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Research article

Atrial fibrillation recurrence after catheter ablation is associated with RAD51 and p63 proteins

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ABSTRACT

Catheter ablation has been demonstrated to reduce atrial fibrillation (AF) recurrence. The mechanisms of AF recurrence after catheter ablation are unknown, and the present study aimed to identify serum proteins associated with AF recurrence.

The present prospective study comprised a cohort of patients with AF, which was divided into two groups after one-year follow-up: group 1 included patients with compensated AF after catheter ablation and group 2 included patients with AF recurrence after catheter ablation. Initial microarray profiling of the serum proteins was performed in small subgroups M1 and M2 recruited from groups 1 and 2, respectively, by an antibody microarray to evaluate potentially relevant proteins. The data of initial proteomic profiling identified candidate proteins in groups 1 and 2, and their levels were then measured by ELISA. The data of profiling suggested an overall increase in the levels of RAD51 and p63 proteins in the M2 subgroup versus that in the M1 subgroup, indicating potential relevance of these two proteins to AF recurrence. The results of ELISA of the levels of RAD51 and p63 in the groups 1 and 2 demonstrated an increase in the levels of RAD51 (11.11 ± 4.36 vs 8.45 ± 4.85 ng/mL; P = 0.009) and p63 (165.73 ± 113.75 vs 100.05 ± 37.56 units of normalized optical density; P = 0.0007) in the group 2 (with AF recurrence or substrate AF) compared with that in the group 1 (compensated AF). Thus, RAD51 and p63 were associated with AF recurrence after catheter ablation and may represent possible etiological factors for subsequent outcomes.

1. Introduction

Catheter ablation is used for efficient treatment of atrial fibrillation (AF) recurrence. The procedure substantially improves the quality of life of patients with symptomatic AF compared with the effects of routine antiarrhythmic therapy [1,2]. Long-term success of AF ablation may be suboptimal in some patients who manifest AF recurrence at the rates ranging from 20 % to 50 % [3,4]. These variabilities between responders and non-responders may be due to the degree of atrial myopathy. Atrial fibrosis is important for stabilization of reentry processes required to maintain AF. Moreover, AF recurrence and resistance to therapy are known to be

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associated with atrial fibrosis [5]. Overall progression of AF is linked to atrial dilatation, atrial myocyte injury, altered collagen turnover, and inflammation, contributing to scarring and fibrosis [6]. These processes of structural and electrical remodeling in patients with long-term AF eventually reduce the likelihood of restoration and subsequent maintenance of restored sinus rhythm [7]. Timely catheter ablation at an early stage of the disease interferes with AF progression to slow various pathological processes leading from paroxysmal to persistent forms of AF [8]. The time interval between initial diagnosis of AF and ablation, which is known as diagnosis-to-ablation time (DAT), may be used to evaluate subsequent long-term beneficial effects of ablation. Additionally, DAT is associated with higher levels of biomarkers of atrial remodeling, including plasma contents of B-type natriuretic peptide and C-reactive protein [9]. The present study aimed to identify serum proteins, which can be used as predictors of AF recurrence after catheter ablation after one-year follow-up, to define the signals involved in AF recurrence.

2. Materials and methods

2.1. Subjects

The cohort of the present study comprised 206 patients, which were selected consecutively. Patients over 18 years of age had symptomatic AF. The score determined using the European Heart Rhythm Association (EHRA) symptom classification for AF [10] was at least A2b, and paroxysmal or persistent AF was diagnosed. The present study has been registered at the ClinicalTrials.gov website (registration number NCT05170607; general protocol has been described in our previous publication [11]). All patients included in the cohort were conducted in the National Research Center for Preventive Medicine (NRCPM), Ministry of Healthcare of Russian Federation, Moscow, Russia. Primary pulmonary vein cryoballoon ablation was performed in all patients using a 28-mm cryoballoon (Arctic Front Advance, Medtronic, USA), and an electrocardiogram (ECG) loop recorder (Reveal Linq, Medtronic, USA and SJM Confirm, Abbott, USA) with simultaneously installed. The procedure was performed from April 2017 to December 2022. Flowchart of the study is presented in Fig. 1. The protocol of the study was approved in accordance with the Declaration of Helsinki and WHO guidelines by the Independent Ethics Committee of NRCPM (number 01–06/17; February 2, 2017). All patients signed a written informed consent to participate in the study.

The appointments at 3, 6, and 12 months after the ablation were scheduled during the total follow up period of 12 months to monitor AF and to discontinue the ongoing antiarrhythmic therapy (excluding beta-blockers) as appropriate after 3 months. The data of implanted ECG loop recorders were retrieved during the visits and used for evaluation. The efficacy of pulmonary vein isolation in patients with recurrent AF was determined by an invasive electrophysiological examination. Patients with ineffective pulmonary vein isolation were treated by redo radiofrequency ablation (RFA). Patients with lesions of the orifices of the pulmonary vein were observed

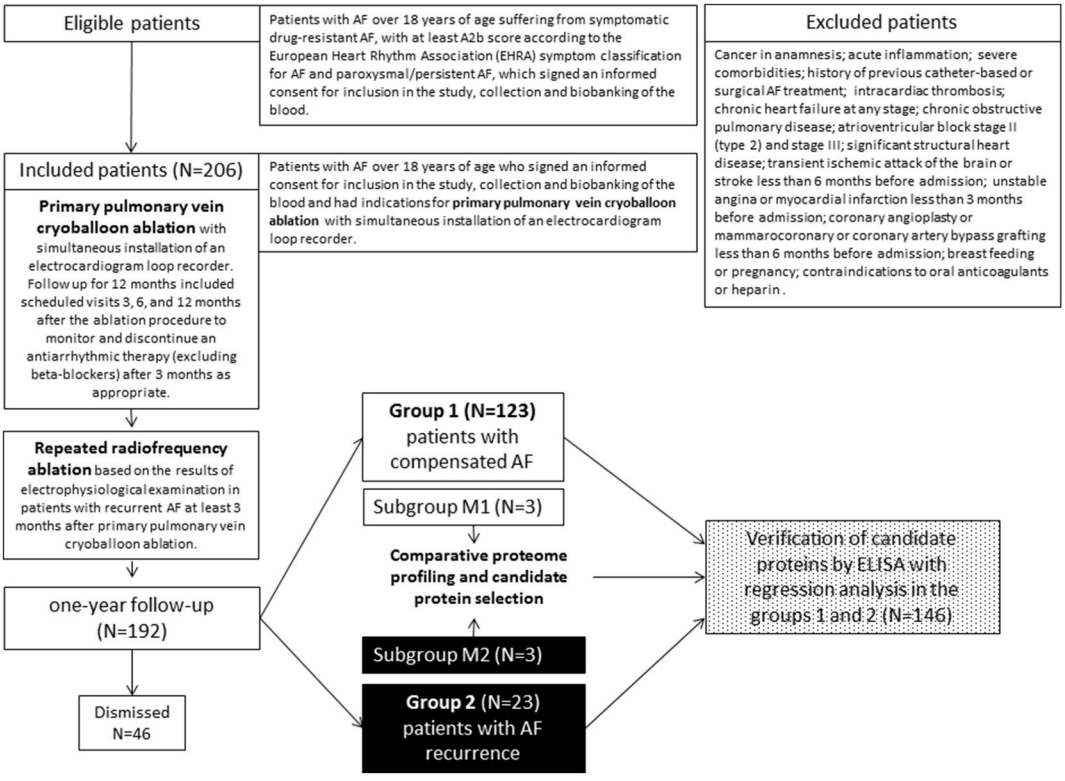

Fig. 1. Flowchart of the study.

for 12 months after the procedure during the visits scheduled at 3, 6, and 12 months time points.

The following inclusion criteria were applied: over 18 years of age; diagnosis of drug-resistant AF based on documented lack of efficacy of medication with at least one antiarrhythmic drug type I or type III, including β-blockers; and diagnosis of symptomatic (≥ EHRA 2b class) paroxysmal/persistent AF manifested in at least two episodes, one of which had to be documented. Diagnosis was compliant with the guidelines of the European Society of Cardiology and Russian Society of Cardiology. Routine examination comprised of a 12-lead or a single-lead ECG tracing over 30 s that demonstrated heart rhythm without distinguishable repeating P waves and irregular RR intervals (if atrioventricular conductivity was unimpaired) [12].

The following exclusion criteria were applied: cancer in anamnesis; history of catheter-based or surgical AF treatment in the past; chronic heart failure of any stage according to the New York Heart Association classification; significant structural heart diseases (anomalous progression of the pulmonary vein, severe coronary artery disease, and valve abnormality, including prosthetic heart valves); decompensated hypo- and hyperthyroidism, severe liver failure, chronic kidney diseases under dialysis; intracardiac thrombosis; oral anticoagulants or heparin cautions; chronic obstructive pulmonary disease with diagnosed pulmonary hypertension; atrioventricular block stages II (type 2) and III; transient cerebral ischemic attack or stroke in previous 6 months; unstable angina/myocardial infarction in previous 90 days; coronary angioplasty/mammary coronary/coronary artery bypass grafting in previous 180 days; acute inflammation; breast feeding or pregnancy.

Groups 1 and 2 were selected after one year follow-up (Fig. 1). Patients with pulmonary compensated AF after repeated ablation at the end of the follow-up were assigned to group 1, and patients with AF recurrence were assigned to group 2. The small subgroups M1 and M2 were picked up from the large groups 1 and 2, respectively, for proteomic profiling using microarrays to investigate candidate proteins by comparison of proteomic profiles. The group M2 was selected from group 2 randomly, and group M1 was selected from the group 1 to match the group M2 in sex and age to minimize the impact of sex and age on potential differences in the corresponding proteomic profiles. The patients of groups 1 and 2 were from the same cohort, and the samples were used for verification of the differences in candidate protein levels, which were identified by proteomic profiling, using ELISA.

2.2. Blood samples

Blood was collected from the cubital vein at the baseline. The serum was separated by centrifugation at 1,000 g for 15 min at 4°C. Aliquots of the serum and plasma samples were stored at −27°C. Before proteomic assays, serum samples were centrifuged at 10,000 g for 15 min at 4°C, and the protein levels were analyzed based on the optical density (OD) at 260–280 nm (NanoDrop One spectrophotometer; Thermo Scientific) with human serum albumin as the reference.

2.3. Routine biochemical tests

The C-reactive protein (CRP; mg/L) was analyzed with high sensitivity quantitative immunoturbidimetric method enhanced with latex particles (0.3–350 mg/L range; highly sensitive range 0.05–20 mg/L) by kits from DiaSys (Germany) on an Architect C 8000 analyzer (Abbot, USA). The level of N-terminal brain natriuretic peptide prohormone (NT-proBNP; pg/mL) in the serum was assayed by chemiluminescent microparticle immunoassay (CV% < 10 %) using a Radiometer AQT90 FLEX (Radiometer Medical ApS, Denmark).

2.4. Analysis of labeled serum proteins by antibody microarrays

The protocol of the assay has been described by us in our previous publications in detail [13,14]. Briefly, serum proteins were diluted to a concentration of 1 mg/mL in phosphate buffered saline (PBS; Arrayit Corp.), mixed with 20 μL of labeling buffer, and labeled by coupling to Green 540 reagent (1 μL; Arrayit Corp.) for 60 min on ice. The coupling reaction was terminated by stop solution (10 μL; Arrayit Corp.). Excess dye was removed by gel filtration. Labeled proteins (500 ng per slide in blocking buffer containing 3 % dry milk) were processed using Explorer antibody microarrays (ASB600, Full Moon Biosystems, USA; 656 antibodies per slide in two replicates; complete list of all antibodies on the microarray is presented at https://www.fullmoonbio.com/datasheets/ASB600_AbList.xls. After 1-h incubation and washing, the slides were incubated with coupling buffer containing 3 % dry milk for 2 h, washed, and dried using a small centrifuge for glass slides.

2.5. Microarray slide scanning and image processing

Microarray slides were scanned and processed as described by us previously [13,14] on an InnoScan microarray scanner 900 (Innopsys, France) to ensure positive matches with the corresponding controls on each slide. Mapix software version 7.0.0 (Innopsys, France) was used to analyze the images, and proteins were identified according to their position using microarray-specific Gal-files to match the UniProtKB/Swiss-Prot database identifiers. The background at the reference wavelength was subtracted, and image intensity was expressed as median pixel intensity. If parallel spots did not match in intensity due to high variability (CV over 25 %), the corresponding proteins were not used in subsequent analysis.

2.6. Indirect ELISA for p63 protein in the serum

Serum samples (100 μL per well at a protein concentration of 10 μg/mL) were diluted with 14 mmol/L carbonate buffer (pH 9.6),

and the solution was used to coat the wells of a 96-well microtiter plate at 4 °C overnight. The wells were washed 3 times with PBS containing 0.05 % v/v Tween 20 and blocked in 150 μL of PBS containing 1 % BSA for 60 min at 37 °C, with subsequent washing. Mouse anti-p63 monoclonal antibody (100 μL; 0.5 μg/mL; MyBioSource, Vancouver, Canada) was incubated with the samples for 1 h at 37 °C, and peroxidase-conjugated secondary anti-mouse antibodies (100 μL; dilution 1:10,000; Invitrogen, Thermofisher) were incubated for 1 h at 37 °C. The wells were washed, and 3,3′,5,5′-tetramethylbenzidine substrate solution (100 μL/well; Bio-Rad) was added for 30-min incubation in the dark. Absorbance was measured at 450 nm after the addition of stop solution (50 μL/well; Bio-Rad). All samples were assayed in duplicate. The corresponding control samples assayed on each microplate were used to normalize the results.

2.7. ELISA of RAD51 in serum

Serum RAD51 levels were analyzed in the samples using an ELISA kit for human RAD51 (DNA repair protein RAD51 homolog 1) (MyBioSource, Vancouver, Canada) based on sandwich ELISA according to the manufacturer instructions within the range of 0.156–10 ng/mL (sensitivity: 0.094 ng/mL; intra-assay precision: CV<8 %; and interassay precision: CV<10 %). All samples were assayed in duplicate.

2.8. Statistical analysis

Statistic analysis was performed using software version 8.0 and SPSS version 23 (IBM, USA). Prior to recruitment, required sample size was estimated using the online calculator Sampsize https://sampsize.sourceforge.net/iface/s2.html#nm (accessed on September 10, 2021). Kolmogorov-Smirnov test was used to assess normality of the distributions. The values were compared between the groups by two-tailed non-parametric analysis of variance (Kruskal-Wallis and Mann-Whitney tests). Categorical variables are presented in percentages, and numerical values are presented as the median and 25th and 75th percentiles. Statistic analysis for independent impact of the variables towards AF recurrence was performed using multivariate logistic regression analysis by a binary logit model with Fisher scoring. *P*-values <0.05 were considered significant.

3. Results

3.1. Characteristics of the groups

The study cohort initially included 206 patients diagnosed with AF and treated in NRCPM. A total of 192 patients completed the one-year follow-up period. Clinical and demographic characteristics of the total cohort have been described previously [15] and are shown in Table 1. A history of AF was 4.0 ± 2.7 years, and the mean age at the AF onset was 53.24 ± 10.55 years. Hypertension (75.63 %) and obesity (53.2 %) were the most common comorbidities. A total of 14.21 % of patients were diagnosed with diabetes mellitus type 2 (fasting plasma glucose levels were equal or up to 126 mg/dL (7.0 mmol/L); 2-h plasma glucose levels were equal or up to 200 mg/dL (11.1 mmol/L) in a oral glucose tolerance test with a 75-g glucose load; and HbA1c was equal or up to 6.5 % (48 mmol/mol)). Additionally, 46 patients were withdrawn due the lack of follow up visits, rejection of repeated procedure, or failure to isolate the pulmonary vein because of anatomical features. Thus, 146 patients remaining in the cohort were assigned into two groups: group 1 (N = 123) with pulmonary vein-associated AF (compensated AF) and group 2 (N = 23) with substrate AF (recurrent AF) (Table 1). AF was considered compensated if there were no AF episodes longer than 2 min within one year after a single ablation or after two ablations. An implanted electrocardiogram loop recorder was used to determine the duration of AF episodes according to the guidelines of the

Table 1
General, clinical, and biochemical characteristics of the groups 1 (compensated AF) and 2 (recurrent AF).

Clinical and demographic parameters	Group 1 (compensated AF; N = 123)	Group 2 (recurrent AF; N = 23)	P
	Median (25; 75 %)	Median (25; 75 %)	
Sex (men, %)	68.0	43.0	0.42
Age (years)	58.00 (50.00; 63.00)	61.00 (51.00; 67.00)	0.31
BMI (kg/m^2)	29.78 (27.90; 32.90)	30.70 (26.30; 32.30)	0.62
Obesity (BMI >30 kg/m^2, %)	46.0	62.0	0.04[a]
Age at the onset of arrhythmia (years)	52.50 (45.00; 59.50)	55.00 (42.00; 61.00)	0.61
Arterial hypertension (%)	68.0	79.0	0.03[a]
Diabetes mellitus type 2 (%)	11.0	11.0	0.95
Coronary heart disease (%)	7.0	0.0	–
Stroke or transient ischemic attack in anamnesis (%)	4.5	5.2	0.78
Serum biomarkers			
NT-proBNP (pg/mL)	78.65 (47.20; 202.00)	828.0 (514.0; 1751.0)	0.00001[a]
C-reactive protein (mg/L)	2.18 (1.01; 7.10)	0.78 (0.61; 2.46)	0.005[a]
RAD51 (ng/mL)	7.35 (4.59; 10.84)	10.86 (8.30; 13.64)	0.003[a]
p63 (%OD_{450} of control samples)	69.06 (59.32; 77.52)	94.36 (70.64; 117.26)	0.00001[a]

[a] P < 0.05 according to Mann-Whitney nonparametric test.

European Society of Cardiology (https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines/Atrial-Fibrillation-Management).

Percentages of patients with arterial hypertension and obesity were significantly lower in group 1 with compensated AF compared with that in group 2 with recurrent AF. The levels of NT-proBNP, p63, and RAD51 were significantly ($P < 0.05$) higher in group 2 compared to that in group 1 (Table 1). However, C-reactive protein levels were decreased in group 2 compared with that in group 1.

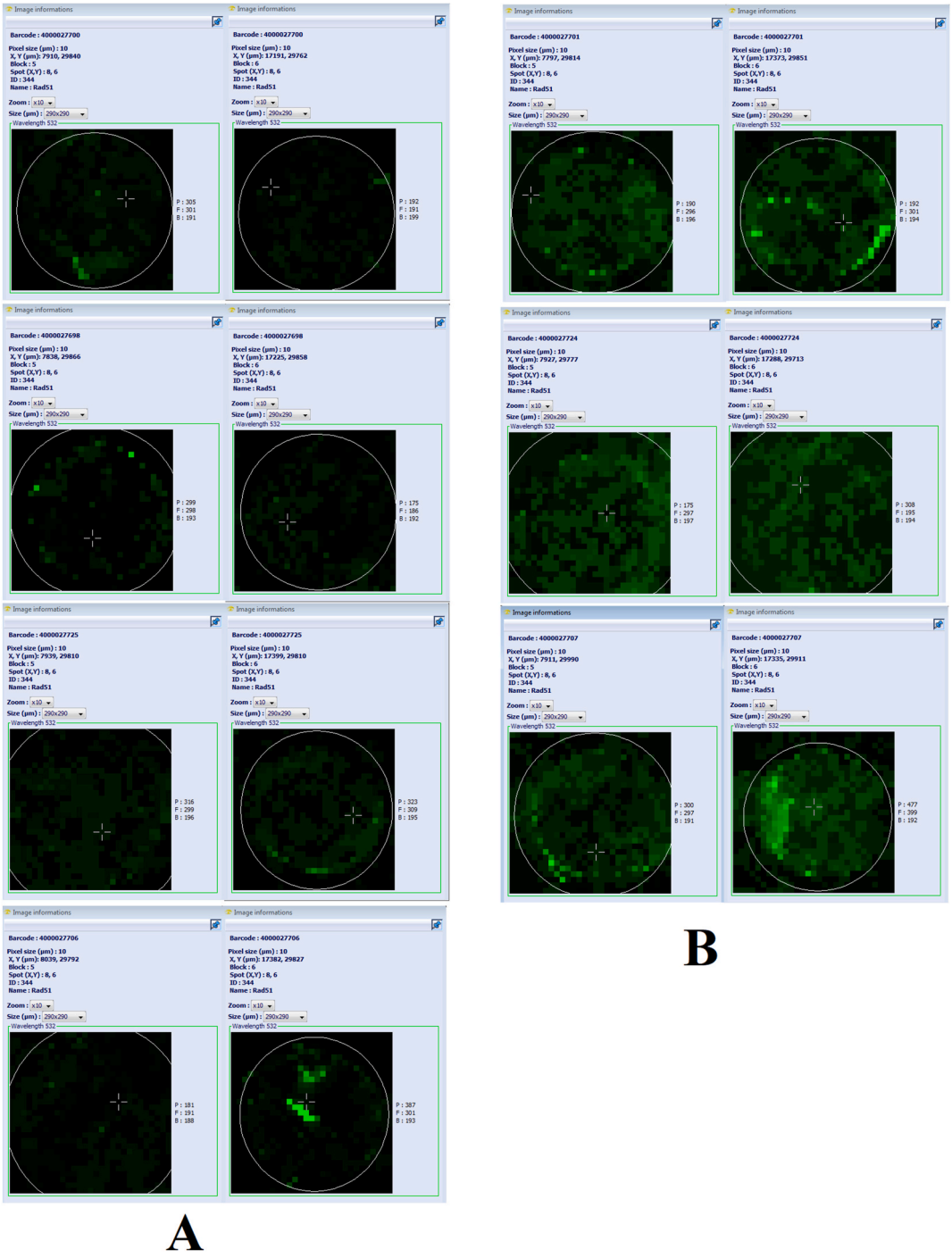

Fig. 2. Images of the microarrays from a representative slide of Explorer antibody microarray (ASB600, Full Moon Biosystems, USA) with 656 antibodies per slide in two replicates for each antibody. Enlarged images of individual samples illustrate the differences in the levels of RAD51 in the serum of a patient with compensated AF of the subgroup M1 (A) versus that in the serum of a patient with AF recurrence of the subgroup M2 (B).

The NT-proBNP and C-reactive protein biomarkers are validated by FDA and were used in the present study as references for evaluation of novel biomarkers, such as RAD51 and p63.

Less than 5 % of patients used the beta blockers, and thus use of beta blockers was not employed as an independent parameter in analysis.

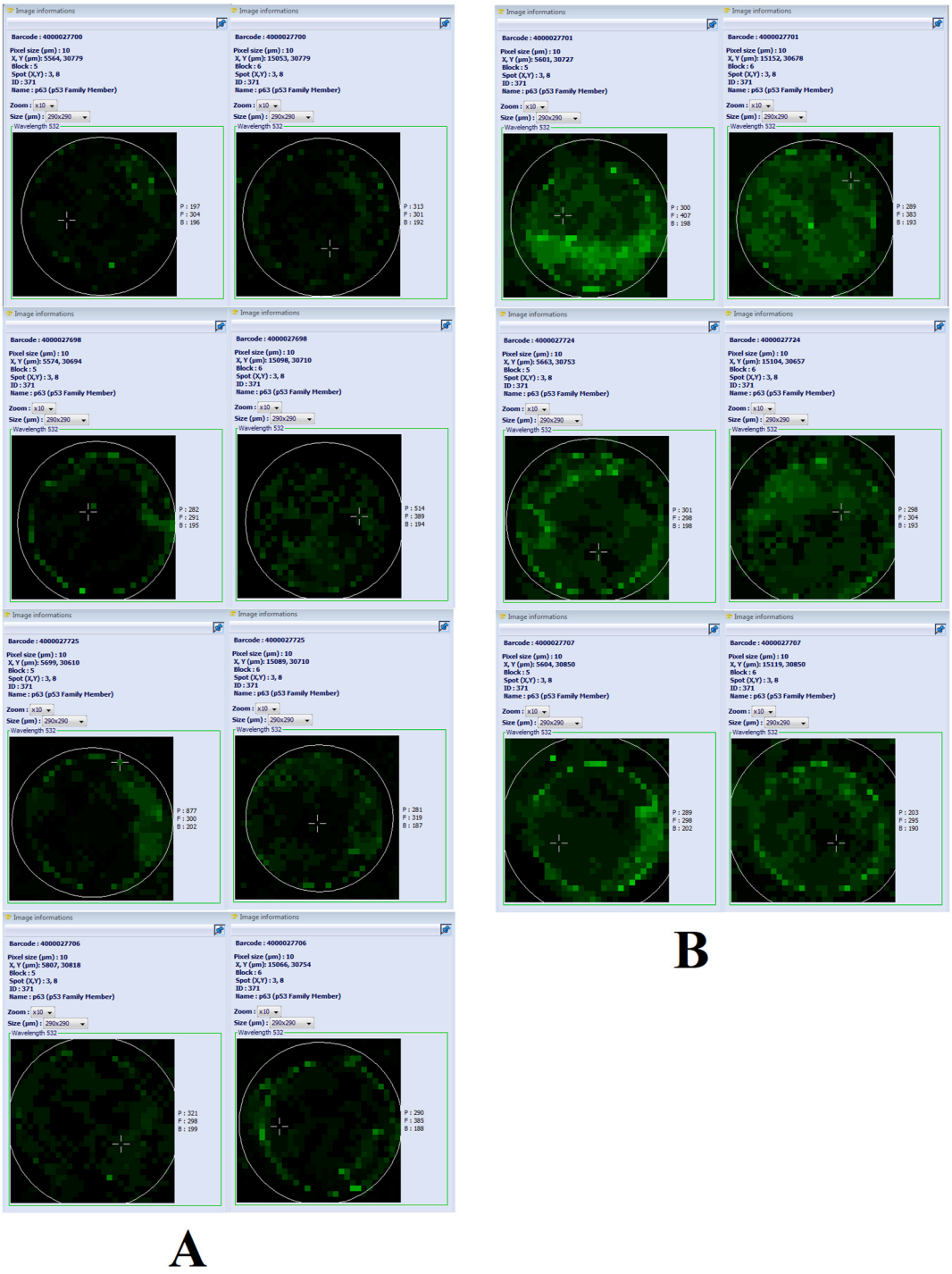

Fig. 3. Images of the microarrays from a representative slide of Explorer antibody microarray (ASB600, Full Moon Biosystems, USA) with 656 antibodies per slide in two replicates for each antibody. Enlarged images of individual samples illustrate the differences in the levels of p63 in the serum of a patient with compensated AF of the subgroup M1 (A) versus that in the serum of a patient with AF recurrence of the subgroup M2 (B).

3.2. Microarray proteome profiling in the subgroups M1 and M2

The M1 subgroup included compensated pulmonary vein-associated AF (N = 3), and the M2 subgroup included recurrent (substrate) AF (N = 3). Comparative microarray proteome profiling for identification of candidate proteins was conducted in these two small subgroups to discriminate between the subgroups M1 and M2. Comparative protein profiling of the serum proteome in the subgroups M1 and M2 using Explorer antibody microarrays was performed in two replicates for 656 antibodies. The data revealed only a few differences between the subgroups. The signals of RAD51 (DNA repair protein RAD51 homolog 1; UniProt ID: Q06609 (Fig. 2 (A, B)) and p63 (tumor protein 63; UniProt ID: Q9H3D4, Fig. 3 (A, B)) were different between the M1 and M2 subgroups. The ratios of the median pixel intensity after background subtraction for RAD51 (Fig. 2 (A, B)) and p63 (Fig. 3 (A, B) in the subgroup M2 to that in the subgroup M1 varied from 2.6 to 4.3. These ratios suggested modest differences in the contents of RAD51 and p63 between the subgroups M1 and M2. Thus, these two proteins were selected as the candidates for validation by ELISA in the main groups 1 and 2. Measurements of the protein contents using antibody microarray technology were expected to correlate with the data of ELISA obtained using antibodies from an alternative source. The Spearman rank correlation coefficient between relative levels measured by ELISA and by antibody microarrays is known to be relatively high ($r = 0.53$, $P < 0.05$) [16].

3.3. ELISA

The levels of RAD51 and p63 were assayed in group 1 (N = 123) with compensated (pulmonary vein-associated AF) AF and group 2 (N = 23) with AF recurrence after catheter ablation (substrate AF) using commercial kits for RAD51 and indirect in-home ELISA for p63. Higher levels of RAD51 (11.11 ± 4.36 vs 8.45 ± 4.85 ng/mL; P = 0.009) and p63 (165.73 ± 113.75 vs 100.05 ± 37.56 normalized OD450; P = 0.0007) were observed in the group with AF recurrence compared with that in the group with compensated AF. Thus, higher serum levels of RAD51 and p63 in the large group 2 were detected by two alternative methods, confirming the results obtained using microarray analysis in the subgroups M1 and M2.

3.4. Regression analysis

Independent associations of C reactive protein, NT-proBNP, p63, and RAD51 with AF recurrence in combined groups 1 and 2 (N = 146) were assessed by binomial regression analysis in four models, which were adjusted for the presence of obesity and arterial hypertension (Table 2) because both obesity and hypertension were associated with AF recurrence in the total cohort (Table 1). Model 1 evaluated associations of NT-proBNP levels (numeric variable) with AF recurrence (binary variable) adjusted for obesity and hypertension. Model 2 evaluated associations of C-reactive protein levels (numeric variable) AF recurrence adjusted for obesity and hypertension. Model 3 evaluated associations of RAD51 levels (numeric variable) with AF recurrence (binary variable) adjusted for obesity and hypertension. Model 4 evaluated associations of p63 levels (numeric variable) with binary AF recurrence (binary variable) adjusted for obesity and hypertension. (Table 2). The results of analysis indicated that all proteins had an independent impact on AF recurrence adjusted for obesity and arterial hypertension according to regression models (Table 2). Then, all variables associated with recurrent AF were included in a single binomial regression model. This expanded model was characterized by the likelihood ratio test to confirm its reliability (Chi-squared = 16.7; df = 6; P = 0.01). Estimated parameters of this model are presented in Table 3. The data of regression analysis indicated the lack of a single variable that provided an independent contribution to AF recurrence, suggesting collinearity between these variables. This observation was consistent with correlation analysis (Table 4). RAD51 serum level was

Table 2
Assessment of explanatory variables for recurrent versus compensated AF using binomial logistic regression in combined groups 1 and 2 (N = 146).

Dependent variable (categorical): AF recurrent or compensated	Covariates in the models	B	Standard error	Wald	P	Exp (B)	95 % confidence interval for Exp (B)	
							lower	upper
Model 1	Obesity (BMI >30 kg/m^2)	−1.05	0.48	4.64	0.03[a]	0.34	0.13	0.91
	Arterial hypertension	0.07	0.49	0.02	0.88	1.07	0.41	2.85
	NT-proBNP (pg/mL)	−0.002	0.000	23.85	0.001[a]	0.99	0.99	0.99
Model 2	Obesity (BMI >30 kg/m^2)	−1.07	0.42	6.56	0.01[a]	0.34	0.15	0.77
	Arterial hypertension	−0.05	0.42	0.02	0.88	0.94	0.41	2.14
	C-reactive protein (mg/L)	0.07	0.03	4.36	0.037[a]	1.07	1.01	1.15
Model 3	Obesity (BMI >30 kg/m^2)	−0.31	0.46	0.44	0.51	0.73	0.29	1.82
	Arterial hypertension	−0.05	0.55	0.007	0.93	0.95	0.32	2.82
	RAD51 (ng/mL)	−0.14	0.05	6.82	0.009[a]	0.87	0.78	0.96
Model 4	Obesity (BMI >30 kg/m^2)	−0.31	0.34	0.82	0.36	0.73	0.38	1.43
	Arterial hypertension	−0.47	0.37	1.60	0.20	0.62	0.29	1.29
	p63 (%OD_{450} of control samples)	0.03	0.006	16.74	0.001[a]	1.02	1.01	1.03

[a] $P < 0.05$. Note that obesity and arterial hypertension are categorical variables (present or absent) and the levels of NT-proBNP, C-reactive protein, RAD51, and p63 are numerical continuous variables.

correlated with the level of p63 with coefficient r = −0.28 (P = 0.04). In turn, the p63 level correlated with obesity and arterial hypertension (Table 4). The level of C-reactive protein was correlated with the level of NT-proBNP and arterial hypertension (Table 4). The level of NT-proBNP is considered a marker of chronic heart failure, and patients with chronic heart failure were excluded from the present study. Thus, NT-proBNP may be a nonspecific marker of AF recurrence. RAD51 and p63 proteins may be implicated in the same or linked signal transduction pathways relevant to AF recurrence.

4. Discussion

Our previous study profiled the proteins in the serum samples using the antibody microarray technology to discriminate patients with AF from normal subjects [15] and patients with coronary stenosis from patients with intact coronary vessels, and differential proteins were subsequently verified using an in-house ELISA [14].

Identification of pathogenic mechanisms of recurrent AF is difficult but is expected to improve the efficacy of current therapies. The present study revealed that increased levels of the RAD51 and p63 proteins were specifically associated with AF recurrence but not with compensated AF adjusted for obesity and arterial hypertension status. Routine biomarkers, such as NT-proBNP and C-reactive protein, were also associated with AF recurrence but were not associated with compensated AF adjusted for obesity and arterial hypertension status. The level of NT-proBNP is considered a marker of chronic heart failure, and patients with chronic heart failure were excluded from the present study. Thus, NT-proBNP may be a nonspecific marker of AF recurrence. Increased levels of the RAD51 [17] and p63 proteins [18] are known to be associated with cancer; however, patients with cancer were not included in the present study. Thus, RAD51 and p63 may be nonspecific markers of AF recurrence. The variables representing the levels of RAD51, p63, NT-proBNP, and C-reactive protein were collinear in the model using in the present study (Table 3; 4). This observation is consistent with physiological functions of RAD51 and p63 proteins.

The p63 protein is the member of the p53 family of transcription factors and is a known regulator of cellular functions, controlling various processes, including genomic stability, proliferation, cell division, senescence, apoptosis, and cell cycle arrest. The p63 protein regulates the DNA damage response, and this regulation is especially important in cancer. Unchecked DNA damage results in mutations, cell cycle halting during the S-phase, and chromosomal breakage [19].

The expression of RAD51 is altered in the absence of p63 in HPV (human papillomavirus)-positive cells [20]. RAD51 is critical for the assembly of nucleoprotein filaments surrounding single-stranded DNA that promote homology recognition in a DNA duplex and subsequent strand exchange. These processes contribute to the activation of DNA repair necessary for genome amplification. ATP-dependent DNA strand transferase effects if RAD51 are involved in the repair of DNA double-strand breaks and single-strand gaps [21]. RAD51 and p63 are known to be associated with cancer. Moreover, boosted RAD51 expression is linked to negative clinical outcomes and adverse events in glioblastoma, non-small-cell lung cancer, and thyroid carcinoma [22]. Additionally, a subtype of p63 (TAp63) is engaged in apoptosis, cell cycle arrest, and DNA repair, promoting cancer cell apoptosis after chemotherapy [19,23].

AF recurrence is assumed to be caused by structural impairment of atrial tissue. Maintenance of the proteome of cardiomyocytes in AF is an energy-consuming process that overloads mitochondrial capacity for energy production. Depletion of NAD^+/NADH and a reduction in electron transport impair mitochondrial energy production and thus lead to an increase in ROS production and DNA damage [24].

Inflammation contributes to AF, and we have previously shown that the presence of anti-adenoviral immunoglobulins is associated with fibrillation and adenoviral infection is one of the possible etiological factors for AF [15]. Moreover, AF recurrence triggers in non-pulmonary veins are highly prevalent in HIV (human immunodeficiency viruses)-positive AF patients. The prevalence of repeated ablations was higher in the HIV-positive group of patients with the substrate or non-pulmonary vein triggers of AF (93.5 % vs. 54 %, p < 0.001) [25]. These observations are consistent with independent and positive associations of systemic immune or inflammatory indices (e.g., platelet × neutrophil/lymphocyte ratio) with AF recurrence after the initial catheter ablation in patients with diabetes [26].

Our data indicated that the levels of C-reactive protein were decreased in the group with AF recurrence compared with that in the group with compensated AF (Table 1). Lower values of C-reactive protein, which is the validated marker of inflammation, in the AF recurrence group suggest the presence of sterile inflammation associated with DNA breaks, which may be an etiological factor for AF recurrence. We propose that RAD51 and p63 are engaged in DNA repair related to AF and that AF recurrence is mediated by sustained DNA damage due to impaired RAD51 activity and inefficient DNA repair eventually triggering p63-mediated apoptosis, thus contributing to structural impairment of atrial tissue.

Table 3
Assessment of explanatory variables in recurrent versus compensated AF using binomial logistic regression in combined groups 1 and 2 (N = 146).

Variables in the model	B	Wald	P
Obesity (BMI >30 kg/m^2)	−428.84	0.00	0.99
Arterial hypertension	−80.61	0.00	0.99
NT-proBNP (pg/mL)	−0.61	0.00	0.99
C-reactive protein (mg/L)	2.18	0.00	0.99
RAD51 (ng/mL)	−2.06	0.00	0.99
p63 (%OD_{450} of control samples)	−0.07	0.00	1.00

Table 4
Correlation analysis of variables in combined groups 1 and 2.

Spearman correlation coefficient		RAD51	p63	C-reactive protein	NT-proBNP
RAD51 (ng/mL)	r		−0.28[a]	0.16	0.039
	P (2-tailed)		**0.04**	0.22	0.76
p63 (%OD_{450} of control samples)	r	−0.28[a]		0.13	−0.14
	P (2-tailed)	**0.04**		0.19	0.15
C-reactive protein (mg/L)	r	0.16	0.13		−0.18[a]
	P (2-tailed)	0.22	0.19		**0.02**
NT-proBNP (pg/mL)	r	0.04	−0.14	−0.18[a]	
	P (2-tailed)	0.78	0.15	**0.02**	
Obesity (BMI >30 kg/m^2)	r	0.02	−0.24[b]	0.18[a]	−0.01
	P (2-tailed)	0.84	**0.001**	**0.02**	0.84
Arterial hypertension	r	0.12	−0.22[b]	0.09	0.05
	P (2-tailed)	0.26	**0.003**	0.21	0.50

[a] $P < 0.05$.
[b] $P < 0.001$.

5. Limitations

Limitations of the present study were as follows: microarray analysis groups comprised of small number of patients due to budgetary constraints. Moreover, the number of patients in group 2 with AF recurrence after one year follow-up was relatively small. This limitation may be resolved in future studies using a larger cohort.

6. Conclusions

The serum levels of RAD51 and p63 were associated with AF recurrence after catheter ablation with one year follow-up and may represent possible etiological factors for subsequent outcomes.

Ethics statement

For participation in the study, all patients had signed an informed consent. The study protocol was approved in accordance with the Declaration of Helsinki and WHO guidelines by the Independent Ethics Committee of NRCPM (number 01–06/17; February 2, 2017).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Nadezhda G. Gumanova: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Polina D. Zlobina:** Formal analysis, Data curation. **Natalya L. Bogdanova:** Investigation, Formal analysis, Data curation. **Hakob A. Brutyan:** Investigation, Data curation. **Elena N. Kalemberg:** Formal analysis, Data curation. **Ya I. Havrichenko:** Visualization, Investigation. **Karapet V. Davtyan:** Writing – review & editing, Resources, Project administration, Data curation, Conceptualization. **Oxana M. Drapkina:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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