Deep sequencing of atrial fibrillation patients with mitral valve regurgitation shows no evidence of mosaicism but reveals novel rare germline variants

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BACKGROUND Atrial fibrillation (AF) is the most common cardiac arrhythmia. Valvular heart disease is a strong predictor, yet the underlying molecular mechanisms are unknown.

OBJECTIVE The purpose of this study was to investigate the prevalence of somatic variants in AF candidate genes in an AF patient population undergoing surgery for mitral valve regurgitation (MVR) to determine whether these patients are genetically predisposed to AF.

METHODS DNA was extracted from blood and left atrial tissue from 44 AF patients with MVR. Using next-generation sequencing, we investigated 110 genes using the HaloPlex Target Enrichment System. MuTect software was used for identification of somatic point variants. We functionally characterized selected variants using electrophysiologic techniques.

RESULTS No somatic variants were identified in the cardiac tissue. Thirty-three patients (75%) had a rare germline variation in ≥1 candidate genes. Fourteen variants were novel. Fifteen variants were predicted damaging or likely damaging in ≥6 *in silico* predictions. We identified rare variants in genes never directly associated with AF: *KCNE4*, *SCN4B*, *NEURL1*, and *CAND2*. Interestingly, 7 patients (16%) had variants in genes involved in cellular potassium handling. The variants *KCNQ1* (p.G272S) and *KCNH2* (p.A913V) resulted in gain of function due to faster activation (*KCNQ1*) and slowed deactivation kinetics (*KCNQ1*, *KCNH2*).

CONCLUSION We did not find any somatic variants in patients with AF and MVR. Surprisingly, we found that our cohort of non–lone AF patients might, like lone AF patients, be predisposed to AF by rare germline variants. Our findings emphasize the extent of still unknown factors in the pathogenesis of AF.

KEYWORDS Atrial fibrillation; Genetics; Bioinformatics; Cardiac tissue; Mitral valve regurgitation; Somatic mutations; Somatic variants

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Introduction

Atrial fibrillation (AF) is a supraventricular cardiac arrhythmia characterized by chaotic contractions of the atria. The mechanism of AF initiation is not fully understood, although it is generally agreed that rapid atrial activation from or near the pulmonary veins (PVs) plays an important role.[1] Moreover, a combination of PV triggering, autonomic innervation by atrial ganglion plexi, and rotors located in the atria might be responsible for maintenance of AF.[2] Several adverse events are related to AF, including stroke, heart failure, reduced quality of life, and increased mortality.[3] AF is the most common clinically significant cardiac arrhythmia, with

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an age-adjusted incidence of 13.4 and 8.6 new cases per 1000 person-years for men and women, respectively. The risk of AF increases with age, and by the age of 80 to 89 the risk of AF is 9 times higher than at age 50 to 59.[4] Moreover, the Framingham Heart Study observed a 4× increase in the age-adjusted prevalence of AF during their 50-year follow-up,[4] likely resulting in a considerable economic challenge in the future. AF has proven difficult to manage, and the optimal treatment strategy is still being investigated.[5] Improving the understanding of AF by delving further into the mechanisms that initiate and sustain AF may give rise to an ideal treatment through individualized therapeutic options according to the patients' clinical and genetic risk profile.[6]

A high prevalence of rare variants in AF-associated genes is well documented in lone AF.[7] Furthermore, somatic variations in atrial appendage tissue of lone AF patients were identified a few years ago.[8,9] The validity of this discovery has recently been questioned, after Roberts et al[10] sequenced 560 AF target genes in 25 AF patients and found no evidence of somatic variations in atrial appendages.

Non–lone forms of AF have been found to contain a genetic component as well, for example, parental AF, which independently predicts an increased risk of AF in offspring.[11] Whether non–lone forms of AF are predisposed by rare germline or somatic variations has not been fully addressed.

Valvular heart disease (VHD) is known to be 1 of the strongest risk factors for AF.[12] However, not all patients with VHD develop AF, suggesting that multiple factors are necessary for its development. This raises the question whether VHD patients who develop AF are genetically predisposed by rare variations.

In this study, we investigated the burden of somatic variations in AF candidate genes in patients with concomitant AF and mitral valve regurgitation (MVR) using left atrial (LA) posterior wall biopsies, that is, the site of the PV inlets.

Methods

Patients and samples

To obtain a homogeneous study population, only Caucasians with concomitant history of AF and MVR requiring surgical repair were included in the study. Exclusion criteria were acute surgery, patients younger than 18 years, women of childbearing age, and congenital heart disease. A total of 44 patients with AF undergoing valve surgery at the University Hospital of Copenhagen, Rigshospitalet, in the period from 2009 to 2014 were included. None of the patients were related. Clinical data were obtained through questionnaires and patient records based on patient contacts to the health care system.

The study was approved by the Ethics Committee of the Capital Region of Copenhagen (protocol reference no. 16238) and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Blood was drawn from the patients just before surgery and stored at –80°C until DNA extraction was performed. During surgery, biopsies were taken from the LA posterior wall (between the PVs), a likely site of predisposing variants. The surgically removed tissue was snap frozen in liquid nitrogen and stored at –80°C until DNA extraction.

Target genes

A total of 110 candidate genes were chosen based on association to AF in genomewide association studies (GWAS), candidate gene studies, cardiac expression levels, function, or association to ECG changes in GWAS. For a list of the 110 chosen candidate genes, see Supplementary Table S1.

The HaloPlex Probe library was custom made using Agilent Technologies' SureDesign tool (Agilent Technologies, Santa Clara, CA).

Next-generation sequencing

Genomic DNA was extracted from blood lymphocytes using Maxwell 16 LEV Blood DNA kit (Promega Corp, Madison, WI, USA) and from LA tissue using Maxwell 16 Tissue DNA purification kit (Promega).

Next-generation sequencing (NGS) of the 110 selected genes was performed using the HaloPlex Target Enrichment System (Agilent Technologies) according to the manufacturer's instructions, prescribing 50 ng DNA per library (corresponding to approximately 7700 cells per sample). Patient DNA was fragmented by endonucleases and hybridized to biotinylated gene-specific probes incorporating Illumina paired-end sequencing and indexed primers (Illumina, San Diego, CA). Hybridized molecules were captured by magnetic beads, amplified by polymerase chain reaction, and sequenced with the MiSeq system (Illumina).

Data analyses and bioinformatics

Processing of NGS data was performed following Broad Institute's (Harvard/MIT) "best practice" guideline. Initial genotyping calls were filtered in order to obtain a set of high-quality genotype calls (see Supplementary Methods and Supplementary Figure S1). Annotation of variants was made by SnpEFF v4.0b,[13] and *in silico* predictions were mined from the dbNSFP2.4 database.[14]

MuTect, a Broad Institute software for identification of somatic point variants in NGS data, was used for identification of somatic point variants in the LA tissue (see Supplementary Methods).[15] Regarding incidental findings of germline variants identified in this study, only rare variants with a minor allele frequency <0.005 in the Exome Aggregation Consortium database,[16] a reference group of 2000 exomes from healthy Danes,[17] and the NHLBI-GO Exome Sequencing Project[18] were considered to be of possible significance.

Molecular biology

Point variants of $K_V7.1$ and $K_V11.1$ were introduced using standard mutated oligonucleotide extension polymerase chain reaction techniques (see Supplementary Methods).