Cardiac levels of NOS1AP RNA from right ventricular tissue recovered during lead extraction

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BACKGROUND There is a scarcity of cardiac tissue available for research.

OBJECTIVE (1) To investigate the feasibility of obtaining myocardial tissue from extracted pacemaker and defibrillator leads for gene expression analysis and (2) to examine the nitric oxide 1 adaptor protein (NOS1AP) RNA expression as a function of patient genotype.

METHODS Seventeen patients (age = 56 ± 20 years; 12 men; 5 pacemakers; 12 defibrillators) undergoing lead extractions for standard indications (5 device erosion; 1 vascular occlusion; 11 lead malfunction or recall) were genotyped for 2 NOS1AP single nucleotide polymorphisms—rs10494366 (T to G) and rs10918594 (C to G)—and had RNA levels measured by real-time polymerase chain reaction for collagen I, troponin I, $Ca_v1.2$, Kv4.3, HERG, KvLQT1, connexin 43, NOS1AP, and sodium–calcium exchanger. Ventricular tissue obtained from 3 failing hearts at transplantation served as reference.

RESULTS A high ratio of cardiac troponin I/collagen I RNA identified 9 of the 17 patient samples (muscle rich), in which the gene expression profile was similar to that of the reference ventricular samples and significantly different ($P < .003$) from the expression profile of samples with a low troponin I/collagen ratio (muscle poor). TT and CC polymorphisms were associated with significantly lower NOS1AP RNA levels ($P < .01$ compared with the GG genotype).

CONCLUSIONS Performing gene expression analyses on right ventricular tissue samples extracted with pacemaker and defibrillator leads is feasible. A significant number of samples contain cardiomyocytes that express troponin I and ion channels at levels comparable to those seen in explanted hearts. Decreased NOS1AP expression in rs10494366 TT and rs10918594 CC homozygotes may underlie shorter repolarization times.

KEYWORDS NOS1AP; Cardiac tissue; Lead extraction; Repolarization time

ABBREVIATIONS **CIED** = cardiac implantable electronic device; **ECG** = electrocardiogram; **NCX** = sodium–calcium exchanger; **NOS1AP** = nitric oxide 1 adaptor protein; **RT-PCR** = real-time polymerase chain reaction; **RV** = right ventricular

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Indications for cardiac implantable electronic device (CIED) implantations have expanded dramatically over the past decade, given the growing clinical indications for pacemakers and defibrillators[1] used for the secondary[2–4] and primary[5–8] prevention of sudden cardiac death as well as in the management of patients with advanced heart failure symptoms.[9–11] It is estimated that there are currently more than 3.5 million CIED leads implanted worldwide, and this number is increasing by about 1 million every year. Paralleling and often exceeding this expansion in implantation indications is the need to explant the CIED and extract the associated leads, which has been rising dramatically in the United States[12] and reaching epidemic proportions.[13,14]

The importance of gene expression in understanding cardiac disease has been recognized for many years, but a good understanding of individual causative genes associated with specific conditions has been elusive. Biomarkers of gene expression have been associated with arrhythmia burden.[15–18] Also, genetic polymorphisms have been associated with altered parameters on the surface electrocardiogram (ECG) and altered risks of sudden cardiac death. An example of this is the modulating effects of single nucleotide polymorphisms in the nitric oxide 1 adaptor protein (NOS1AP) gene on the repolarization time by surface ECG.[19–24]

A limiting factor in the progress of our understanding of cardiac genetics has been the paucity of available cardiac tissue, the source of which is usually being restricted to the very sick end-stage heart failure patients, such as those undergoing ventricular assist device placement or cardiac transplantation. This is particularly true now that performing cardiac biopsies has fallen out of favor clinically because of an unfavorable risk–benefit ratio,[25] except in the post transplant population. Given that cardiac tissue recovered with extracted leads contains fibrotic strands from various parts of the heart and variable quantities of myocardium, we investigated in this study the feasibility of using cardiac tissue recovered with extracted pacemaker

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Table 1 Primers used in real-time polymerase chain reaction

Target	Forward primer (reference nucleotides)	Reverse primer (reference nucleotides)	Genbank reference
GAPDH	AATTGAGCCCGCAGCCTCCC (2–21)	GAGCGATGTGGCTCGGCTGG (95–76)	NM_002046.3
Cardiac troponin I	CAAGCAGGTGAAGAAGGAGG (662–681)	CAGTAGGCAGGAAGGCTCAG(792–773)	NM_000363.4
Collagen I	GGGATTCCCTGGACCTAAAG (1866–1885)	GGAACACCTCGCTCTCCA (1928–1911)	NM_000088.3
KV4.3	ACCATTGTCACCATGACCAC (1561–1580)	AATGACCAGGACGCCACT (1665–1648)	NM_004980.4
CaV1.2	GCCTACCTCCGCAACGGCTG (878–897)	CGGCCCCTTTCCCTCCGAGA (1001–982)	NM_199460.2
HERG	TCGCGCCGCAGAACACCTTC (424–443)	GACGGCGCAGTTCTCCACCC (524–505)	NM_172056.2
KvLQT1	GTCCCACCGGCTGGAAATGCT (455–475)	GTGGCCAGGGCGGCATACTG (546–547)	NM_000218.2
Connexin 43	GGTCTGAGTGCCTGAACTTGCCT (144–166)	GGCGCTCCAGTCACCCATGT (268–249)	NM_000165.3
NOS1AP	CCATGCGCCGGATACGGTATGA (548–569)	CCCACGTCCATTCCTTTTTCTGCAA (691–667)	NM_014697.2
NCX	ACCTGTTTGGCCAACCTGTCTTCA (1985–2008)	TGCTGGTCAGTGGCTGCTTGT (2095–2075)	NM_021097.2

GAPDH = glyceraldehyde phosphate dehydrogenase; NCX = sodium–calcium exchanger; NOS1AP = nitric oxide 1 adaptor protein.

and defibrillator leads for gene expression testing and, as a proof of concept, applied this new method to compare right ventricular (RV) tissue levels of NOS1AP mRNA between patients grouped according to their genotype expression of 2 NOS1AP single nucleotide polymorphisms.

Methods

Population characteristics

Patients undergoing lead extraction for established clinical indications were enrolled in this study between January 1 and November 12 of 2009. The study protocol was approved by the institutional review board of the University of Pittsburgh. All enrolled patients signed written informed consent prior to the lead extraction procedure, allowing the investigators to collect 15 cm^3 of blood for genetic analysis as well as freezing any tissue recovered with the extracted leads. Eligible patients had to be older than 18 years of age and have an established clinical indication for lead extraction, according to published guidelines.[12] The dwell time of the leads had to be no less than 6 months for eligibility in order to increase the yield of recovering tissue during the extraction procedure.

Enrolled patients underwent lead extraction and device explantation as per standard of care. Implantation of a replacement device or a temporary pacemaker during the procedure was dictated by clinical need and left to the discretion of the operating physician. As per standard practice, all patients had a 5F arterial line for continuous hemodynamic monitoring and a 7F venous sheath placed in the femoral vessels. Lead extraction was achieved by means of a combination of telescoping mechanical and powered sheaths (Spectranetics, Colorado Springs, CO). All enrolled patients underwent analysis of a 12-lead surface ECG for repolarization times.

A total of 62 patients signed written informed consent. Of the overall group, patients were excluded from the analysis if they had systemic infection as the indication for lead extraction (n = 17) or if they did not have an RV lead extracted (n = 2). Of the remaining 43 patients, 32 were utilized to attempt RNA extraction. Of these 32, 17 yielded sufficient tissue so as to allow adequate RNA recovery for real-time polymerase chain reaction (RT-PCR) analysis. The final analysis focused on those 17 patients.

NOS1AP genotyping

All participants were genotyped for the NOS1AP single nucleotide polymorphisms rs10494366 (T to G) and rs10918594 (C to G), previously shown to be associated with QT interval.[19–24] Genotyping was done with predesigned TaqMan assays C_1777074_10 and C_1777009_10 and analyzed by Applied Biosystems Prism 7000 (Applied Biosystems, Foster City, CA) in 1ng genomic DNA extracted from leukocytes by using the PureGene Kit (Qiagen, Vallencia, CA), as previously reported.[26]

Cardiac tissue gene expression

Tissue recovered from extracted RV leads was frozen with liquid nitrogen in the electrophysiology laboratory and then stored at −80°C in a freezer. Tissue mass on the RV leads ranged from an estimated 10 to >200 mg. In larger samples, tissue within 5 mm of the lead tip was utilized. Ventricular tissue obtained from 3 explanted hearts during heart transplantation served as reference.

All samples (17 from extracted leads and 3 reference samples from explanted hearts) were processed similarly. As previously described,[27–29] total RNA was extracted from cardiac tissue by using the RNeasy kit (QIAGEN, Cat# 74134, Qiagen, Valencia, CA). The complementary DNA was synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. An SYBR Green–based formulation (Absolute Sybr-Green, Thermo Fischer Scientific, Waltham, MA) was utilized for fluorescence-based kinetic RT-PCR using a Perkin-Elmer Applied Biosystems model 7000 sequence detection system (Applied Biosystems, Foster City, CA). The expression levels of RNA encoding 9 cardiac tissue profiling genes (cardiac troponin I, collagen I, $Ca_v1.2$, Kv4.3, HERG, KvLQT1, connexin 43, and sodium–calcium exchanger [NCX], NOS1AP) were normalized to that of glyceraldehyde phosphate dehydrogenase. Primers utilized for RT-PCR are presented in Table 1.

Data and statistical analysis

RNA expression was normalized to the mean expression of each gene in the reference treatment (TX) group. The ratio of cardiac troponin I to collagen I was used to empirically separate lead samples with a ratio >0.1 (muscle rich) from