired. Only late imaging was followed by single-photon emission computed tomography (SPECT).

An experienced nuclear medicine technologist, blinded for the subject’s group status, processed the planar images to determine the early (15 min post-injection) and late (4 hrs post-injection) heart (H)/mediastinal (M) ratios (HERMES Medical Solutions, Stockholm, Sweden). Early H/M indicates myocardial norepinephrine transporter uptake function, whereas late H/M gives a measure of overall neuronal function including information from uptake to release through the storage mechanism (17). In addition, myocardial washout, which indicates sympathetic drive, was calculated as:

\[
\frac{(\text{early H/M} - \text{late H/M})}{\text{early H/M}} \times 100\%
\]

(early H/M - late H/M)
early H/M *100%
Regional differences in $^{123}$I-mIBG uptake were assessed using a 17-segments bull’s-eye model. Two experienced nuclear medicine physicians, both blinded for the subject’s group status, scored each segment on a 0-4 scale (0=normal $^{123}$I-mIBG uptake, 4=no $^{123}$I-mIBG uptake). The SPECT-score was calculated as the sum of individual SPECT segment scores. Heterogeneous myocardial $^{123}$I-mIBG uptake was defined as reduced tracer uptake (segment score >0) in at least three adjacent segments (SPECT-score ≥3). A total SPECT-score of 1-2 was considered as homogeneous $^{123}$I-mIBG uptake.

**Statistics**

Statistical analyses were performed with SPSS 18.0 for Mac. Results are expressed as mean±standard deviation (SD). Data normality was determined using the Kolmogorov-Smirnov test. Logarithmic transformation was performed on not-normally distributed data to obtain normal distribution. Testing for significant differences between groups (carriers vs. controls and symptomatic vs. asymptomatic patients) was performed with a Student’s t-test, a chi-square test or a Fisher’s Exact test where appropriate. P-values <0.05 were considered statistically significant.
Results

Clinical characteristics

We studied 14 carriers of the risk haplotype (nine men, five women), aged 39.2±10.0 years, range 28-62 years, median 36 years (Table 1). The six symptomatic carriers suffered 17 VF episodes prior to this study. Sixteen VF episodes occurred at rest (unknown circumstances surrounding one VF episode), including four after mild alcohol use (Table 2). All symptomatic carriers had an ICD; four previously had appropriate ICD shocks (54 appropriate shocks for 11 VF episodes). While VF risk in carriers is age-dependent (increasing with advancing age), the ages of the symptomatic and asymptomatic carriers did not differ (9). The non-carrier control group comprised seven relatives of the carriers (four men, aged 33.6±5.8 years). In all subjects, HRV and \(^{123}\text{I}-\text{mIBG}\) scintigraphy were studied in the absence of \(\beta\)-adrenoceptor blocking or antiarrhythmic medication.

Analysis of HRV, BRS, and pre-VF heart rate changes

Carriers had longer mean IBI than controls (Table 3). In line with this finding, the power spectral HRV data in supine position showed that carriers had lower normalized LF activity, higher normalized HF activity, and lower mean LF/HF ratio (Figure 1). During active standing, no significant between-group differences in mean IBI, normalized LF, normalized HF or LF/HF ratio occurred (Figure 1). Analysis of standing-to-supine ratios revealed that the sympathetic trigger of standing up elicited a stronger decrease in mean IBI, a stronger increase in normalized LF (\(p=0.022\)), and a larger (although not statistically significant) decrease in normalized HF in carriers compared to controls. Consequently, carriers had a larger increase in LF/HF ratio than controls, although this difference just failed to reach statistical significance.
There were no statistically significant differences in HRV parameters between symptomatic and asymptomatic carriers (Table 4, Figure 1).

In one symptomatic carrier (31 year-old man), the heart rate accelerated suddenly after nine minutes of measurement in supine position. This patient became anxious, fearing his ICD would deliver a shock, as he recognized this feeling from a previous period which occurred immediately before an ICD shock. After his heart rate had started to return to normal, the measurement was continued according to protocol. The recording showed that his sudden acceleration in heart rate was attended by small decreases in blood pressure, stroke volume, and maximal dP/dt of the pulse wave, all suggestive of parasympathetic withdrawal rather than sympathetic activation (Figure 2).

Of the ICD shocks prior to this study, ICD recordings could be retrieved from three patients, yielding seven analysable VF episodes. The mean recorded time before VF onset was 7.4±1.0 seconds, and mean heart rate immediately before VF was 110±25 beats/min (range 80-162).

Planar image analysis revealed no statistically significant differences between carriers and controls in early H/M, late H/M or washout (Table 5). Quantitative segmental analysis of the SPECT images showed no differences in SPECT-score between carriers and controls (Figure 3, left). However, symptomatic carriers had less spatially heterogeneous $^{123}$I-mIBG distribution in the left ventricle when compared to asymptomatic carriers. Heterogeneous $^{123}$I-mIBG SPECT (SPECT-score $\geq$3) occurred in seven of eight asymptomatic carriers, and in one of six symptomatic carriers ($p=0.008$, Figure 3, right).
Clinical analysis revealed that, although VF in risk haplotype carriers appeared to occur under conditions of increased parasympathetic tone (rest, sleep, after mild alcohol use), heart rates immediately preceding VF can also be elevated, consistent with a sudden swing from a parasympathetic state into a sympathetic state. One symptomatic carrier exhibited such a swing during HRV recording, when heart rate suddenly accelerated in a manner consistent with vagal withdrawal. These observations spawn the notion that carriership is associated with more labile autonomic tone, i.e., stronger fluctuation between parasympathetic and sympathetic status. This notion is supported by HRV analysis, which showed that carriers had higher parasympathetic tone at baseline (higher IBI, lower LF, higher HF, lower mean LF/HF in supine position), but stronger response to sympathetic stimulation (more decrease in IBI and HF, more increase in LF and LF/HF ratio in response to standing up), than controls. Using 123I-mIBG scintigraphy, we found no evidence that increased sympathetic responsiveness in carriers was due to higher cardiac sympathetic activity, as early H/M, late H/M, and washout did not differ between carriers and controls. This suggests that increased (para)sympathetic responsiveness in carriers is due to extra-cardiac factors, e.g., efferent signalling pathways of the ANS. This notion is consistent with the finding that DPP6, the putative causative gene, is expressed in neurons and plays an important functional role there, e.g., by modulating gating function of neuronal ion channels (18). 123I-mIBG scintigraphy revealed that symptomatic carriers are distinguishable from non-symptomatic carriers by a more spatially homogeneous distribution of 123I-mIBG uptake. This observation goes against the generally accepted concept that more heterogeneity in functional properties (e.g., autonomic activity) increases arrhythmia risk. We fully subscribe to this concept and would certainly not propose the opposite (more heterogeneity in functional properties reducing arrhythmia risk). In an effort
to better understand our observation, we can only provide a speculative explanation, as follows. Our finding could reflect the clinical observation that VF in carriers seems to be often initiated by premature beats originating from specific areas within the heart (19). Such specific arrhythmogenic areas in the heart were also found in other inherited SCA syndromes. For instance, in Brugada syndrome (20), the right ventricular outflow tract and free wall are focal points for arrhythmia, and ablation of these areas drastically reduces VF incidence. In DPP6 haplotype carriers, it is conceivable that, while sympathetic signalling in symptomatic carriers, similar to controls, is spread evenly throughout the heart (as indicated by SPECT-\textsuperscript{123I}-mIBG scintigraphy), asymptomatic carriers may be protected from VF occurrence by the fact that they happen to have reduced sympathetic signalling (which is critical for arrhythmia onset) in the regions where premature beats in carriers trigger VF. Whatever the reason for localized reduced sympathetic signalling in these carriers, this scintigraphic parameter may have clinical relevance, as it may be used to distinguish carriers with increased VF risk from carriers without. Such a distinction is crucial for risk stratification and may have important therapeutic consequences. At present, ICD implantation is considered the only viable therapy option in carriers, because predictors of VF occurrence are lacking at present, and carriers with increased VF risk cannot be distinguished from carriers without elevated risk (6). Consequently, carriers without increased VF risk receive unnecessary ICD implantation, and derive no benefit from it, but possible harm stemming from the ICDs' potentially serious adverse effects (21).

The study of heart rate and blood pressure variability (Tables 3-4) gave some unexpected results. Since BRS and IBI are normally correlated (higher BRS at longer IBI), one would have expected carriers to have higher BRS values than controls, since their supine IBIs were considerably longer. BRS by Fourier analysis is computed as the square root of interval
power over systolic pressure power in the LF-band (ms/mmHg, see Methods). The LF-numbers for systolic variances (averages 13.3 and 6.8 mmHg² in controls and carriers, respectively) tally with the HRV-LF variances of 2098 and 1330 ms² ending up in the computed almost equal BRSs. In view of the large standard deviations and the number of comparisons, we consider only the dominance of parasympathetic control in the supine position a result that distinguishes carriers from controls, as proven by the lower heart rates and lower LF/HF. Therefore, our study seems to indicate that the DPP6 haplotype carriers have a higher vagal outflow at rest, which can suddenly, without apparent cause, drop. So compared to LQTS patients, these patients are not per se characterized by an absolute higher sympathetic activity but more by a relative higher parasympathetic activity (22,23).

We can only speculate about the mechanisms that underlie the increased vulnerability to VF of risk haplotype carriers. In the heart, the putative increase in DPP6 activity would result in increased $I_{to}$. This current is present in atrial and ventricular cardiomyocytes, and causes initial (phase 1) repolarization of the action potential (24), thereby setting the initial plateau potential of phase 2 and modulating inward and outward currents during phase 2, in particular, the voltage-gated L-type calcium current $I_{Ca-L}$. In this way, $I_{to}$ controls action potential duration and amplitude. $I_{to}$ is carried by a voltage-gated transmembrane ion channel consisting of four Kv4.3 $\alpha$-subunits (25). Cell surface expression and channel properties are influenced by several accessory subunits, including DPP6 (7,8) (in addition to KChIP2 (26), Kvβ (27), and MinK or MinK related peptide (28)). Kv4 $\alpha$-subunit expression on the cell surface increases when co-expressed with DPP6. (7) DPP6 also modifies $I_{to}$ gating properties. (7,8,18)

Of note, $I_{to}$ density and properties are modulated by $\alpha$-adrenergic and $\beta$-adrenergic stimulation through G-protein coupled pathways (29). This regulation links $I_{to}$ to control by the ANS. One possible mechanism by which net increase in $I_{to}$ may facilitate VF occurrence is
through its effect to counteract $I_{Ca-L}$ activation, resulting in action potential abbreviation and increased susceptibility to re-entrant excitation, analogous to the mechanism proposed to underlie arrhythmia occurrence in Brugada syndrome (30). Accordingly, the $I_{to}$ blocker quinidine prevents VF in risk haplotype carriers. While DPP6 and $I_{to}$ also impact neuronal excitability, the mechanisms by which abnormal (increased) function of DPP6 in neurons (e.g., of the ANS) may elicit cardiac arrhythmia is less clear.

A study limitation is the small size of the study population. To compound this difficulty, inclusion of symptomatic carriers, i.e., those who survived VF, is extremely difficult as survival rate after out-of-hospital VF is low (~20%) (31). Moreover, potential study subjects cannot be included if they use β-adrenoceptor or antiarrhythmic drugs, as these drugs impact on HRV and $^{123}$I-mIBG scintigraphy. Yet, these drugs (quinidine) are often prescribed to these individuals to prevent VF recurrence. Another limitation is that, while analysis of QT variability or other indicators of heterogeneity of repolarization might give valuable information (in particular, information on sympathetic control possibly superior to information gained from HRV analysis (32)) we could not conduct such analyses, because data needed for these analyses were not systematically collected. Despite these limitations, we believe that this study is of significant novelty value, because it provides indications that an inherited SCA syndrome is based on primary derangements in two organ systems (heart and ANS) which conspire to increase VF risk.

**Conclusions**

Although in part speculative this study provides clinical functional suggestions that the primary pathophysiologic basis of an inherited SCA syndrome may lie in combined disruptions in the heart and in the ANS. This novel insight suggests that studies aimed at discovering genes
underlying inherited SCA syndromes should focus on genes which are expressed both in the heart and in neurons (33). Moreover, our findings suggest that $^{123}$I-mIBG scintigraphy may be used for risk stratification and identification of risk haplotype carriers with elevated VF risk in whom ICD implantation should be considered.
Acknowledgements

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References


Figure legends

**Figure 1:** Heart rate variability

Top left: In supine position the normalized LF (filled squares) is significantly lower in carriers than controls \((p=0.001)\). The normalized HF (open squares) is significantly higher in carriers than controls \((p=0.004)\). Top middle: During active standing there were no significant differences in LF or HF between carriers and controls. Top right: only the standing-to-supine ratios of normalized LF were higher in carriers than controls \((p=0.022)\).

Bottom row: There were no significant differences in normalized LF, normalized HF or standing-to-supine ratios between symptomatic and asymptomatic carriers.

**Figure 2:** Sudden drop in interbeat interval in supine position with attendant changes in stroke volume and mean arterial pressure in one patient just before standing up.

**Figure 3:** \(^{123}\text{I}-\text{mIBG}\) SPECT.

There were no differences in SPECT-score between carriers and controls (left). Symptomatic carriers more often had a more homogeneous distribution of \(^{123}\text{I}-\text{mIBG}\) on SPECT (right).
Figure 1: Heart rate variability (normalized units).
Figure 2: Patient example.
Figure 3: $^{123}$I-mIBG SPECT.
Table 1: Characteristics of study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Carriers (n=14)</th>
<th>p-value</th>
<th>Carriers (n=6)</th>
<th>Asymptomatic (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>4</td>
<td>9</td>
<td>0.75</td>
<td>4</td>
<td>5</td>
<td>0.87</td>
</tr>
<tr>
<td>Age (SD), years</td>
<td>33.6±5.8</td>
<td>39.2±10.0</td>
<td>0.19</td>
<td>38.2±9.7</td>
<td>40.0±10.9</td>
<td>0.75</td>
</tr>
<tr>
<td>BMI (SD), kg/m²</td>
<td>26.2±4.4</td>
<td>28.7±5.5</td>
<td>0.31</td>
<td>27.0±3.7</td>
<td>29.9±6.5</td>
<td>0.35</td>
</tr>
<tr>
<td>ICD</td>
<td>0</td>
<td>12</td>
<td>n.a.</td>
<td>6</td>
<td>6</td>
<td>n.a.</td>
</tr>
<tr>
<td>ICD shocks *</td>
<td>0</td>
<td>4</td>
<td>n.a.</td>
<td>4</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aborted VF</td>
<td>0</td>
<td>6</td>
<td>n.a.</td>
<td>6</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>Syncope</td>
<td>0</td>
<td>2</td>
<td>n.a.</td>
<td>2</td>
<td>0</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean±standard deviation and tested with an unpaired Student’s t-test. Binary variables are presented as absolute numbers and tested with a chi-square test. * Number of patients who had received appropriate ICD shocks prior to inclusion.
Table 2: Numbers and circumstances of VF episodes.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Age at first VF episode</th>
<th>Number of VF episodes before inclusion</th>
<th>VF during vagal condition</th>
<th>Circumstances of VF episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>32</td>
<td>30</td>
<td>1</td>
<td>Yes</td>
<td>Alcohol intake</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>38</td>
<td>34</td>
<td>3</td>
<td>Yes</td>
<td>Passenger in a car (1), sleep (2)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>31</td>
<td>30</td>
<td>4</td>
<td>Yes</td>
<td>Alcohol intake (1), office (2), unknown (1)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>38</td>
<td>33</td>
<td>5</td>
<td>Yes</td>
<td>Alcohol intake (2), fever (1), rest (2)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>57</td>
<td>52</td>
<td>3</td>
<td>Yes</td>
<td>Sleep (3)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>33</td>
<td>32</td>
<td>1</td>
<td>Yes</td>
<td>One hour after exercise</td>
</tr>
</tbody>
</table>

M=male, F=female. Age (at first VF episode) in years.
The number of VF episodes during a described condition is given behind the description.
Table 3: Heart rate variability and blood pressure measures in carriers (symptomatic and asymptomatic combined) and controls.

<table>
<thead>
<tr>
<th></th>
<th>SUPINE POSTION</th>
<th>STANDING POSTION</th>
<th>STANDING TO SUPINE RATIOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=7)</td>
<td>Carriers (n=14)</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Heart rate variability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean IBI (SD)</td>
<td>0.81±0.07</td>
<td>1.03±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD-IBI (SD)</td>
<td>0.06±0.02</td>
<td>0.06±0.03</td>
<td>0.993</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td>3,687±2,773</td>
<td>4,248±4,414</td>
<td>0.764</td>
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<tr>
<td>VLF (ms²)</td>
<td>571±352</td>
<td>696±618</td>
<td>0.627</td>
</tr>
<tr>
<td>LF norm (n.u.)</td>
<td>56.9±16.5</td>
<td>31.3±13.8</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>HF norm (n.u.)</td>
<td>32.0±13.5</td>
<td>57.317.9</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>LF/HF</td>
<td>2.11±1.10</td>
<td>0.68±0.50</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>BRS (ms/mmHg)</td>
<td>12.55±5.16</td>
<td>13.95±8.00</td>
<td>0.681</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Systolic (mmHg)</td>
<td>126±17</td>
<td>125±16</td>
<td>0.885</td>
</tr>
<tr>
<td>SD</td>
<td>5.0±1.4</td>
<td>4.5±1.0</td>
<td>0.369</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean±standard deviation and tested with an unpaired Student’s t-test.
Table 4: Heart rate variability and blood pressure measures in symptomatic and asymptomatic carriers.

<table>
<thead>
<tr>
<th></th>
<th>SUPINE POSITION</th>
<th></th>
<th>STANDING POSITION</th>
<th></th>
<th>STANDING TO SUPINE RATIOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic (n=6)</td>
<td>Asymptomatic (n=8)</td>
<td>p-value</td>
<td>Symptomatic (n=6)</td>
<td>Asymptomatic (n=8)</td>
</tr>
<tr>
<td><strong>Heart rate variability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean IBI (SD)</td>
<td>1.07±0.11</td>
<td>1.0±0.10</td>
<td>0.322</td>
<td>0.83±0.13</td>
<td>0.79±0.10</td>
</tr>
<tr>
<td>SD-IBI (SD)</td>
<td>0.07±0.03</td>
<td>0.05±0.03</td>
<td>0.417</td>
<td>0.06±0.02</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Total power (ms²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF (ms²)</td>
<td>694±431</td>
<td>697±760</td>
<td>0.444</td>
<td>927±461</td>
<td>376±327</td>
</tr>
<tr>
<td>LF norm (n.u.)</td>
<td>36.0±15.7</td>
<td>27.9±12.0</td>
<td>0.294</td>
<td>57.7±24.5</td>
<td>49.3±17.5</td>
</tr>
<tr>
<td>HF norm (n.u.)</td>
<td>59.2±14.5</td>
<td>55.8±21.0</td>
<td>0.740</td>
<td>28.0±22.07</td>
<td>38.0±19.07</td>
</tr>
<tr>
<td>LF/HF</td>
<td>0.73±0.60</td>
<td>0.64±0.46</td>
<td>0.774</td>
<td>3.86±3.46</td>
<td>1.76±1.28</td>
</tr>
<tr>
<td>BRS (ms/mmHg)</td>
<td>16.2±8.78</td>
<td>12.26±7.49</td>
<td>0.382</td>
<td>8.04±2.31</td>
<td>5.65±2.45</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Systolic (mmHg)</td>
<td>131±15</td>
<td>121±16</td>
<td>0.295</td>
<td>128±18</td>
<td>126±9</td>
</tr>
<tr>
<td>SD</td>
<td>4.3±1.4</td>
<td>4.6±0.7</td>
<td>0.641</td>
<td>6.5±2.7</td>
<td>4.8±0.7</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean±standard deviation and tested with an unpaired Student’s t-test.
Table 5: ¹²³I-mIBG scintigraphy.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=7)</th>
<th>Carriers (n=14)</th>
<th>Symptomatic (n=6)</th>
<th>Asymptomatic (n=8)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹²³I-mIBG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M (SD)</td>
<td>2.73±0.28</td>
<td>2.61±0.46</td>
<td>2.56±0.35</td>
<td>2.65±0.55</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Late H/M (SD)</td>
<td>3.08±0.70</td>
<td>2.74±0.59</td>
<td>2.68±0.27</td>
<td>2.78±0.77</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Washout, % (SD)</td>
<td>-12.2±16.2</td>
<td>-4.9±12.3</td>
<td>-5.7±14.6</td>
<td>-4.2±11.3</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>¹²³I-mIBG SPECT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median score (range)</td>
<td>2 (0-5)</td>
<td>3 (0-7)</td>
<td>1 (0-3)</td>
<td>4 (2-7)</td>
<td>0.25</td>
<td>0.13</td>
</tr>
<tr>
<td>Heterogeneous SPECT (%)</td>
<td>3</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>0.66</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean±standard deviation or median (range) and tested with an unpaired Student’s t-test. Categorical variables are presented as absolute numbers and tested with a Fisher’s Exact test.