GENETIC EPIDEMIOLOGY OF VENTRICULAR TACHYCARDIA IN PATIENTS WITH CARDIOMYOPATHY IN KAZAKHSTAN: A TARGETED SEQUENCING STUDY

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ABSTRACT

Objective. Ventricular tachycardia (VT) is a common symptom in cardiac disorders of different etiology. Abnormalities of cardiac ion channels are attributed to mutations in the genes encoding the channel protein and cause altered function of channels, which can predispose to arrhythmias. Molecular alterations of cardiac ion channels proteins are known to cause VT in several congenital cardiomyopathies.

Aim: to investigate genetic basis of VT in patients with cardiomyopathy in Kazakhstan using targeted high throughput next generation sequencing (NGS) (design of new gene panel).

Methods. We have enrolled 95 patients with sporadic (64/95) or familial (26/95) cardiomyopathies (DCM: 37.3%, idiopathic VT: 38.9%, coronary artery disease with severe episodes of VT: 24.2%, and others: 3%). Using a predesigned customized HaloPlex Target Enrichment System™ (Agilent Technologies, USA) 96 cardiomyopathy associated candidate-genes were sequenced on Illumina HiSeq2000.

Results: 173 mutations previously associated with cardiomyopathies (disease-associated variants listed in the HGMD) were identified. On average, each patient carried ≥1.6 mutations, irrespective of the initial clinical diagnosis. Furthermore, 215 private (unique) non-synonymous variants were observed in the patient cohort. Prediction scores of the private variants indicated high probability of disease association. Including the newly identified high-probability variants, each patient carried on average >4.8 genetic variants. Interestingly, there was no difference in frequency of genetic variants between the CAD and the DCM subgroup of patients.

Conclusions: Our study indicates that CAD patients carry an overlapping pattern of genetic variants as observed for DCM patients or other forms of inheritable cardiac disorders. Multiple genetic mutations and novel variants were observed for most of the patients. Because of the wide overlap of the pattern of genetic variants between CAD and DCM patients we have to assume a polygenic effect and a common molecular basis for CAD and DCM or challenge the causal relation of a multitude of genetic mutations.

AIM

To investigate genetic basis of VT in patients with cardiomyopathy in Kazakhstan using targeted NGS (design of new HaloPlex gene panel)

MATERIALS & METHODS

Study population

The study was performed in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Nazarbayev University. Informed written consent was obtained from all participants.

The study cohort consisted of 92 unrelated patients with ventricular tachycardia (VT) which was the result of different background conditions: DCM (n=32, 33.7%), CHD (n=23, 24.2%), Idiopathic VT (n=37, 38.9%)
Patients were from Kazakhstan, with Asian and/or Caucasian ancestry (Table 1).

Target enrichment, sequencing and variant annotation

SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA, US) was used for capturing of the designed regions.
Design of the capture baits was done using eArray (Agilent Technologies, Santa Clara, CA, US). Gene panel consists of 96 genes associated with cardiac disorders (Table 2).

Gene enrichment and next-generation sequencing

Using a customized HaloPlex Target Enrichment System™ (Agilent Technologies, USA) 96 cardiomyopathy associated candidate-genes were enriched and then sequenced on HiSeq2000 platform using 2x150bp paired-end standard sequencing conditions.

Sequence data processing and variant calling was done using Agilent NGS data analysis software SureCall version 2.0.7.0 (Agilent Technologies, Santa Clara, CA, USA).

Resulting variants were further matched with entries in the Human Gene Mutation Database (HGMD) and annotated with ANNOVAR.
Additionally, to achieve better scoring we also included the predictions from the database of human non-synonymous SNVs dbNSFP.

Statistical analysis was performed using SPSS, 21 version, USA.

Prediction of pathogenicity classes

The most commonly used tools (SIFT_score/pred, Polyphen2_HDIVscore/pred, Polyphen2_HVAR_score/pred, LRT_score/pred, MutationTaster_score/pred, MutationAssessor_score/pred, FATHMM_score/pred, RadialSVM_score/pred, LR_score/pred, and MetaSVM_score/pred were used to predict the pathogenicity class of each variant, independent, if listed in the HGMD, novel or rare.

Table 1. Patient characteristics.

NYHA functional class and functional parameters	CHD (n=23)	DCM (n=32)	iVT (n=37)
I	1 (4.3%)	1 (3.1%)	25 (67.6%)
II	6 (26.1%)	1 (3.1%)	11 (29.7%)
III	16 (69.6%)	20 (62.5%)	1 (2.7%)
IV	0 (0%)	10 (31.2%)	0 (0%)
LVEF (%)	36.6%	25.5%	60.9%
LA (mm)	42.9	47.31	30.59
LV ESD (cm)	6.24	6.85	4.62
LV LEDD (cm)	5.05	5.99	3.12
Family history of CM or SCD			
familial	6 (26.1%)	8 (25%)	11 (29.7%)
sporadic	17 (73.9%)	24 (75%)	24 (64.9%)
unknown	0	0	2 (5.4%)

CHD, coronary heart disease; DCM, dilated cardiomyopathy; iVT, idiopathic ventricular tachycardia; CM, cardiomyopathy; SCD, sudden cardiac death; LVEF, left ventricle ejection fraction; LA, Left atrial dimension; LV-EDD, Left ventricular end-diastolic dimension; LV-ESD, Left ventricular end-systolic dimension.

Table 2. Selected cardiac disorders and associated genes. List of 96 target genes.

Arrhythmogenic syndromes	associated genes
Long QT syndrome	KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, ANK2, KCNJ2, CACNA1c, Cav3, SCN4b, AKAP9, KCNJ5, SNTA1
Shortened QT syndrome	KCNQ1, KCNH2, KCNJ2
Brugada syndrome (BrS)	SCN5A, CACNB2, GPD1L, SCN1b, KCNE3, SCN3b, CACNA1c, MOG1, KCNE5, KCND3, HCN4
Idiopathic ventricular arrhythmia	KCNA5, KCNE2, KCNQ1, NPPA, NUP155, LMNA, SCN5A, KCNJ8, ABCC9, GJA5, KCNJ2
Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)	RyR2, CASQ2, KCNJ2
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	PKP2, DSG2, DSC2, DSP, JUP, TMEM43, TGFB3, RyR2
Dilated cardiomyopathy	LMNA, LDB3, TNNT2, PLN, MYH7, MYBPC3, SCN5A, DES, SGCD, CSRP3, TCAP, ACTC, TNNC1, TNNI3, TTR, ILK, EMD, CRYAB, BAG3, CHRM2, SGCB, DSP, TPM1, NEBL, DSG2, TTN, EYA4, ABCC9, TMPO, PSEN1, PSEN2, ACTN2, TAZ, VCL, ANKRD1, FKTN, LAMP2, NEXN, TBX20, DTNA, MYPN, LAMA4, FHL2, LAMA2, DMD, RBM20, SERCA2A, MYH6, CRYAB, BAG3, CHRM2, SGCB, NEBL, DSG2
Hypertrophic Cardiomyopathies	MYH7, TNNT2, TPM1, MYBPC3, TNNI3, PRKAG2, MYL3, TNNC1, MYL2, ACTC, LAMP2, GLA, Cav3, TTR, FHL1, TTN, CSRP3, MYOZ2, MYH6, MYLK2, MYO6, LDB3, TCAP, VCL, ACTN2, PLN, JPH2, CRYAB, NDUFV2, GAA, CALR3, CTF1, NEXN
Restrictive Cardiomyopathies	MYH7, TNNI3, MYBPC3, TPM1, TNNT2, TNNC, ACTC, MYL2, MYL3, TCAP, LMNA, DES, CSRP3, TAZ, LDB3, MYOZ2, PLN, GLA

RESULTS

The candidate gene library design covers a total target region of 463.767kbp with 406.062 analyzable target bases including all exonic and proximal intronic (+/-10bp) sequence and representing by 2,017 target loci. The mean coverage of all 92 samples at the target loci was 707.62-fold.

Targeted sequencing and stepwise filtering of the annotated variants identified a total of 307 unique variants in 74 genes totaling up in 456 variants for the overall study group (HGMD listed) (Fig .1).

More than 50% of the patients carried at least two mutations, irrespective of the clinical phenotype (Table 3). Furthermore, 215 private (unique) non-synonymous variants were observed in the patient cohort.

Prediction scores of the private variants indicated high probability of disease association (Fig.2,3). Including the newly identified high-probability variants, each patient carried on average >4.8 genetic variants (Fig.1, Table 3).

Interestingly, statistical evaluation revealed no difference in the frequency of genetic variants (HGMD or rare variants) observed for the CHD and the DCM subgroup (Fig 2,3,4).

The most abundant mutations of the CHD were observed in *MYBPC3, DMD, LAMA2, MYH6* and *GAA*. *PRKAG2* mutations were overrepresented in the CHD subgroup (Fig.4).

CONCLUSIONS

Our study indicates that the majority of the investigated patients with cardiomyopathies and VT, irrespective of disease phenotype or subgroup carry multiple disease-linked mutations and additional potentially functional rare variants in cardiac disease genes. We thus have to consider a molecular disease-overlap with converging phenotypes.

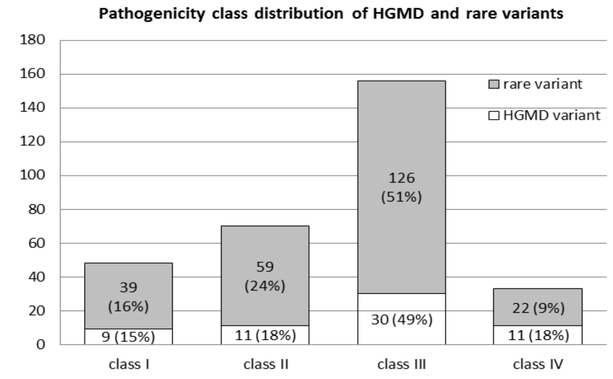

Figure 1. Distribution of HGMD listed variants and rare variants with respect to their pathogenetic potential (class I: high potential, class II: intermediate potential, class III: low potential and class IV: benign).

Table 3. Frequency of patients positive for pathogenetic variants in the specific clinical subgroups (CHD, DCM, and iVT). Variation per positive patient was calculated by dividing the (cumulating) number of variants by the number of positive patients.

	patients carrying ≥ 1 class I variant	% positive	cumulative number of variants	% of all variants	var. per pos. patient
CHD (n=23)	10	43.5%	13	11.8%	1.3
DCM (n=32)	10	31.3%	13	8.2%	1.3
iVT (n=37)	20	54.1%	26	13.9%	1.3
	≥ 1 class I/II variant				
CHD (n=23)	16	69.6%	31	28.2%	1.94
DCM (n=32)	24	75.0%	49	30.8%	2.04
iVT (n=37)	30	81.1%	70	37.4%	2.33
	≥ 1 class I/II/III variant				
CHD (n=23)	22	95.7%	89	80.9%	4.05
DCM (n=32)	31	96.9%	131	82.4%	4.23
iVT (n=37)	36	97.3%	160	85.6%	4.44

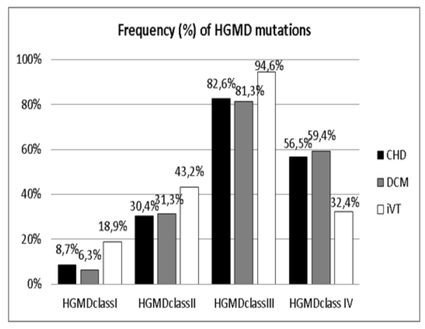

Figure 2. Incidence of HGMD variants per clinical subgroup.

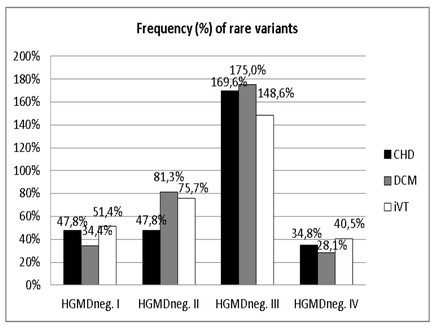

Figure 3. Incidence of rare variants (HGMD variants excluded) per clinical subgroup.

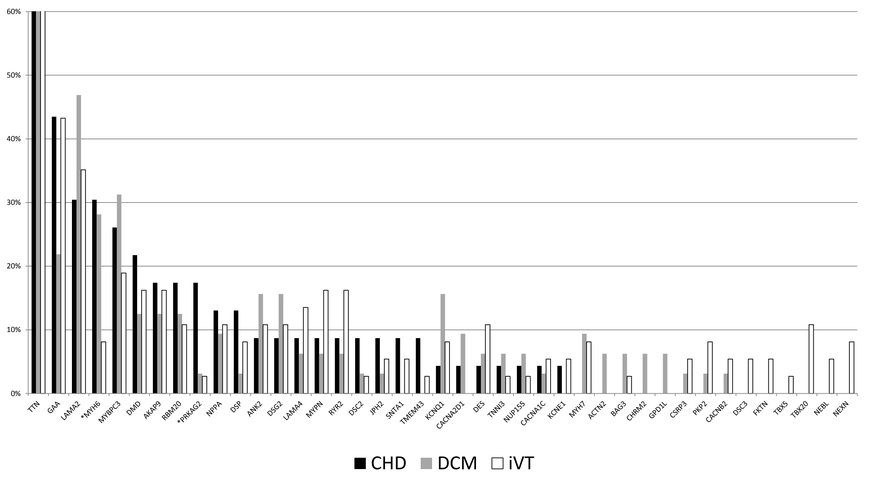

Figure 4. Combined frequency of mutations and rare variants. *Statistical testing using Fishers exact t-test suggests a trend for a difference in the frequency of MYH6 and PRKAG2 variants (p-value of 0.053 and 0.054. respectively) between CHD and the other subgroups.

TRANSLATIONAL PERSPECTIVE

We were able to show that patients with coronary heart disease carry a similar genetic burden (qualitatively and quantitatively) in cardiac risk genes as known for dilated cardiomyopathy.

These common genetic alterations might be applied in improving diagnostic and therapeutic strategies to the individual patient in daily clinical practice.

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