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Research Article

Utility of a Novel Biomarker Panel in the Prediction of QT Dynamicity

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Abstract

Background: The QT-heart rate (QT-HR) slope is a predictor of mortality in heart failure patients. A biomarker panel that correlates with QT-HR slope could serve as a predictor of mortality in this population.

Methods: QT intervals (both the QT-peak and QT-end) were analyzed and QT-HR slopes were calculated from Holter monitors worn for greater than 48 hours in 64 patients with heart failure. Discovery analysis was performed to assess which biomarkers correlate well with the QT-HR slope.

Results: The correlation coefficient between the true QTp-HR and QT-HR slopes and those estimated by the 12-protein (QTp-HR) and 2-protein (QT-HR) biomarker panels were 0.72 ($p<0.00001$) and 0.46 ($p<0.001$), respectively. The correlation between the true QTp-HR and QT-HR slopes and those estimated by the genomic biomarker panel were 0.86 ($p<0.00001$) and 0.86($p<0.00001$), respectively.

Conclusions: We have found protein- and gene-based biomarker panels that correlate highly with the QT-heart rate slope.

Keywords: Genomics; Proteomics; QT; Electrocardiography

Introduction

The QT interval is the electrocardiographic summation of the cardiac action potential. Its duration correlates with the risk of sudden death due to Torsades de Pointes. The QT duration is affected by many factors such as the relative activity of inward and outward cardiac ion channels and circulating epinephrine and electrolyte concentrations [1].

QT dynamicity refers to the degree of QT lengthening with decreases in heart rate (QT-heart rate slope). This QT-heart rate (QT-HR) slope varies between and within individuals and is not predicted by the baseline QT interval [2,3]. It is well known that a steeper QT-HR slope correlates with an increased risk of arrhythmia and mortality in patients with heart failure attributed to both ischemic and non-ischemic heart disease [4-7]. QT-HR slope analysis is not feasible in most clinical settings as it is time intensive and, in certain cases (dynamic t wave changes or alterations in depolarization time), not always possible to achieve an accurate QT measurement [8-10].

Untargeted "omics" biomarker discovery is the process of analyzing, using high-throughput transcriptomics, proteomics, and other omics technologies to generate large-scale datasets that can be deeply interrogated. After the relative expression values of genes/proteins are obtained, their relationship with a clinical condition of interest is assessed systematically using both univariate and multivariate statistical methods and informatical tools. As such, biomarker discovery analysis allows for the identification of genes/proteins that may diagnose, prognose, or predict disease states or measure the degree of abnormality in physiological, pathological or other phenotype.

By harnessing an established biomarker discovery strategy, we sought to determine whether we could predict the QT-HR slope using signatures derived from blood proteins and genes in a heart failure population [11,12]. The intent was not only to identify a set of genes and/or proteins that will predict QT-HR, but also to provide insights into the mechanisms of death in certain subsets of the heart failure population.

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Methods

Patient Population

Patients attending the St. Paul's Hospital Heart Function Clinic were included if they met the inclusion/exclusion criteria. The inclusion criteria included current or previously documented but recovered heart failure with reduced ejection fraction (HFREF) (left ventricular ejection fraction (LVEF) ≤ 35% and New York Heart Association (NYHA) class ≥ II) and age > 19 years. The exclusion criteria included permanent atrial fibrillation, high burden of ventricular and/or atrial pacing or mechanical circulatory assist device as these factors interfere with Holter QT assessment.

Holter Analysis

Holter monitors (SEER Light Extend; GE Healthcare, Little Chalfont, United Kingdom) were worn. To assess the intra-person variability in QT-heart rate slope seen in a 48 hour Holter monitoring period, patients wore Holter monitors for 14 days (48 hour Holter monitoring repeated for a total of 7 times). Holter monitor data was then processed using MARS (version 6, GE Medical Systems Inc., Milwaukee, WI) [8] and analyzed by an expert cardiovascular technologist, blinded to the discovery analysis results, at the St. Paul's Hospital, Electrocardiography Laboratory. The QTp interval was measured from the QRS onset to the peak of the T wave. The QT was measured from the QRS onset to the end of the T wave [10]. Each of the three channels was screened to ensure the QT interval measurements were possible (the T wave morphology was not isoelectric and there was no artifact), the QRS and T wave morphology were the same (no intermittent pacing, bundle branch block or alteration in the T wave axis), and accurate (the measurements were between the start of the QRS and the end of the T wave). All QT analysis data was over-read by two experienced cardiologists (KI and MB).

Genomic (Transcriptomic) and Proteomic Analyses

Blood was collected at the time of enrollment in EDTA (BD, Franklin Lake, NJ) and PAXgene Blood RNA (PreAnalytiX, Hombrechtikon, Switzerland) tubes. The EDTA tubes were placed on ice and centrifuged within 2 hours of collection. Plasma aliquots and the PAXgene tubes were stored at -80°C until selected for 'omic' analyses. Plasma samples were trypsin digested and analyzed with multiple reaction monitoring (MRM) mass spectrometry at the UVic Genome BC Proteomics Centre, Victoria, Canada. A total of 306 peptides, corresponding to 130 proteins, were measured. These proteins had MRM assays developed already and were related to HF either based on our previous data (not shown here) or based on published literature [13-16].

Total RNA was extracted on QIAcube (Qiagen Inc) from the 64 PAXgene blood samples using the PAXgene Blood miRNA kit from PreAnalytix (Cat. #763134) according to manufacturer's instructions. RNA was amplified and hybridized overnight to the Affymetrix Human Gene 1.1 ST array plates at TSRI DNA Array Core Facility, Scripps Research Institute (La Jolla, CA). Array plates were scanned using the Affymetrix GeneTitan MC Scanner (Affymetrix Inc.) with recommended settings.

Statistical Analysis

Statistical analysis was performed with R (www.r-project.org) and Bioconductor (www.bioconductor.org). The quality of the MRM data was evaluated and pre-filtered and those peptides with median relative ratio <0.0005, median response <100, and more than two standards' accuracy being out of the 80-120 range were eliminated from further analyses as previously described [17]. Peptides present in less than 75% of the patients were eliminated from analysis. At the next step, the levels of the peptides' not detected in a sample were replaced with half of the minimum peptide level detected in the rest of the patients. Following this, the MRM data was log 2 transformed and standardized. For proteins with multiple peptides measured by MRM, the level of the protein was calculated based on the peptide with highest relative ratio in the majority of the samples analyzed.

The whole blood Affymetrix Human Gene 1.1 ST chips were checked for quality problems using the oligo[1] Bioconductor package, and those that did not pass the quality check were repeated using RNA from the same PAXgene tube. Arrays were background corrected, normalized, and summarized separately using the robust multi-array average (RMA) method with the FARMS Bioconductor package. Non-informative probe sets were eliminated using the FARMS I/NI Bioconductor package [18].

QT-Heart Rate Slope

To assess the most appropriate curve to analyze the QT slopes, a linear model was fit for each sample. The adjusted R-squares of all the fitted models were all greater than 0.8, indicating that the QT-HR slope found on the Holter monitor was linear. To check the variability of the QT-HR slope, a linear mixed effect (LME) model was applied to the QT-HR slope across all seven reports.

Correspondence of Gene and Protein Biomarkers and QT-Heart Rate Slope

A univariate-linear regression model was developed on each gene or protein to assess which genes or proteins were best correlated with the QTp-HR and QT-HR slope. A stepwise selection, based on the Akaike Information Criterion (AIC), was applied to the 20 most correlated features to identify those which together best correlated with QTp-HR and QT-HR slopes. A multivariate classification model was built using elastic net and the features were selected by AIC in order to minimize overfitting.

This study was approved by the Providence Health Care Research Ethics Board and conforms to the principles outlined in the Declaration of Helsinki. All included subjects consented to their enrollment. All authors had full access to the data and take full responsibility for its integrity.

Results

Sixty-four patients were included in the analysis (Table 1): 40 patients with current HFREF (LVEF ≤ 35% and NYHA class ≥II) and 24 patients with recovered HFREF (LVEF ≥ 40% and NYHA class I or II).

Correlation of Gene and Protein Biomarkers and QT-Heart Rate Slope

The genes and protein markers found to have the highest correlation with the measured QTp-HR and QT-HR slopes are listed in Table 2. The 20 proteins with highest Pearson's correlation coefficient between the estimated and measured QTp-HR and QT-HR slopes were included in a stepwise model

Table 1. Demographics of Patients. Continuous variables were expressed as mean ± standard deviation

	Patients (n=64)
Age (years)	63 ± 14
Male gender	47 (73)
Hypertension	41 (64)
Diabetes mellitus	28 (44)
Chronic renal impairment	9 (14)
Current smoker	8 (13)
HF etiology	
Ischemic	38 (59)
Non-ischemic	26 (41)
NYHA	
I	16 (25)
II	29 (45)
III	18 (28)
IV	1 (2)
SBP (mmHg)	115 ± 19
DBP (mmHg)	65 ± 9
HR (bpm)	68 ± 14
Medications	
ACE-I or ARB	51 (80)
Beta blocker	58 (91)
Spironolactone	31 (48)
Creatinine (μmol/L)	117 ± 102
BNP (pg/ml)	252 ± 371
Ejection fraction (%)	36 ± 12
ICD	18 (28)
Pacemaker	7 (11)
GFR	64 ± 26
QT (ms)	436 ± 42
QT-slope	-2.30 ± 0.80
QTp (ms)	344 ± 41
QTp-slope	-2.54 ± 0.87

Categorical variables were expressed as number (percentage). ACE-I: Angiotensin Converting Enzyme Inhibitor, ARB: Angiotensin II Receptor Blocker, BNP: b-type Natriuretic Peptide, DBP: Diastolic Blood Pressure, HF: Heart Failure, HR: Heart Rate: ICD: Implantable Cardiac Defibrillator, NYHA: New York Heart Association, SBP: Systolic Blood Pressure, QT –slope=slope of linear regression analysis of QT and heart rate, QTp-slope=slope of linear regression analysis of QTp and heart rate.

based on AIC. Twelve proteins were selected for the model for QTp-HR slope, and two were selected for the QT-HR slope. An elastic net model was built by using the 12-protein and 2-protein panel for QTp-HR and QT-HR slopes, respectively. The correlation between the measured QTp-HR and QT-HR slopes and those estimated based on the 12-protein QTp-HR and 2-protein QT-HR biomarker panel were 0.72 ($p<0.00001$) and 0.46 ($p<0.001$), respectively (Figure 1A and B).

The 20 genomic probe sets with highest Pearson's correlation between the estimated and measured QTp-HR and QT-HR slopes were included in a stepwise model based on AIC. Nine out of the 20 probe sets were selected for QTp-HR slope based on their correlation with the measured QTp-HR slope. An elastic net model was built with the 9-probe sets. The correlation between the measured QTp-HR slope and that estimated based on this 9-probe set biomarker panel was 0.86 ($p<0.00001$) (Figure 2A). A different 9 probe set biomarker panel was used for the QT analysis. The correlation between the measured QT-HR slope and that estimated based was 0.86 ($p<0.00001$) (Figure 2B).

We have tested how a random 9 probe set biomarker panel would correlate with QTp-HR and QT-HR slope, respectively. We randomly selected 9 probe sets 1,000 times. Using elastic net we built a model with each of the 1,000 sets and calculated the correlation between the measured QTp-HR and QT-HR slope and the one estimated based on these 9-probe set biomarker panels. The distribution of the correlations is shown on Figure 3. The random panels' correlation was considerably lower than the biomarker panels; the highest of the 1,000 sets was 0.65 and 0.58, for QTP-R and QT slopes, respectively. Therefore, our panel performs significantly better than what one would expect by chance using this microarray data.

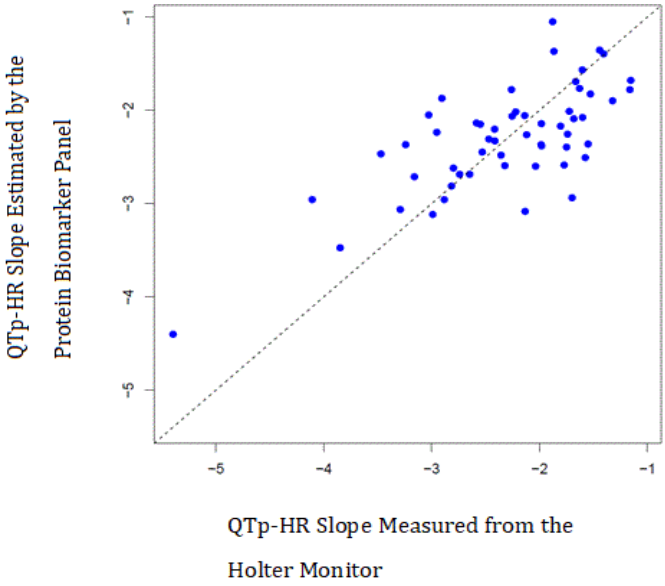

Figure 1A. Measured QT-HR slope versus QT-HR slope estimated by plasma protein panel. The correlation between the measured QTp-HR slope obtained from the Holter monitor and the QTp-HR slope estimated by the 13-protein biomarker panel was found to be 0.72 (p-value < 0.00001)).

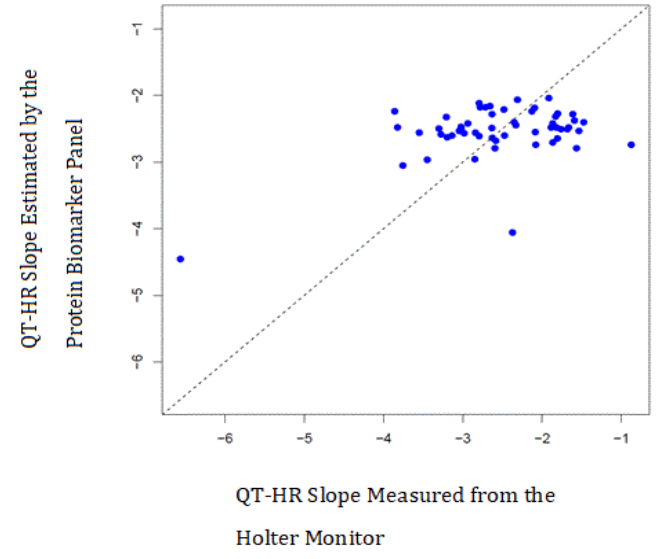

Figure 1B. Measured QT-HR slope versus QT-HR slope estimated by plasma protein panel. The correlation between the measured QT-HR slope obtained from the Holter monitor and the estimated QT-HR slope estimated by the 2-protein biomarker panel was 0.46 (p-value < 0.001).

Table 2. Biomarker panel genes and proteins. QT refers to the correlation of measured QT-HR slope and that estimated by the biomarker; QTp refers to the correlation of measured QTp-HR slope and that estimated by the biomarker.

Panel Type	Probe Set ID	In Biomarker Panel	Gene Symbol	Name	Panel	Correlation	P-value of the correlation
Proteomic	NA	No	AACT	Alpha 1 Antichymotrypsin	QT	0.14	0.30
		No	A2AP	Alpha 2 Antiplasmin	QTp	0.23	0.10
		No			QT	0.15	0.26
		No	ANT3	Antithrombin III	QT	-0.12	0.39
		No	APOE	Apolipoprotein E	QTp	0.18	0.20
		Yes	APOM	Apolipoprotein M	QTp	0.28	0.04
		No			QT	0.15	0.28
		No	CRP	C Reactive Protein	QT	0.20	0.14
		No	C4BPA	C4b Binding Protein Alpha Chain	QT	0.14	0.29
		No	CAH1	Carbonic Anhydrase 1	QTp	0.16	0.26
		Yes	CAMP	Cathelicidin Antimicrobial Peptide	QTp	0.25	0.06
		No	CAMP	Cathelicidin Antimicrobial Peptide	QT	0.17	0.21
		No	CD5L	CD5 Antigen Like	QT	0.13	0.35
		Yes	CERU	Ceruloplasmin	QTp	-0.16	0.24
		No			QT	-0.18	0.18
		No	FA9	Coagulation Factor IX	QT	0.14	0.31
		No	FA12	Coagulation Factor XII	QTp	0.22	0.11
		No		Complement C1q Subcomponent Subunit A	QT	0.21	0.12
		Yes	C1QC	Complement C1q Subcomponent Subunit C	QTp	-0.18	0.18
		No	CO8G	Complement component C8 gamma chain	QT	0.14	0.31
		Yes	CO8A	Complement Component C8 Alpha Chain	QTp	0.16	0.26
		No			QT	0.14	0.32
		Yes	CBG	Corticosteroid Binding Globulin	QTp	0.37	0.01
		Yes			QT	0.39	0.003
		No	CYTC	Cystatin C	QTp	-0.18	0.18
		No	LG3BP	Galectin 3 Binding Protein	QTp	0.17	0.22
		No			QT	0.13	0.35
		No	GELS	Gelsolin	QT	-0.17	0.21
		Yes	IBP3	Insulin Like Growth Factor Binding Protein 3	QTp	0.16	0.23
		No	PLTP	Phospholipid Transfer Protein	QTp	-0.16	0.24
		Yes	PRG4	Proteoglycan 4	QTp	0.17	0.21
		Yes	SHBG	Sex Hormone Binding Globulin	QTp	-0.24	0.08
		Yes			QT	-0.29	0.03
		Yes	TENA	Tenascin	QTp	-0.17	0.22
		No			QT	-0.12	0.38
		Yes	VASN	Vasorin	QTp	-0.24	0.08
		No			QT	-0.14	0.30
		No	PROS	Vitamin K Dependent Protein S	QT	0.12	0.38
		Yes	VTNC	Vitronectin	QTp	0.23	0.09
		No	VWF	Von Willebrand Factor	QTp	-0.27	0.05

Genomic	7892576	Yes	---		QT	0.43	0.0012
	7892588	Yes	---		QTp	0.40	0.0028
	7893032	Yes	---		QTp	0.52	0.0001
		No	---		QT	0.38	0.0047
	7893354	No	---		QTp	-0.40	0.0026
		No			QT	-0.42	0.0014
	7893363	No	---		QT	-0.38	0.0045
	7893883	No	---		QTp	-0.39	0.0038
	7894186	No	---		QT	-0.39	0.0034
	7895029	Yes	---		QTp	-0.44	0.0010
		Yes			QT	-0.39	0.0037
	7896249	No	---		QT	-0.44	0.0009
	7896588	Yes	---		QTp	-0.40	0.0024
		No			QT	-0.41	0.0022
	7896684	No	---		QTP	-0.39	0.0032
	7902023	Yes	RAVER2	ribonucleoprotein, PTB-binding 2	QTp	-0.45	0.0007
		Yes			QT	-0.49	0.0001
	7902883	Yes	LRRC8D	leucine rich repeat containing 8 family, member D	QTp	-0.44	0.0009
	7960933	Yes	M6PR	mannose-6-phosphate receptor (cation dependent)	QTp	-0.39	0.0040
	7991367	Yes	C15orf38	chromosome 15 open reading frame 38	QT	0.42	0.0015
	8006746	Yes	TBC1D3	TBC1 domain family, member 3	QT	-0.38	0.0043
	8010924	No	VPS53	vacuolar protein sorting 53 homolog (S. cerevisiae)	QTP	-0.38	0.0051
	8014376	Yes	TBC1D3	TBC1 domain family, member 3	QT	-0.38	0.0051
	8014437	No	TBC1D3	TBC1 domain family, member 3	QT	-0.37	0.0056
	8014603	No	TBC1D3	TBC1 domain family, member 3	QT	-0.38	0.0045
	8014633	Yes	TBC1D3	TBC1 domain family, member 3	QT	-0.38	0.0051
	8019655	Yes	TBC1D3	TBC1 domain family, member 3	QT	-0.38	0.0052
	8019716	No	TBC1D3	TBC1 domain family, member 3	QT	-0.37	0.0056
	8023481	No	NARS	asparaginyl-tRNA synthetase	QTP	-0.38	0.0045
	8047788	No	ADAM23	ADAM metallopeptidase domain 23	QT	-0.39	0.0032
	8051298	No	GALNT14	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminy ltransferase 14 (GalNAc-T14)	QTP	0.40	0.0030
	8071920	No	SNRPD3	small nuclear ribonucleoprotein D3 polypeptide 18kDa	QTP	-0.38	0.0045
	8090509	No	RAB7A	RAB7A, member RAS oncogene family	QTP	0.39	0.0033
	8093494	Yes	CRIPAK	cysteine-rich Pak1 inhibitor	QTp	-0.41	0.0018
	8095139	Yes	SRD5A3	steroid 5 alpha-reductase 3	QT	-0.42	0.0016
	8107470	No	PTMA	prothymosin, alpha	QTP	-0.39	0.0034
	8111210	Yes	FTH1P3	ferritin, heavy polypeptide 1 pseudogene 3	QTp	0.40	0.0026
	8149809	No	---		QTP	0.45	0.0007
		No			QT	0.49	0.0002
	8165700	No	---		QTP	0.38	0.0050

Discussion

We have found genomic and proteomic panels that predict the QT-HR slope. When analyzed separately, the genomic panel demonstrated the highest ability to predict the QT-HR slope as compared to the tested proteins. As QT-HR slope assessment is cumbersome and often impossible to be performed accurately, it is not currently being used commonly in clinical practice. The ability to predict the QT-HR slope with a blood test may allow clinicians to identify which heart failure patients are likely to have the steepest slope and, as such, are at highest risk of death.

The QT-HR slope is a measure of the rate adaptation of ventricular repolarization. This appears to be primarily modulated by the relative activity of myocyte ion channels in particular I_{KS}, I_{KR}, and $I_{Na\text{-}L[19]}$, and in the heart failure population, by alterations in autonomic tone. Multiple previous studies have shown that an increase in QT-HR slope correlates with

increases in arrhythmia and all cause mortality in patients with and without heart failure [4,6,7]. Cygankiewicz et al. showed an increase in mortality (HR=1.57 and 1.58 for QTp-HR and QT-HR, respectively) in patients with cardiomyopathy when those with the steepest slopes were compared to the rest of the group [4]. Iacoviello et al. furthered these findings in 179 patients with non-ischemic cardiomyopathy. They found that there was an increase in arrhythmic events (HR=1.38; p<0.001) and mortality (HR=1.86 for a 0.05 increase; p<0.001) in patients with an increased QT/HR slope [6]. Despite this overwhelming evidence for prediction of risk in these populations, QT-HR slope analysis is not used clinically as, although the current Holter software has attempted

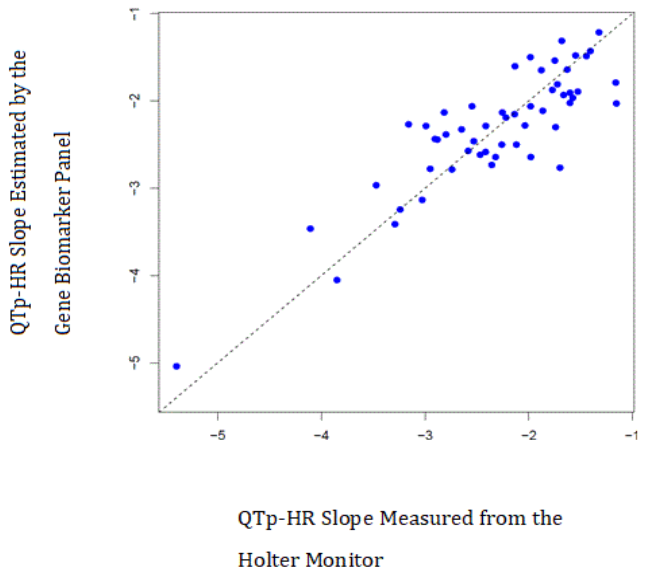

Figure 2A. True QT slope versus QT slope estimated by the whole-blood RNA panel. The correlation between the measured QTp-HR slope obtained from the Holter monitor and the QTp-HR slope estimated by the 9-probe set biomarker panel was 0.86 (p-value < 0.00001).

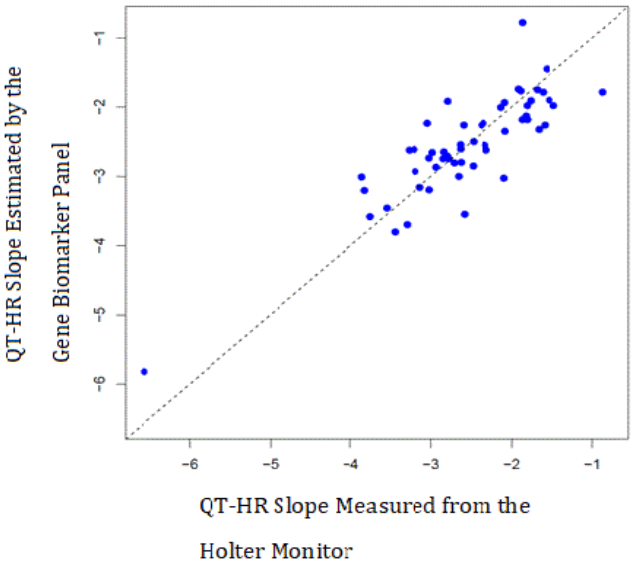

Figure 2B. True QT slope versus QT slope estimated by the whole-blood RNA panel. The correlation between the measured QT-HR slope obtained from the Holter monitor and the QT-HR slope estimated by the 9-probe set biomarker panel was 0.86 (p-value < 0.00001).

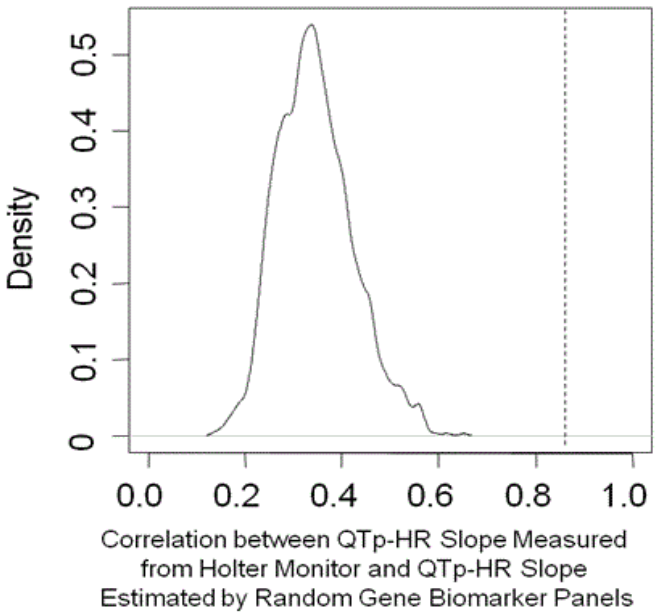

Figure 3A. Density plot of 1,000 random 9 probe set panels correlation with QTp (A) and QT (B) slopes. The dotted lines indicate the correlation of the biomarker panel with QTp (A) and QT(B) slopes.

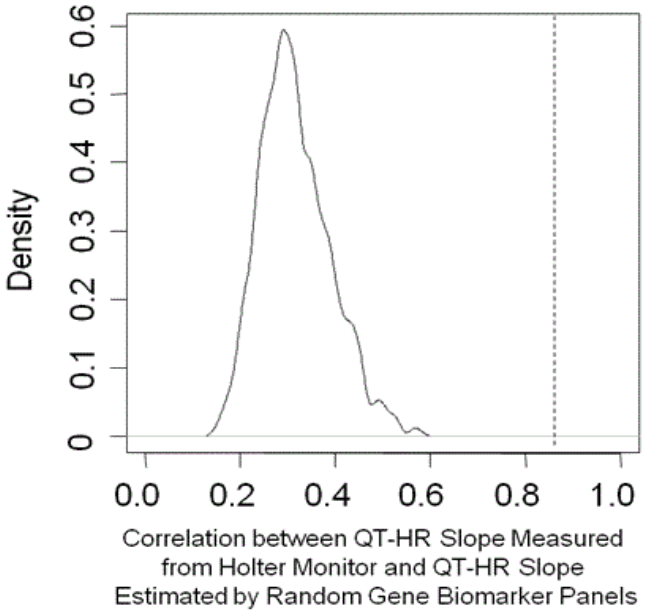

Figure 3B. Density plot of 1,000 random 9 probe set panels correlation with QTp (A) and QT (B) slopes. The dotted lines indicate the correlation of the biomarker panel with QTp (A) and QT(B) slopes.

to automate this process, QT-HR assessment requires significant man-power during the Holter post-processing to ensure a reliable analysis [8-10]. Although our analysis requires validation, the discovery of a blood test that would be fast and reliable would further the risk assessment in the above populations. Furthermore, the one can use the blood markers implicated to affect QT-HR slope to provide insight into the putative physiological mechanisms of QT-HR dynamicity.

Discovery analysis is complementary to targeted analysis in the linkage of biomarkers to pathophysiological mechanisms

and disease diagnosis and prognosis. Where targeted analysis assesses the association between mutations or biomarker levels in one or few markers based on known mechanisms, discovery analysis assesses for associations without being limited to those that are known to be implicated in the pathophysiological processes.

The baseline QT interval is predicted by common variants at ten specific loci and it increases proportionally to the number of QT prolonging alleles within these variants [20,21]. These alleles are responsible for many cellular mechanisms which would have biologically plausible QT effects. The pathways postulated to be affected by these loci include nitric oxide synthase (NOS1AP), I_{KS}(KCNQ1), I_{KR} (KCNH2), SCN5A (I_{NA}), ATP1B1 (Na^+/K^+ ATPase beta subunit 1), sarcoplasmic reticulum Ca^{2+} ATPase (phospholamban) and possibly TNFA expression (LITAF), thioredoxin domain-containing protein 11(TXNDC11), RING-type zinc finger protein of unknown function [20,21]. Although these alleles have been demonstrated to correlate well with the QT interval, in our analysis there was no correlation between their associated genomic/proteomic markers and the QT-HR slope. Such an observation opens the door to additional contributions to the electrical abnormalities underlying the relationship with arrhythmogenesis.

Protein Levels and QT-Heart Rate Slope: Positive Correlation

We found several proteins whose blood level correlated with the QT-HR slope. The protein expression of apolipoprotein M (ApoM), cathelicidin antimicrobial peptide (CAMP) and corticosteroid binding globulin (CBG) all correlated positively with QT-HR slope. ApoM is mainly found within HDL, triglyceride rich lipoproteins and low density lipoprotein [22]. Although polymorphisms in ApoM may predispose to coronary artery disease it has not been known to have effects on QT interval or QT-HR [22]. CAMP is an anti-microbial protein released primarily by neutrophils in a response to micro-organisms [23]. CAMP has not been known to have effects on the QT interval or QT-HR. CBG is a transport glycoprotein for cortisol within the cytosol and is primarily produced in the liver [24]. CBG levels are known to increase with pregnancy and type 1 diabetes mellitus and decrease liver cirrhosis and with significant stress such as burn injury, cardiac surgery, trauma and sepsis [24]. The QT interval and QT-HR are not known to be affected by CBG [24,25].

Protein Levels and QT-Heart Rate Slope: Negative Correlation

Sex hormone binding globulin (SHBG) activity was found to have a negative correlation with QT-HR slope. SHBG is a transport protein for testosterone and estradiol. Secretion of SHBG is stimulated in a dose dependent fashion by estradiol [26]. Testosterone has a biphasic response to SHBG with both low and high concentrations of testosterone being associated with reduced SHBG secretion [26]. Although hormonal concentrations significantly alter the QT interval [27], there appears to be no association between the baseline QT interval and serum levels of SHBG [28]. Furthermore, there is no clear relationship of SHBG to the QT-HR slope.

Gene Expression and QT-Heart Rate Slope: Positive Correlation

Chromosome 15 open reading frame 38 (C15orf38) and ferritin, heavy polypeptide 1 pseudogene 3 (FTH1P3) expression correlated positively with the QT-HR slope.

Expression of both C15orf38 and FTH1P3 do not have any known cardiac manifestations, nor have been described to be associated with alterations in the QT interval or QT-HR slope.

Gene Expression and QT-Heart Rate Slope: Negative Correlation

We found that the blood RNA levels of ribonucleoprotein PTB-binding 2 (RAVER2), leucine rich repeat containing 8 family, member D (LRRC8D), cation dependent mannose-6-phoshate receptor (M6PR), cysteine-rich Pak1 inhibitor (CRIPak), steroid 5 alpha-reductase 3(SRD5A3) and 4 TBC1 domain genes were negatively correlated with the QT-HR slope. RAVER2 is a RNA binding protein which serves to not only bind RNA but to mediate protein-protein interaction [29]. In humans, RNA binding protein defects have been associated with cancer and neuromuscular disorders. RAVER2 has been associated with an increased susceptibility to ulcerative colitis and has no known association with QT interval effects [30].

Leucine rich repeat proteins affect hormone-receptor interactions, enzyme inhibition, cell adhesion and cellular trafficking. In mammals, LRR proteins have been implicated in neural development, regulation of gene expression and apoptosis signaling [31]. The function of LRR8 is unclear, but may have a significant role in B cell development [32]. There is no known association between LRR8 and the QT interval or QT-HR.

M6PR delivers newly synthesized acid hydolases for the trans-Golgi network to endosomes, which are then transferred to lysosomes [33]. There is no known association between the QT interval or QT-HR and M6PR.

TBC1D3 is an oncogene, which alters growth factor receptor signaling and has been found to be over-expressed in breast and prostate cancers [34]. There is no known association between TBC1D3 and QT dynamics. p21-activated protein kinase 1 (Pak1) is involved in many cellular processes including estrogen receptor signaling, cytoskeleton reorganization and promotion of cell survival. CRIPak inhibits the Pak1-mediated enhancement of estrogen receptor transactivation [35]. The effects on the estrogen receptor may account for the observed QT-RR slope effects. 5-alpha reductase converts testosterone to dihydrotestosterone, which binds the androgen receptor. Mutations in type 3 5-alpha reductase (SRD5A3) have been implicated in increases in the frequency of prostate cancer [36]. The observed effects of SRD5A3 on QT-HR slope could be as a result of these hormonal effects.

Limitations

Our biomarker discovery work was performed in patients with heart failure who were taking heart failure medical therapy. It is unclear if these findings would be generalizable to other populations. Our analysis revealed a poor correlation between the blood genes [RNAs]/proteins that would be expected to alter the QT interval [21]. It is not clear if this was as a result of the

included patients or because, although these biomarkers affect the baseline QT interval, they do not affect the QT-HR slope.

It is known that the QT-HR slope is dependent on the degree of heart rate acceleration, and whether the heart rate is increasing or decreasing as in exercise and recovery. The analysis of the Holter monitors used the mean QT interval at each heart rate and did not correct for heart rate acceleration nor for phase of exercise. It is unclear to what degree this impacted our results.

Our analysis did not include clinical events such as arrhythmia burden or mortality. Although not the focus of the current study, such will be an important next step in evaluating the utility of these promising novel observations.

Conclusion

We have found a blood-derived biomarker panel that predicts the QT-Heart Rate slope. As steeper QT-Heart rate slopes have been associated with increases in ventricular arrhythmias and mortality, our biomarker panel may allow for the identification of a high-risk heart failure group. Furthermore, we have identified a novel set of biomarkers that, if found to be associated with increased mortality, and if validated in other populations, may provide a simple risk stratification tool. The markers provide further insights into the mechanism of death in patients with heart failure. Further study to confirm that this novel biomarker panel is predictive of mortality in similar patient cohorts is necessary.

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Disclosures

None

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