Chapter 11

Critical QRS Prolongation after Premature Stimulation is Associated with Polymorphic Ventricular Tachycardia in Nonischemic Cardiomyopathy: Results from the Leiden Nonischemic Cardiomyopathy Study

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Accepted for publication in Heart Rhythm

ABSTRACT

Background

Progressive activation delay after premature stimulation has been associated with ventricular fibrillation in nonischemic cardiomyopathy (NICM). The purpose of this study was to (1) investigate prolongation of the paced QRS duration (QRSd) after premature stimulation as a marker of activation delay in NICM, (2) assess its relation to induced ventricular arrhythmias, and (3) analyze its underlying substrate by late gadolinium enhancement CMR (LGE-CMR) and endomyocardial biopsy.

Methods and results

Forty patients with NICM (age 57±14years, 83% male, LVEF 30±13%) were prospectively enrolled in the Leiden Nonischemic Cardiomyopathy Study and underwent a comprehensive evaluation including LGE-CMR, electrophysiological study (EPS) and endomyocardial biopsy. Twenty patients without structural heart disease served as controls for EPS. After the 400 ms drive train and progressively premature stimulation, the maximum increase in QRSd was larger in NICM than in controls (35±18ms vs. 23±12ms, p=0.005) and the coupling interval window with QRSd prolongation was wider (47±23ms vs. 31±14ms, p=0.005). The maximum paced QRSd exceeded the ventricular refractory period allowing for pacing before the QRS offset in 20/39 NICM patients vs. 1/20 controls (p<0.001). In NICM, QRSd prolongation was associated with polymorphic VT inducibility (16/39 patients), and was related to long thick strands of fibrosis in biopsies, but not to focal enhancement on LGE-CMR (all p>0.05).

Conclusions

QRSd is a simple parameter to quantify activation delay after premature stimulation and its prolongation is associated with the inducibility of polymorphic VT and with the pattern of myocardial fibrosis in biopsies.

Clinical Trial Registration—clinicaltrials.gov. Identifier: NCT01940081.

INTRODUCTION

Implantable cardioverter defibrillator (ICD) therapy can terminate, but does not prevent potentially life-threatening ventricular arrhythmias. Although progress has been made to treat monomorphic ventricular tachycardia (VT), therapies that prevent polymorphic VT and ventricular fibrillation (VF) in nonischemic cardiomyopathy (NICM) are lacking. Improved understanding and identification of the underlying substrate for these arrhythmias may allow personalized risk stratification and development of tailored treatment.

Activation delay following premature stimulation has been associated with a history of VF in patients with various non-coronary heart diseases based on intracardiac recordings from the right ventricle (RV).[1] Total biventricular activation delay may be quantified by measuring paced QRS duration (QRSd) on surface ECGs, which is easy to apply and may better reflect activation delay in left dominant myocardial disease.

The purpose of this study was to (1) investigate prolongation of the paced QRSd following decremental premature stimulation as a marker of activation delay in NICM, (2) assess its relation to induced ventricular arrhythmias, and (3) analyze its association with the extent and distribution of fibrosis in endomyocardial biopsy specimens and on late gadolinium enhancement CMR (LGE-CMR).

METHODS

Patients

The Leiden Cardiomyopathy Study is a single-center, prospective cohort study designed to analyze the substrate and mechanisms of ventricular arrhythmias in NICM (ClinicalTrials.gov Identifier: NCT01940081). From October 2011, patients 18-80 years old with NICM and documented sustained ventricular arrhythmia, suspected sustained ventricular arrhythmia (e.g. because of out-of-hospital cardiac arrest, palpitations or syncope) or considered to be at intermediate or high risk for ventricular arrhythmias were enrolled. A high risk for ventricular arrhythmias was defined as a left ventricular ejection fraction (LVEF) ≤ 35%; an intermediate risk as LVEF ≤ 50% and late enhancement on LGE-CMR. Exclusion criteria were inability to understand the nature and risks of the study procedures, pregnancy, inability to comply with the protocol due to hemodynamic instability, and other cardiomyopathies (e.g. prior myocardial infarction, sarcoidosis, infiltrative cardiac disease such as amyloidosis, Chagasic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy, non-compaction cardiomyopathy and congenital heart disease).

Patients underwent a comprehensive evaluation including transthoracic echocardiography, LGE-CMR, 24-hour Holter monitoring, exercise testing, blood sampling for NT-proBNP levels, an invasive electrophysiological study, endomyocardial biopsy, iodine-123 metaiodobenzylguanidine (MIBG) scan and genetic analysis of 55 cardiomyopathy-related genes. Targeted next generation sequencing of these 55 genes, a list of which is provided in the Supplemental Methods, was performed analyzing 151 bp paired-end reads on an Illumina MiSeq sequencer, as previously described.[2] The (likely) pathogenicity of single nucleotide polymorphisms and small indels was based on the nature and location of the variant, conservation of the affected amino acid residue(s) and the surrounding region, frequency of the variant in (healthy) population databases, its predicted pathogenicity according to multiple available prediction programs, the scientific literature and/or variant databases.

Premature ventricular contraction (PVC) or VT ablation and ICD implantation were performed if clinically indicated. The study protocol was approved by the local ethics committee and by the appropriate national ethics committee. All patients provided written informed consent.

The control group for electrophysiologic study measurements consisted of 20 patients referred for catheter ablation of atrioventricular nodal reentrant tachycardia or a concealed bypass tract in the absence of structural heart disease, who underwent routine electrophysiological study according to our institutional protocol.

LGE-CMR acquisition

In patients who had not undergone LGE-CMR previously, CMR was performed on a 1.5T Gyroscan ACS-NT/Intera MR system, or on a 3.0T Ingenia MR system (both Philips Medical Systems, Best, the Netherlands). A standardized protocol was followed, including cine images in long-axis (two- and four-chamber) and short-axis views covering the complete LV. Approximately 15 minutes after bolus injection of gadolinium (Magnevist; Schering, Berlin, Germany; 0.15 mmol/kg) a look-locker sequence was acquired in short axis orientation at mid-ventricular level. T1-weighted LGE images were acquired with an inversion-recovery 3D turbo-field echo sequence with parallel imaging. The inversion time was optimized to null normal appearing myocardium. The heart was imaged in long-axis two- and four-chamber views (between 5-10 slices), and short-axis views (between 20-24 slices). Signal outside the field of view was suppressed using two saturation slabs to avoid fold-over artifacts.

In patients who had already undergone CMR previously, the scan was acquired on various systems, but always included cine acquisitions in long-axis (two- and four-chamber) and short-axis views, covering the complete left ventricle (LV). LGE images were acquired in long-axis (two- and four-chamber) and short-axis views. CMR was not performed in patients with an implanted device.

LGE-CMR analysis

All CMR analyses were performed using MASS software (research version 2012; LKEB; Leiden University Medical Center, the Netherlands). The LV and RV end-diastolic and end-systolic endocardial contours were traced on short-axis cine images to calculate the LV end-diastolic and end-systolic volumes, LVEF, LV mass, RV end-diastolic and end-systolic volumes, and RV ejection fraction. The LV and RV volumes and the LV mass were indexed to body surface area.[3] Myocardial scar was only considered to be present if LGE was visible in 2 orthogonal views. LGE was defined by signal intensity ≥35% of maximal myocardial signal intensity, and subdivided into core (≥50% of maximal signal intensity) and border zone (35-50% of maximal signal intensity).[4]

Electrophysiological study

Anti-arrhythmic drugs were discontinued for at least 5 half-lives, if possible. Programmed electrical stimulation consisted of 3 drive trains of 8 beats (S1) (cycle lengths (CL) 600, 500 and 400ms) with 1-3 ventricular extrastimuli and burst pacing at twice diastolic threshold from the RV apex and the RV outflow tract using the distal electrode pair of a quadripolar catheter. The first extrastimulus (S2) was applied at a coupling interval identical to the CL of the drive train, followed by a coupling interval (CI) of 350ms, which was then progressively decreased by steps of 10ms until the ventricular effective refractory period (VERP) was reached. Consecutive extrastimuli (S3 and S4) and burst pacing were applied from a coupling interval of 350ms down to 200ms. Polymorphic VT was defined by ≥5 consecutive ventricular beats at ≥ 120 bpm with continuously changing QRS morphology,[5,6] and was regarded as sustained if lasting > 30s or requiring termination because of hemodynamic compromise. Sustained monomorphic VT was defined by similar beat-to-beat QRS morphology and duration >30s or the requirement for termination because of hemodynamic compromise. If no sustained ventricular arrhythmia was induced, stimulation was repeated with isoprenaline (2-10µg/min).

In the control group for electrophysiology study measurements, drive trains of 600 and 400 ms with single extrastimuli were applied from the RV apex as part of the routine electrophysiological study protocol. All studies were recorded on an electrophysiology recording system for offline analysis, with a sweeping speed of 200 mm/second and bipolar electrograms filtered at 30-500 Hz (Prucka Cardiolab recording system, Houston, Texas).

Electrophysiological study analysis

The paced QRSd was measured from the pacing artifact to the latest offset of the QRS in any lead (examples in Figure 1) using electronic calipers. For the 600ms and 400ms drive trains with single extrastimuli from the RV apex, QRSd restitution curves were constructed and the following parameters were derived:

(1) CI at onset of QRSd prolongation (CI_{onset}): the CI of the first premature extrastimulus that results in a ≥5 ms increase in QRSd compared to the previous step.
(2) CI window with QRSd prolongation: the interval between CI_{onset} and the VERP.
(3) Maximal ΔQRSd: maximum increase in QRSd after premature stimulation.
(4) Critical QRSd prolongation: the QRSd that exceeds the length of the VERP, which allows to pace before the offset of the QRS (calculated as QRSd-VERP).
(5) Critical CI ($CI_{critical}$) window: the CI window at which the paced QRSd exceeds the length of the VERP.

Examples of QRS restitution curves and calculations of the derived parameters are displayed in Figure 1.

Endomyocardial biopsy

After the electrophysiological study, endomyocardial biopsies were obtained – immediately thereafter, two were stored in liquid nitrogen and one specimen was fixed in 4% formaldehyde and embedded in paraffin. Cross-sections were cut at 4 μm and stained with picro-Sirius Red to evaluate collagen content and collagen distribution patterns. Mayer's haematoxylin was used to counterstain cell nuclei. Using a fluorescent microscope, high-resolution photomicrographs were taken with a digital camera (Nikon Eclipse, Nikon Europe, Badhoevedorp, the Netherlands) and analyzed using dedicated software (ImageJ 1.49c, National Institutes of Health, USA). All staining and subsequent analyses were performed by an independent investigator blinded to all clinical and procedural data. Fibrosis content was quantified by calculating the collagen area percentage. Distribution of fibrosis was categorized according to predefined patterns:

1) Interstitial: collagen strand thickness exceeds 10% of the width of adjacent cardiomyocytes without interrupting myocardial fiber continuity.
2) Short thin strands: dispersed presence of two or more fibrotic areas smaller than the width of five cardiomyocytes that disrupt myocardial fiber continuity.
3) Long thick strands: one or more fibrotic areas exceeding the width of five cardiomyocytes that disrupt the myocardial fiber continuity.

Underlying substrate of QRS prolongation

The relation between QRSd restitution curve parameters and the following parameters was analyzed: 1) CMR-derived LV and RV volumes and ejection fraction, 2) LGE on CMR, 3) NT-proBNP levels, 4) collagen area percentages in biopsy specimens, 5) pattern of fibrosis in biopsy specimens and 6) the presence of a pathogenic or likely pathogenic mutation.

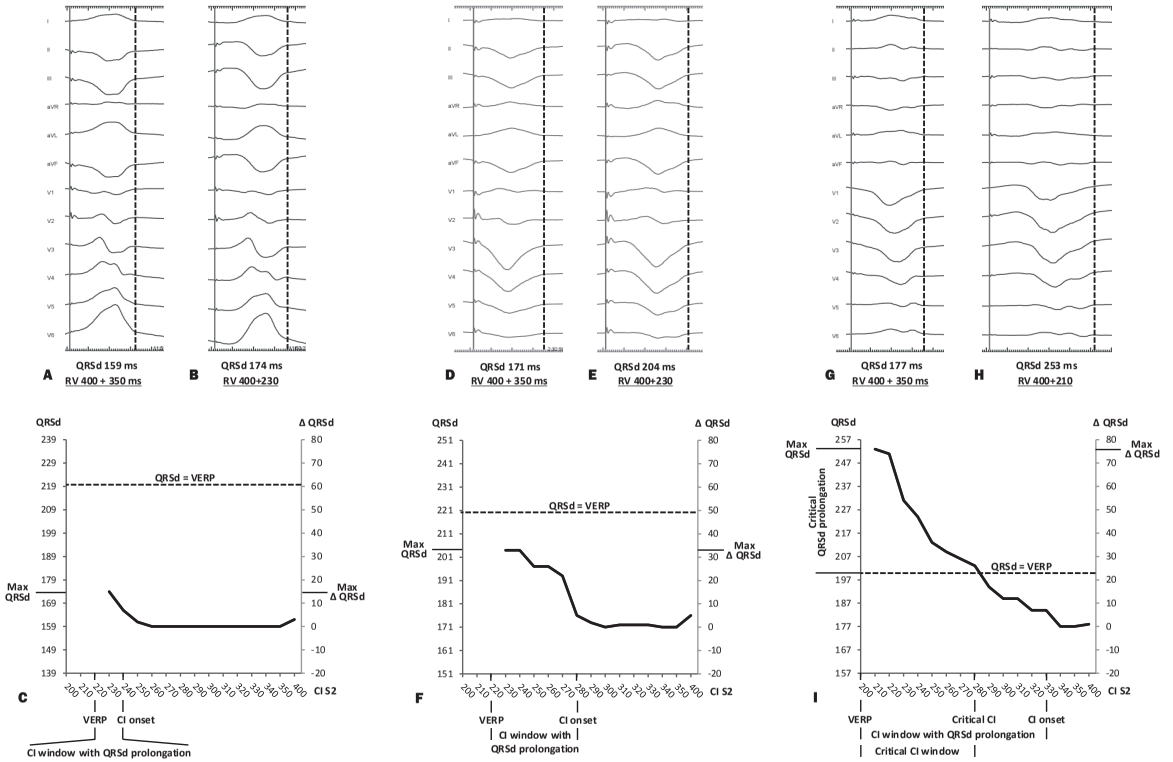

Figure 1. Examples of QRS duration restitution curves and derived parameters

QRS duration measurements and restitution curves in a control without structural heart disease after premature stimulation (panels A-C), on a patient with cardiomyopathy and more pronounced QRSd prolongation, but without *critical* QRSd prolongation (panels D-F), and in a patient with cardiomyopathy and marked *critical* QRSd prolongation after premature stimulation (panels G-I). In this patient the maximal QRSd exceeds the VERP (indicated by horizontal dashed line) and therefore S2 can be applied within the QRS complex of S1. CI indicates coupling interval; VERP, ventricular effective refractory period.

Statistical analysis

Categorical variables are displayed as number (percentage) and continuous variables are expressed as mean ± standard deviation or median (interquartile range, IQR). Categorical variables were compared using the χ^2 test or the Fisher's exact test. Continuous variables were compared using the Mann-Whitney *U* test because of the limited sample size. For paired variables the Wilcoxon signed-rank-test was used. The association between QRS restitution curve parameters and patient characteristics was analyzed by univariate linear regression analyses. For this purpose, NT-proBNP levels were log-transformed because of the skewed distribution. All analyses were performed with SPSS version 20.0 (IBM, Somers, New York, USA). All tests are two-sided and probability values <0.05 were considered significant.

RESULTS

Patients

Between October 2011 and August 2013, 40 patients (age 57 ± 14 years, 83% male) were enrolled in the Leiden Nonischemic Cardiomyopathy Study. Baseline characteristics are summarized in Table 1. Eight patients (20%) had documented sustained ventricular arrhythmia, 6 (15%) had suspected sustained ventricular arrhythmia, and 26 (65%) were considered to be at intermediate or high risk for ventricular arrhythmia. Cine CMR was performed in 36 patients (90%), while the remaining 4 (10%) had an implanted device and underwent echocardiography. Based on combined CMR and echo data the LVEF was reduced in all patients and the LV end-diastolic volume index was increased in 34 patients (85%) compared with published reference ranges normalized for gender, age and body surface area.[7] LGE was observed in 26 of 34 (77%) patients with LGE images and the median LGE mass was 8.8 g (IQR, 1.1–19.3 g). CMR and biopsy data are summarized in Table 2. Genetic analysis of 55 cardiomyopathy-related genes was performed in all but one patient and revealed a pathogenic or likely pathogenic mutation in 19 (49%) – details are listed in supplemental Table 1.

QRSd prolongation in NICM patients and controls

Both in NICM and in controls, the QRSd increased after premature stimulation (examples in Figure 1). Differences between NICM patients and controls were more pronounced after the 400 ms drive train than after the 600 ms drive train (supplemental Figure 1 and supplemental Table 2) and therefore, the 400 ms drive train was used for further analyses. After the 400 ms drive train, the onset of QRSd prolongation occurred at longer CI (CI_{onset}) in NICM compared to controls (275±23 vs. 235±16 ms, p<0.001, Figure 2), the CI window with QRSd prolongation was wider (47±23 vs. 31±14 ms, p=0.005), and the

Table 1. Baseline characteristics

	n=40
Age, years	57 ± 14
Male	33 (83%)
BMI, kg/m^2	27 ± 4
Diabetes mellitus	6 (15%)
Hypertension	10 (25%)
NYHA functional class	
I	7 (18%)
II	20 (50%)
III or IV	13 (33%)
History of AF / atrial flutter	16 (40%)
ECG	
Heart rate, bpm	73 ± 13
PR interval, ms	182 ± 32
QRS duration, ms	129 ± 34
eGFR, mL/min/1.73m^2	86 ± 25
NT-proBNP, pg/mL	932 (499 - 1773)
Exercise test (n=29)	
Peak oxygen consumption, mL/kg/min	20 ± 6
Peak oxygen consumption, % of predicted	80 ± 22
AAD during electrophysiology study	
Class I	0 (0%)
Sotalol	1 (3%)
Amiodarone	2 (5%)
Device at discharge	
CRT-defibrillator	20 (50%)
Implantable cardioverter-defibrillator	13 (33%)
None	7 (18%)
Genetic screening (n=39)	
Pathogenic or likely pathogenic mutation	19/39 (49%)

Variables are expressed as number (percentage), mean ± standard deviation, or median (interquartile range). AAD indicates anti-arrhythmic drugs; AF, atrial fibrillation; BMI, body mass index; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate; NYHA, New York Heart Association

maximum Δ QRSd was larger (35±18 vs. 23±12 ms, p=0.005). Critical QRS prolongation (i.e., QRSd exceeding VERP, which allowed to apply an extrastimulus within the paced QRS complex) was observed in 20 of 39 patients with NICM (51%) compared with only one of 20 controls (5%, p<0.001) (Figure 1 panels G-I, Figure 2, example Figure 3). The critical CI window, with QRSd exceeding VERP, ranged from 10 to 130 ms in NICM, compared with only one case with a critical CI window of 10 ms in controls (p<0.001, Figure

Table 2. CMR and biopsy data

Parameter	Value
CMR - cine images (n=36)	
LVEDV, mL	303 ± 90
LVEDV index, mL/m^2	147 ± 42
LVESV, mL	221 ± 93
LVESV index, mL/m^2	107 ± 44
LVEF, %	30 ± 13
LV mass, g	143 ± 34
LV mass index, g/m^2	70 ± 16
RVEDV, mL	189 ± 45
RVEDV index, mL/m^2	92 ± 19
RVESV, mL	105 ± 39
RVESV index, mL/m^2	51 ± 18
RVEF, %	46 ± 11
CMR - LGE images (n=34)	
LGE presence	26/34 (77%)
LGE total, g	8.8 (1.1 - 19.3)
Core mass, g	3.3 (0.4 - 8.7)
Border zone mass, g	4.9 (0.6 - 13.4)
Endomyocardial biopsy (n=23)	
Collagen area percentage, %	33 (19 - 42)
Collagen distribution type	
Interstitial	8/23 (35%)
Short thin strands	7/23 (30%)
Long thick strands	8/23 (35%)

Variables are expressed as number (percentage), mean ± standard deviation, or median (interquartile range). LGE denotes late gadolinium enhancement; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVESV, LV end-systolic volume; RVEDV, RV end-diastolic volume; RVEF, RV ejection fraction; RVESV, RV end-systolic volume.

2). Results were similar when excluding the 3 cardiomyopathy patients who were on class III anti-arrhythmic drugs.

QRSd prolongation and inducibility of polymorphic VT

The programmed electrical stimulation protocol could be successfully completed, or resulted in reproducible induction of sustained ventricular arrhythmias in 39 of 40 NICM patients (98%). Sustained monomorphic VT was induced in 11 of 39 patients (28%) and polymorphic VT ≥ 5 beats in 16 of 39 (41%) (Figure 3). The longest polymorphic VT was induced with triple extrastimuli in all 16 patients. None of the baseline characteristics were associated with polymorphic VT inducibility (Table 3).

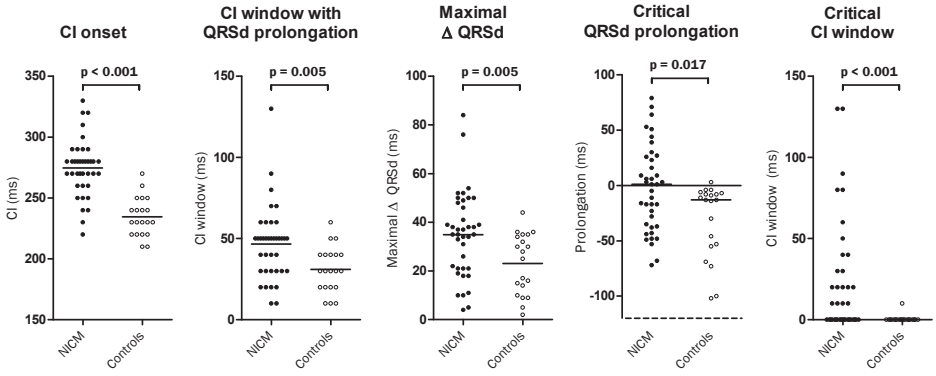

Figure 2. QRS duration prolongation in nonischemic cardiomyopathy and controls
The QRS duration restitution curve parameters based on 400 ms drive trains with single extrastimuli differed significantly between patients with cardiomyopathy and controls.

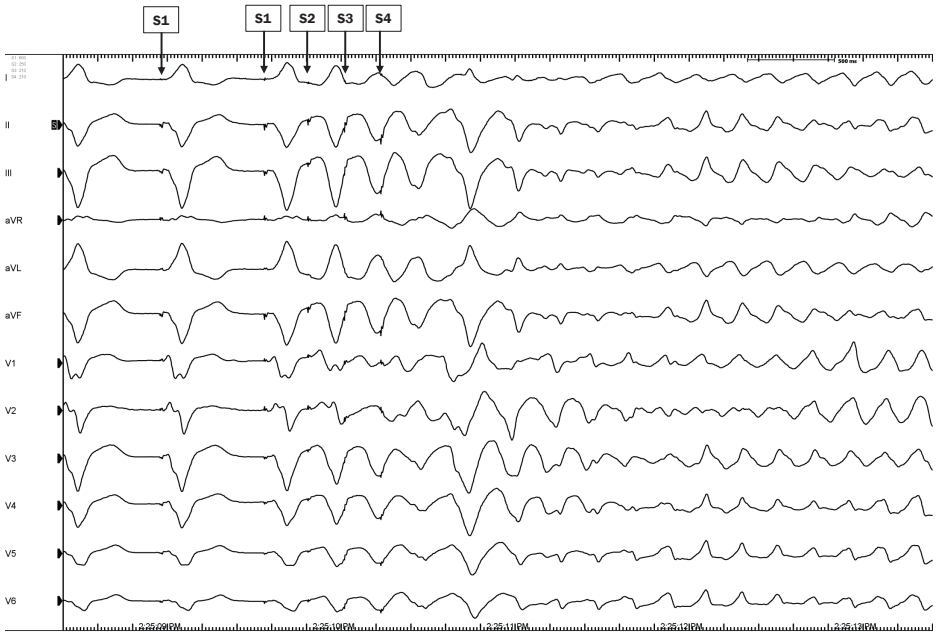

Figure 3. Example of pacing within the QRS complex and induction of polymorphic VT
During application of triple extrastimuli after a 400 ms drive train, the QRS complex prolongs after premature stimulation. S3 and S4 are applied within the paced preceding QRS complexes, resulting in the induction of polymorphic VT.

In contrast, four of five QRSd restitution curve parameters were associated with the inducibility of polymorphic VT (Figure 4 and supplemental Table 3). The CI at onset of QRS prolongation was similar in patients with vs. without inducible polymorphic VT (276 ± 20 ms vs 274 ± 26 ms, p=0.92), but the CI window with QRSd prolongation was wider

Table 3. Baseline characteristics and inducible polymorphic VT

	PVT (n=16)	No PVT (n=23)	P
Age, years	60 ± 13	56 ± 13	0.40
Male	13 (81%)	19 (83%)	1.00
BMI, kg/m^2	27 ± 4	28 ± 4	0.47
Diabetes mellitus	9 (56%)	7 (30%)	0.11
Hypertension	4 (25%)	6 (26%)	1.00
NYHA functional class III or IV	4 (25%)	9 (39%)	0.36
History of AF / atrial flutter	2 (13%)	4 (17%)	1.00
eGFR, mL/min/1.73m^2	87 ± 24	86 ± 27	1.00
NT-proBNP, pg/mL	1058 (487 - 2232)	872 (498 - 1646)	0.64
ECG			
Heart rate	72 ± 15	73 ± 12	0.77
PR interval	188 ± 33	181 ± 31	0.49
QRS duration	126 ± 32	133 ± 36	0.69
Exercise test			
Peak oxygen consumption, mL/kg/min	19 ± 7	20 ± 5	0.65
Peak oxygen consumption, % of predicted	81 ± 28	80 ± 16	0.91
Genetic screening			
Pathogenic or likely pathogenic mutation	8/16 (50%)	10/22 (46%)	0.78
MRI - cine images			
LVEDV index, mL/m^2	136 ± 44	153 ± 41	0.27
LVEF, %	35 ± 15	27 ± 12	0.19
LV mass index, g/m^2	67 ± 7	71 ± 19	0.51
RVEDV index, mL/m^2	96 ± 22	89 ± 18	0.63
RVEF, %	41 ± 13	49 ± 10	0.057
MRI - LGE images			
LGE presence	11/13 (85%)	15/20 (75%)	0.68
LGE total, g	15.0 (4.3 - 22.7)	8.2 (0.4 - 22.6)	0.32
LGE core, g	5.5 (1.9 - 11.1)	2.9 (0.1 - 8.4)	0.41
LGE BZ, g	7.7 (2.4 - 14.0)	4.3 (0.2 - 14.0)	0.39
Endomyocardial biopsy specimens			
Collagen area percentage, %	22 (10 - 39)	38 (23 - 51)	0.093
Interstitial fibrosis	3/10 (30%)	4/12 (33%)	
Short thin strands	2/10 (20%)	5/12 (42%)	0.42
Long thick strands	5/10 (50%)	3/12 (25%)	

PVT denotes polymorphic ventricular tachycardia. Other abbreviations as in tables 1 and 2.

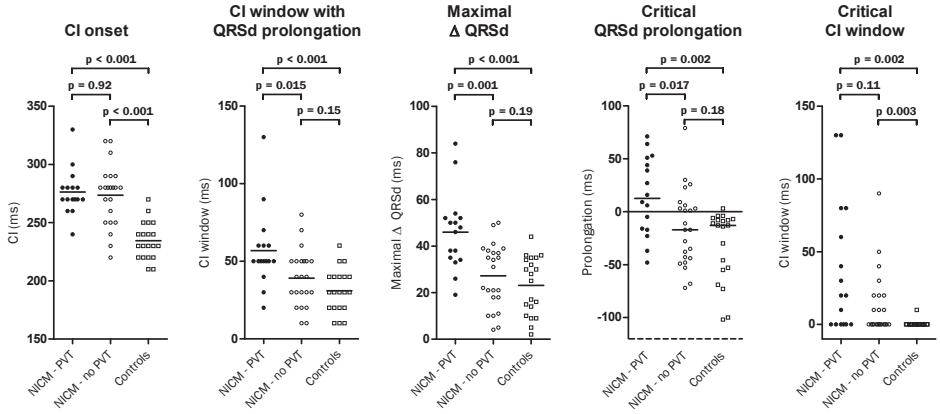

Figure 4. QRS duration prolongation and inducibility of polymorphic VT
Three QRS duration restitution curve parameters were associated with the inducibility of polymorphic VT in patients with cardiomyopathy.

(57 ± 25 ms vs. 39 ± 18 ms, p=0.015) and the maximum Δ QRSd was larger (46 ± 17 ms vs 27 ± 14 ms, p=0.001). The critical QRS prolongation was significantly longer, allowing to pace further within the QRS (15 ± 37 ms vs -15 ± 36 ms, p=0.017) and the critical CI window tended to be wider, thereby allowing to pace within the QRS over a wider range of CIs (median 20 ms, IQR 0-75 ms vs. median 0 ms, IQR 0-20 ms, p=0.11).

CMR-derived data, NT-proBNP levels and genetics: relation to QRSd prolongation

Larger end-diastolic LV volume indexes were associated with a wider CI window with QRSd prolongation, and larger end-systolic LV volume indexes and lower LVEF were associated with a smaller maximal Δ QRSd, but neither was associated with any of the other QRSd restitution curve parameters (Supplemental Table 4). The extent of LGE (total LGE, core and border zone), NT-proBNP levels and the presence of a (likely) pathogenic mutation were not associated with any QRSd restitution curve parameter (all p>0.05).

Extent and distribution of fibrosis in biopsy specimens and association with QRSd prolongation

Endomyocardial biopsy was performed in 24 patients (60%; LV in 23 patients, RV side of septum in 1 patient); no biopsies were obtained in the remaining 16 patients (40%) because of a potential risk for complications or patient's preference. One set of biopsies was excluded due to poor quality. In the remaining 23 patients, the median collagen area percentage was 33% (IQR, 19 – 42%) and the collagen distribution type was interstitial in 8 (35%), short thin strands in 7 (30%) and long thick strands in 8 (35%) (examples in Figure 5). The density of fibrosis quantified as area percentages was weakly

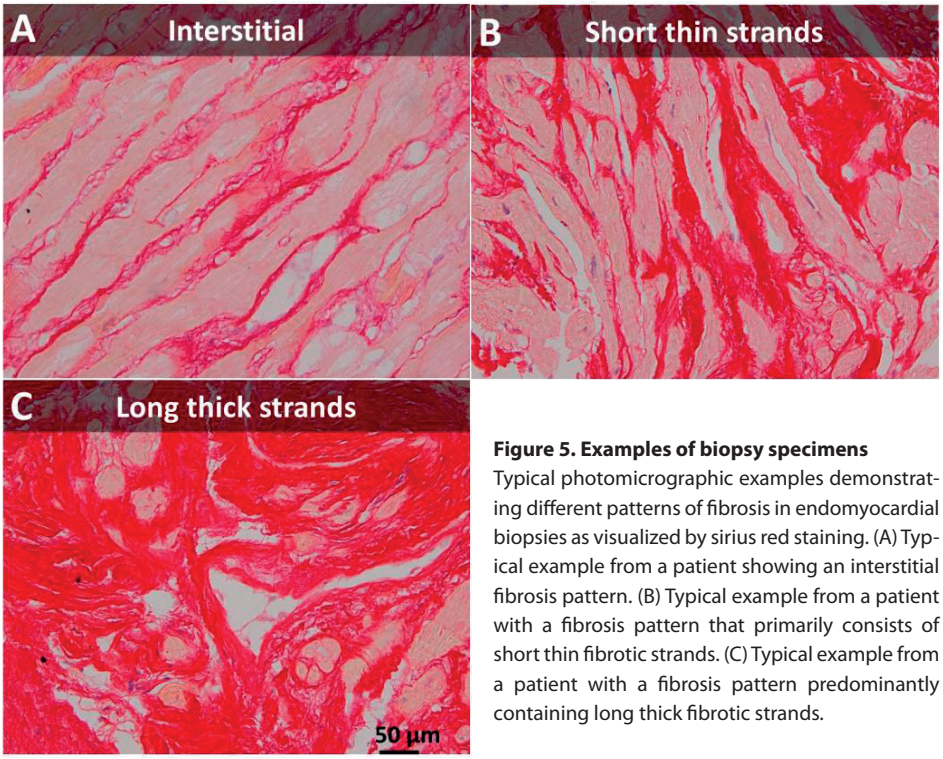

Figure 5. Examples of biopsy specimens
Typical photomicrographic examples demonstrating different patterns of fibrosis in endomyocardial biopsies as visualized by sirius red staining. (A) Typical example from a patient showing an interstitial fibrosis pattern. (B) Typical example from a patient with a fibrosis pattern that primarily consists of short thin fibrotic strands. (C) Typical example from a patient with a fibrosis pattern predominantly containing long thick fibrotic strands.

associated with a smaller maximal increase in QRSd but not with any other parameter of QRSd prolongation (Supplemental Table 4). However, long thick strands of fibrosis were associated with a wider CI window with QRSd prolongation, and with a wider critical CI window with the ability to pace within QRSd.

DISCUSSION

The key findings of this study are: (1) In patients with NICM and in controls, the paced QRSd increased after premature stimulation, (2) in NICM, the QRSd started to increase earlier, at longer CI than in controls, the maximum increase in QRSd was larger and the paced QRSd frequently exceeded the VERP (i.e., critical QRS prolongation), which allowed to apply an extrastimulus within the paced QRS complex, (3) the CI window with QRSd prolongation, the maximal ΔQRSd, and critical QRS prolongation were associated with the inducibility of polymorphic VT in NICM, (4) not the density of fibrosis but the presence of long thick strands of fibrosis was associated with a wider CI window of progressive QRS prolongation and a wider critical CI window at which the QRSd exceeds VERP, which may indicate a substrate for polymorphic VT or VF, and (5) none of the QRSd

restitution curve parameters was related to the presence or extent of LGE on CMR, NT-pro BNP levels, nor the presence of (likely) pathogenic mutations.

Quantification of activation delay after premature stimulation

Activation delay after premature stimulation has previously been quantified and related to VF in humans with various cardiac diseases, in particular hypertrophic cardiomyopathy, by applying stimulation protocols sequentially from 4 different RV catheters.[1] Left ventricular activation could not be assessed as no catheters were placed in the LV. Of interest, both normal and abnormal electrogram responses were frequently observed at the different positions of the 4 RV catheters, suggesting that conduction delay may not be uniform and therefore, multiple recording sites may be required. In contrast, the paced QRSd is easily available, can be measured during a standard programmed electrical stimulation protocol, does not require multiple intracardiac catheters and reflects both RV and LV activation.

Activation delay and polymorphic VT

In the current study we could demonstrate that the CI window with QRSd prolongation, the maximum Δ QRSd and critical QRS prolongation were associated with the inducibility of polymorphic VT. In contrast, none of the baseline characteristics, including CMR-derived volumes, ejection fraction and LGE, NT-proBNP levels and the presence of a (likely) pathogenic mutation, were associated with polymorphic VT inducibility.

A larger CI window with QRSd prolongation may increase the likelihood that a spontaneous PVC is discontinuously propagated, resulting in progressive activation delay, wave break and increased dispersion of repolarization. The maximal Δ QRSd may be considered as a marker of the cumulative activation delay throughout the heart after the shortest-coupled (VERP + 10 ms) premature stimulus. Prolonged and pronounced activation delay may reflect non-uniform anisotropic conduction which, together with dispersion of repolarization, may predispose for polymorphic VT and VF.[8]

These findings are consistent with a prior study using extensive stimulation protocols and multiple RV catheters in patients with various non-coronary heart diseases (but only 5 patients with dilated cardiomyopathy), which demonstrated that the maximum increase in RV intracardiac electrogram duration was larger, but also that the S1S2 coupling intervals at which the electrograms started to increase were longer in patients with a history of VF.[1] In a retrospective study in consecutive patients with and without structural heart disease who underwent EPS for various reasons, activation delay, measured from the distal stimulation electrodes to proximal electrodes located at 35 mm distance, was substantially longer in patients with vs. without inducible VF.[9] Finally, in a retrospective study conducted in borderline NICM patients with LVEF >50% and LV end-diastolic dimension < 65 mm, 15 patients resuscitated from polymorphic VT/VF had

longer paced QRSd after S3 (at CI 10 ms above VERP) compared to patients without documented ventricular arrhythmias.[10]

Substrate and mechanisms of activation delay and polymorphic VT

The QRSd restitution curve parameters that were associated with the inducibility of polymorphic VT did not correlate with CMR-derived volumes, ejection fraction or LGE, and were not related to larger fibrosis area percentages on biopsy, but tended to be related to long thick strands of fibrosis in biopsy. This is in line with two studies in explanted hearts from patients with end-stage heart failure, which suggested that activation delay after premature stimulation may be related to the presence of long fibrotic strands.[11,12] In one of these studies, even large amounts of diffusely distributed, short-stranded fibrosis only marginally affected conduction velocity, whereas large fibrotic strands appeared to serve as inexcitable barriers with consecutive conduction delay, supporting that the geometry rather than the total amount of fibrosis creates a potential substrate for arrhythmias.[12] The authors elegantly demonstrated how such barriers may contribute to activation delay, namely by increased path length, transverse instead of longitudinal conduction, wave front curvature and reduced conduction velocity.[12] In addition, activation delay caused by long strands of fibrosis may promote wave break and thereby predispose for polymorphic VT/VF.[13] Of interest, treatment with eplerenone, losartan, or both could reduce the development of age-related interstitial and patchy fibrosis in adult mice.[14] Importantly, only the reduction of patchy fibrosis correlated with reduced inducibility of ventricular arrhythmias based on anisotropic reentry, further supporting the link between type of fibrosis and arrhythmogeneity.

Other potential mechanisms of activation delay after premature stimulation have been described in patients with various structural heart disease.[15] In particular early applied extrastimuli that capture at an earlier and less repolarized level of the preceding beat correlated with delayed propagation. Mechanisms are not necessarily mutually exclusive and the observed critical QRS prolongation may be dependent on the combination of different underlying structural and functional changes resulting in an increased vulnerability for polymorphic VT.

Clinical implications

Activation delay after premature stimulation, which was associated with ventricular arrhythmias in the present and in prior studies, can be easily quantified by any clinical electrophysiologist by measuring the QRSd after 400 ms drive trains with single extrastimuli. The protocol and calculation of the suggested parameters can be performed in a couple of minutes and can be obtained non-invasively in patients with pacemakers or implantable cardioverter-defibrillators. Whether QRSd restitution curve parameters

predict spontaneous ventricular arrhythmias requires further studies with larger sample sizes and long follow-up duration.

Limitations

The number of subjects is small. As this study focused on identifying electrophysiological parameters associated with a predisposition towards polymorphic VT, several factors such as dispersion of repolarization after premature stimulation not caused by activation delay,[8] connexin 43 expression, distribution and co-localization with N-cadherin[11] were not analyzed. Also, the current study did not analyze the association between activation delay and spontaneous polymorphic VT/VF as such analyses require larger patient cohorts and long follow-up.

CONCLUSION

Premature stimulation in patients with NICM causes progressive activation delay, which can be easily quantified by measuring the QRSd after a 400 ms drive train with single extrastimuli. The CI window with QRSd prolongation was wider in patients with NICM than in controls, the maximum increase in QRSd was larger, and a pronounced critical QRS prolongation was demonstrated allowing for extrastimuli being applied within the QRS complex. These 3 phenomena, which are easily assessed during a 3-minute stimulation protocol, were associated with the inducibility of polymorphic VT. QRSd prolongation after premature stimulation tended to be related to long thick strands of fibrosis, but not to overall fibrosis area percentages in biopsy specimens, supporting the link between the geometry of fibrosis and arrhythmogeneity. Measuring the QRSd may be a simple tool to quantify progressive activation delay after premature stimulation as a risk factor for polymorphic VT/VF.

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Supplemental Table 1. Genetic screening results

Gene with likely pathogenic or pathogenic mutation	Frequency
LMNA	5 (12.8%)
TTN	4 (10.3%)
SCN5A	2 (5.1%)
MYH6	2 (5.1%)
LDB3	1 (2.6%)
PKP2	1 (2.6%)
PLN	1 (2.6%)
CALR3	1 (2.6%)
DES	1 (2.6%)
Multiple*	1 (2.6%)
None	20 (51.3%)

* 3 mutations: DSP, MYPN and MYH7

Supplemental Table 2. Comparison of 600 ms and 400 ms drive trains between NICM and controls

	NICM	Controls	p (Mann-Whitney)
Regular 12-lead ECG, 25 mm/second			
QRSd	129 ± 34	90 ± 12	< 0.001
EPS - drive train 600 ms from RV apex			
QRSd during drive train	195 ± 18	157 ± 12	< 0.001
QRSd after extrastimulus 350 ms	191 ± 25	147 ± 14	< 0.001
QRSd plateau	191 ± 25	148 ± 13	< 0.001
CI at onset of QRSd prolongation	311 ± 17	262 ± 15	< 0.001
Local VERP	259 ± 25	228 ± 26	< 0.001
CI window with QRSd prolongation	54 ± 23	34 ± 20	0.003
Maximal QRSd	229 ± 28	176 ± 15	< 0.001
Maximal Δ QRSd	38 ± 20	29 ± 12	0.21
Critical QRSd prolongation	-28 (-56 – -8)	-51 (-77 – -28)	0.053
Critical CI window	0 (0 – 0)	0 (0 – 0)	0.41
EPS - drive train 400 ms from RV apex			
QRSd during drive train	192 ± 23	153 ± 13	< 0.001
QRSd after extrastimulus 350 ms	192 ± 26	150 ± 15	< 0.001
QRSd plateau	191 ± 27	149 ± 15	< 0.001
CI at onset of QRSd prolongation	275 ± 23	235 ± 16	< 0.001
Local VERP	228 ± 23	204 ± 21	< 0.001
CI window with QRSd prolongation	47 ± 23	31 ± 14	0.005
Maximal QRSd	226 ± 31	172 ± 18	< 0.001
Maximal Δ QRSd	35 ± 18	23 ± 12	0.005
Critical QRSd prolongation	+1 (-37 – +26)	-13 (-55 – -7)	0.017
Critical CI window	10 (0 – 30)	0 (0 – 0)	< 0.001

Variables are expressed as mean ± standard deviation or median (interquartile range).
CI indicates coupling interval; EPS, electrophysiology study; QRSd, QRS duration; RV, right ventricle; VERP, ventricular effective refractory period

Supplemental Table 3. QRSd prolongation and inducibility of polymorphic VT

	Polymorphic VT	No Polymorphic VT	p (Mann-Whitney)
Regular 12-lead ECG, 25 mm/second			
QRSd	126 ± 32	133 ± 36	0.69
RVA - Drive train 600 ms with 1 extrastimulus			
QRSd during drive train	193 ± 15	197 ± 20	0.63
QRSd after extrastimulus 350 ms	188 ± 15	194 ± 32	0.85
QRSd plateau	188 ± 15	194 ± 32	0.85
CI at onset of QRSd prolongation	308 ± 18	314 ± 17	0.34
Local VERP	252 ± 23	266 ± 25	0.11
CI window with QRSd prolongation	60 ± 23	48 ± 23	0.14
Maximal QRSd	234 ± 24	225 ± 31	0.23
Maximal Δ QRSd	45 ± 21	31 ± 17	0.058
Critical QRSd prolongation	-18 (-44 – +10)	-38 (-73 – -11)	0.13
Critical CI window	0 (0 – 20)	0 (0 – 0)	0.18
RVA - Drive train 400 ms with 1 extrastimulus			
QRSd during drive train	190 ± 17	193 ± 27	0.88
QRSd after extrastimulus 350 ms	189 ± 19	194 ± 31	0.53
QRSd plateau	188 ± 19	193 ± 32	0.68
CI at onset of QRSd prolongation	276 ± 20	274 ± 26	0.92
Local VERP	219 ± 19	235 ± 23	0.061
CI window with QRSd prolongation	57 ± 25	39 ± 18	0.015
Maximal QRSd	234 ± 26	220 ± 34	0.030
Maximal Δ QRSd	46 ± 17	27 ± 14	0.001
Critical QRSd prolongation	+13 (-17 – +49)	-17 (-44 – +6)	0.017
Critical CI window	20 (0 – 75)	0 (0 – 20)	0.11

Variables are expressed as mean ± standard deviation or median (interquartile range). Abbreviations as in supplemental Table 1.

Supplemental Table 4. Parameters affecting QRSd prolongation

All RV 400 train	Effect on CI at onset QRSd prolongation, ms		Effect on CI window with QRSd prolongation, ms		Effect on maximal Δ QRSd, ms		Effect on critical QRSd prolongation, ms		Effect on critical CI window, ms	
	(95% CI)	p	(95% CI)	p	(95% CI)	p	(95% CI)	p	(95% CI)	p
CMR - cine images (n=36)										
LVEDV index, per 10 mL/m^2 ↑	2.2 (0.3 - 4.0)	**0.023**	-0.1 (-2.1 - 1.9)	0.92	-1.0 (-2.4 - 0.5)	0.19	-0.9 (-3.8 - +2.0)	0.54	0.5 (-2.0 - 3.0)	0.68
LVESV index, per 10 mL/m^2 ‹ ↑	1.6 (-0.2 - 3.4)	0.076	-0.7 (-2.6 - 1.1)	0.44	-1.4 (-2.7 - 0.0)	**0.044**	-1.4 (-4.1 - +1.4)	0.32	0.1 (-2.3 - 2.5)	0.93
LVEF, per 10 % ↓	2.4 (-3.9 - 8.7)	0.45	-4.4 (-10.4 - 1.7)	0.15	-5.7 (-10.0 - -1.4)	**0.011**	-5.6 (-14.6 - +3.5)	0.22	-2.4 (-10.2 - 5.4)	0.54
RVEDV index, per 10 mL/m^2 ↑	1.5 (-2.9 - 6.0)	0.49	1.1 (-3.3 - 5.4)	0.62	0.1 (-3.2 - 3.4)	0.93	0.0 (-6.4 - +6.3)	0.99	0.7 (-4.7 - 6.2)	0.79
RVESV index, per 10 mL/ ↑	0.4 (-4.3 - 5.1)	0.86	-0.5 (-5.1 - 4.1)	0.84	0.5 (-3.1 - 4.0)	0.79	+0.8 (-6.0 - +7.6)	0.81	-0.2 (-6.1 - 5.6)	0.93
RVEF, per 10% ↓	-0.9 (-8.5 - 6.7)	0.82	-2.3 (-9.7 - 5.1)	0.54	0.9 (-4.8 - 6.5)	0.76	+2.1 (-8.9 - +13.0)	0.70	-2.1 (-11.5 - 7.2)	0.64
CMR - LGE images (n=34)										
Total LGE mass, per 10 g ↑	-3.8 (-9.2 - 1.5)	0.15	0.5 (-5.1 - 6.0)	0.86	-1.0 (-4.9 - 3.0)	0.63	+0.4 (-7.7 - +8.4)	0.93	-1.5 (-8.4 - 5.5)	0.67
Core mass, per 10 g ↑	-8.2 (-17.6 - 1.2)	0.086	1.3 (-8.7 - 11.2)	0.80	-0.7 (-7.8 - 6.5)	0.85	+2.3 (-12.1 - +16.7)	0.75	-1.3 (-13.7 - 11.2)	0.83
Border zone mass, per 10 g ↑	-5.4 (-16.7 - 5.8)	0.33	0.4 (-11.0 - 11.9)	0.94	-3.2 (-11.3 - 5.0)	0.44	-1.5 (-18.1 - +15.1)	0.86	-4.5 (-18.7 - 9.8)	0.52
Laboratory										
Log NT-proBNP	6.2 (-0.3 - 12.7)	0.060	1.1 (-5.4 - 7.6)	0.73	-0.8 (-5.9 - 4.3)	0.76	-1.1 (-12.3 - +10.1)	0.84	3.6 (-6.5 - 13.8)	0.47
Biopsy (n=23)										
Collagen area percentage, per 10% ↑	-0.4 (-5.5 - 4.7)	0.87	-4.7 (-10.3 - 0.9)	0.096	-4.4 (-8.5 - -0.3)	**0.036**	-7.4 (-15.9 - +1.0)	0.081	-3.6 (-12.6 - 5.4)	0.42
Collagen distribution pattern										
Long strands vs. interstitial	12.9 (-11.8 - 37.5)	0.28	18.6 (-10.9 - 48.0)	0.20	3.8 (-22.9 - 30.5)	0.76	+15.1 (-29.0 - +59.4)	0.47	35.9 (-12.2 - 84.0)	0.13
Long strands vs. short strands	4.3 (-26.9 - 35.5)	0.77	41.4 (12.7 - 70.1)	**0.008**	15.5 (-11.4 - 42.5)	0.24	+35.4 (-18.3 - +89.2)	0.18	44.5 (-6.9 - 96.9)	0.084
Long strands vs. others	8.6 (-13.8 - 31.0)	0.43	30.0 (6.7 - 53.3)	**0.014**	9.7 (-9.9 - 29.2)	0.31	+25.3 (-13.6 - +64.1)	0.19	40.2 (3.7 - 76.6)	**0.032**
Genetics (n=39)										
Pathogenic or likely pathogenic mutation	11.1 (-4.6 - 26.7)	0.16	1.5 (-14.1 - 17.0)	0.85	2.7 (-9.1 - 14.6)	0.64	+3.7 (-22.1 - +29.4)	0.77	6.7 (-16.9 - 30.4)	0.57

Effect in ms (95%) is displayed.