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Mitochondrial DNA 4977bp Deletion Mutation in Peripheral Blood Reflects Atrial Remodeling in Patients with Non-Valvular Atrial Fibrillation

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Purpose: Recently, mitochondrial DNA 4977bp deletion (mtDNA*4977-mut*), a somatic mutation related to oxidative stress, has been shown to be associated with atrial fibrillation (AF). We hypothesized that patient age, as well as electroanatomical characteristics of fibrillating left atrial (LA), vary depending on the presence of mtDNA*4977-mut* in peripheral blood among patients with non-valvular AF. **Materials and Methods:** Analyzing clinical and electroanatomical characteristics, we investigated the presence of the mtDNA*4977-mut* in peripheral blood of 212 patients (51.1±13.2 years old, 83.5% male) undergoing catheter ablation for non-valvular AF, as well as 212 age-matched control subjects. **Results:** The overall frequency of peripheral blood mtDNA*4977-mut* in patients with AF and controls was not significantly different (24.5% vs. 19.3%, p=0.197). When the AF patient group was stratified according to age, mtDNA*4977-mut* was more common (47.4% vs. 20.0%, p=0.019) in AF patients older than 65 years than their age-matched controls. Among AF patients, those with mtDNA*4977-mut* were older (58.1±11.9 years old vs. 48.8±11.9 years old, p<0.001). AF patients positive for the mtDNA mutation had greater LA dimension (p=0.014), higher mitral inflow peak velocity (E)/diastolic mitral annular velocity (Em) ratio (p<0.001), as well as lower endocardial voltage (p=0.035), and slower conduction velocity (p=0.048) in the posterior LA than those without the mutation. In multivariate analysis, E/Em ratio was found to be significantly associated with the presence of mtDNA*4977-mut* in peripheral blood. **Conclusion:** mtDNA*4977-mut*, an age-related somatic mutation detected in the peripheral blood, is associated with advanced age and electro-anatomical remodeling of the atrium in non-valvular AF.

Key Words: Atrial fibrillation, mitochondrial DNA, 4977bp deletion mutation, atrial remodeling

INTRODUCTION

Atrial fibrillation (AF) is the most common form of cardiac arrhythmia in clinical practice and is associated with significant morbidity.[1] Many studies have investi-

gated mechanisms underlying AF development, and growing evidence supports that genetic variations play a role in its pathogenesis.[2] For instance, mutations in mitochondrial DNA (mtDNA) have been shown to be associated with the same factors that are considered critical in development of AF: aging process and oxidative stress. So far, a greater number of mtDNA mutations have been found in individuals with greater age,[3] heart failure,[4] and ischemic heart disease.[5] Among the known mutations in mtDNA, 4977bp deletion mutation (mtDNA*4977-mut*) is one of the most frequently detected genetic alterations,[6,7] and it has been identified in various human tissues, including skeletal muscle,[8] brain,[9] and heart.[10,11] Fittingly, the mtDNA*4977-mut* has been increasingly associated with AF. While a number of studies have been conducted to establish a relationship between mtDNA*4977-mut* and AF, characterizations of AF patients positive for this mutation are still inadequate and applicability of peripheral mtDNA*4977-mut* as a biomarker in AF remains untested. Therefore, in this study, we set out to analyze the frequency of somatic mtDNA*4977-mut* in Korean patients with non-valvular AF and in age-matched controls to outline an association between AF and mtDNA*4977-mut* in peripheral blood, to correlate the pathophysiological characteristics of AF patients with mtDNA*4977-mut*, and finally, to validate the utility of the mutation as a biomarker for atrial remodeling.

MATERIALS AND METHODS

Patient selection

The study protocol was approved by the Institutional Review Board of Severance Cardiovascular Hospital, Yonsei University Health System, and adhered to the Declaration of Helsinki. All patients provided written informed consent. The study enrolled 212 consecutive patients with AF included in the Yonsei AF Ablation Cohort (83.5% male, 51.1±12.5 years old) and 212 age-matched controls (50.0% male, 51.1±13.2 years old). All AF patients underwent radiofrequency catheter ablation (RFCA). Of the AF patients, 153 had paroxysmal AF (PAF) and 59 had persistent AF (PeAF). We examined all 212 AF patients with 3D spiral computed tomography (CT; 64 Channel, Light Speed Volume CT, Philips, Brilliance 63, Eindhoven, the Netherlands) to visually define the anatomy of left atrium (LA), and used trans-thoracic echocardiography (Sonos 5500, Philips Medical System, Andover, MA, USA or Vivid 7, GE Vingmed Ultrasound, Horten, Norway) to evaluate patients for additional structural heart disease, LA remodeling, and ventricular function. The control group consisted of 212 healthy subjects without structural heart disease who were age-matched with the patient group. We confirmed the absence of any potential structural heart disease in the control group with echocardiography and treadmill exercise ECG. Whole blood samples, collected using EDTA as an anticoagulant, were taken for DNA extraction and genetic analyses.

Electrophysiological mapping and radiofrequency catheter ablation

We conducted electrophysiologic mapping in the AF group. For guidance during the RFCA procedure, a 3D electroanatomical map was produced. By double trans-septal puncture, multi-view pulmonary venograms were obtained. Systemic anti-coagulation was achieved with intravenous heparin by maintaining an activated clotting time of 350–400 seconds. The 3D spiral CT images were merged with NavX-generated 3D geometry of the LA and pulmonary veins (PV) (NavX, St. Jude Medical Inc., Minnetonka, MN, USA) to acquire an electroanatomical map. Also, intracardiac electrograms were collected with the Prucka Cardio Lab™ electrophysiology system (General Electric Medical Systems Inc., Milwaukee, WI, USA). We generated an LA voltage map by obtaining contact bipolar electrograms from 350–500 points on the LA endocardium during atrial pacing at 500 ms and calculated mean LA voltage as previously described.[12,13] To calculate local conduction velocity, conduction distance was measured on the anterior-posterior and posterior-anterior views of the isochronal map and then divided by the time difference as described before.[13] In all patients, the RFCA was conducted with an open irrigation 3.5 mm-tip deflectable catheter (Celsius, Johnson & Johnson Inc., Diamond Bar, CA, USA; Coolflex, St. Jude Medical Inc., Minnetonka, MN, USA; 30–35 W; 47°C). All patients initially underwent circumferential PV isolation and cavo-tricuspid isthmus block. For those patients with PeAF, we added a roof line, posterior inferior line, and anterior line[14] as a standard lesion set. At the operator's discretion, additional ablations of the superior vena cava, a non-PV foci or complex fractionated electrogram was conducted.

Detection of mtDNA*4977-mutation* in whole blood

Total DNA was extracted from the whole blood using the commercially available QIAamp DNA Mini kit (Qiagen, Va-

lencia, CA, USA). Two primer sets were designed using Primer3 (http://frodo.wi.mit.edu/primer3/), and each forward primer was labeled with the fluorescent dye 6-FAM (Macrogen Inc., Seoul, Korea) for PCR amplification of mtDNA*4977 mut* (Fig. 1). The primer sequences were as follows: mtDNA4977bp-Forward 1:5'-FAM-CAGTGAAATGCCCCAACTAAA-3', mtDNA4977bp-Reverse 1: 5'-TCGATGATGTGGTCTTTGGA-3', and mtDNA4977-bp-Forward 2:5'-FAM-ATGGCCCACCATAATTACCC-3', mtDNA4977bp-Reverse 2:5'-GATAGGGCTCAGGCGTTTGT-3'. PCR amplification was performed with a final volume of 10 μL that contained 1.0 μL Gold ST*R buffer (Promega, Madison, WI, USA), 1.0 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 0.6 μM of each of the primers, and 10 ng of total DNA as the template. Thermal cycling was conducted on a PCR machine (Bio-Rad Laboratories, Hercules, CA, USA) under the following conditions: 95ºC for 11 min, followed by 33 cycles at 94°C for 20 sec, 60°C for 30 sec, and 72°C for 30 sec, and a final extension at 72°C for 7 min. After PCR had finished, 1.0 μL aliquots of each of the PCR products and 0.2 μL of GeneScan 500 LIZ size standard (Applied Biosystems, Foster City, CA, USA) were added to 20 μL de-ionized formamide. The mixture was denaturated and separated by capillary electrophoresis on a 3130xI Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the size and area of the specific fragments were displayed as peaks on an electropherogram that was generated using the GeneScan Analysis Software 3.1.2 (Applied Biosystems, Foster City, CA, USA).

Biochemical analysis

Peripheral blood samples were taken before RFCA and the plasma levels of the following protein markers were measured using enzyme-linked immunosorbent assay kits: tissue inhibitor of metalloproteinases-1 (TIMP-1; R&D Systems, Minneapolis, MN, USA), transforming growth factor-β (R&D Systems, Minneapolis, MN, USA), and pro-atrial natriuretic peptide (pro-ANP; Biomedica, Antony, France).

Statistical analysis

Multiple parameters including clinical features, echocardiographic parameters, electro-anatomical remodeling of the LA, and the plasma levels of protein biomarkers were compared between patients with AF and their age-matched controls. These parameters were also compared within the AF patient group between those with and without the mtDNA*4977 mut*. Comparisons between groups were analyzed using the t-test for continuous variables or the chi-squared test for nominal variables. All continuous variables were expressed as mean±SD, whereas all categorical variables were expressed as absolute and relative frequencies (%). In order to examine the association between the parameters and mtDNA*4977 mut* in AF, both univariate and multivariate logistic regression analyses were performed. All statistical analyses were conducted using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA), and all *p*-values <0.05 were considered statistically significant.

RESULTS

Frequency of peripheral mtDNA*4977 mut* in patients with AF vs. control

The somatic mutation associated with oxidative stress mtDNA*4977 mut* was detectable in the peripheral blood of both

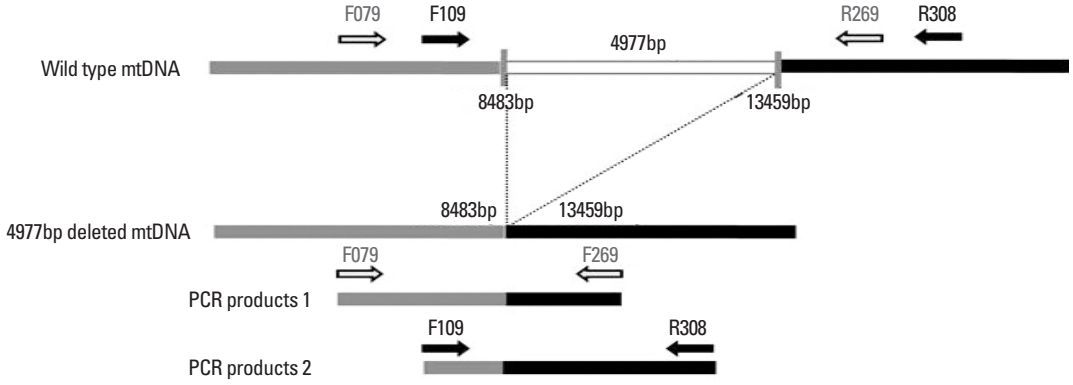

Fig. 1. A schematic representation of the method of mtDNA 4977bp deletion detection. Two different deletion primers (forward and reverse sequences, F079-R269 and F109-R308) were used to amplify DNA fragments containing mtDNA*4977 mut*, ensuring the accuracy of PCR amplification and automatic fragment analysis.

AF patients and their age-matched controls. Overall, 21.9% (93/424) of patients included in the study tested positive for mtDNA*4977*-*mut*. When AF patients were compared to the control group, the prevalence of peripheral mtDNA*4977*-*mut* was not significantly different overall (24.5% for AF vs. 19.3% for control, p=0.197). As further outlined in Table 1, body mass index (p=0.003), frequency of hypertension (p=0.007), and LA dimension (p<0.001) were all greater and left ventricular ejection fraction (p=0.001) was smaller in AF patients than their age-matched controls.

Peripheral mtDNA*4977*-*mut* in AF

While the AF and control groups overall did not show significant differences in the frequency of mtDNA*4977*-*mut* in peripheral blood, the two groups did significantly differ when they were further broken down according to age (Fig. 2A). Patients were first divided according to the median age of 51 years. Within the AF patient group, the prevalence of mtDNA*4977*-*mut* was significantly higher in those 51 years and older (35.5% vs. 12.7%, p<0.001) than in those younger than 51 years old. The mutation was still more frequent in older AF patients when they were compared to their age-matched controls (35.5% AF vs. 22.7% control, p=0.038). These findings were significant when we divided the AF patient population between those younger than 65 or those older than 65 years old, where the cut-off point was suggested by a commonly utilized risk score for stroke, CHA_2DS_2-VASc score (Fig. 2A). Within the AF group, AF patients with mtDNA*4977*-*mut* were on average older (p<0.001), exhibited a higher frequency of diabetes

Table 1. Baseline Characteristics of Patients with AF and Their Age-Matched Controls

	AF (n=212)	Control (n=212)	p value
Age, yrs	51.1±12.5	51.1±13.2	0.991
PAF, n (%)	153 (72.2)	0 (0.0)	-
BMI, kg/m^2	25.3±3.6	23.8±2.9	0.003
CHF, n (%)	11 (5.2)	0 (0.0)	-
Hypertension, n (%)	77 (36.3)	21 (9.9)	0.007
DM, n (%)	19 (9.0)	9 (4.2)	0.999
Stroke, n (%)	24 (11.3)	0 (0.0)	-
TIA, n (%)	9 (4.2)	0 (0.0)	-
$CHADS_2$ score	0.7±0.9	-	-
mtDNA*4977*-*mut*, n (%)	52 (24.5)	41 (19.3)	0.197
TTE: 2D and Doppler parameters			
LA size, mm	40.7±5.6	34.6±4.8	<0.001
LVEF, %	62.6±7.3	66.2±7.2	0.001
E/Em	9.5±4.0	9.1±4.2	0.228

AF, atrial fibrillation; PAF, paroxysmal AF; BMI, body mass index; CHF, congestive heart failure; DM, diabetes mellitus; TIA, transient ischemic attack; mtDNA*4977*-*mut*, mitochondrial DNA 4977bp deletion mutation; TTE, trans-thoracic echocardiography; LA, left atrium; LVEF, left ventricular ejection fraction; E, mitral inflow early diastolic velocity; Em, mitral annulus early diastolic velocity.

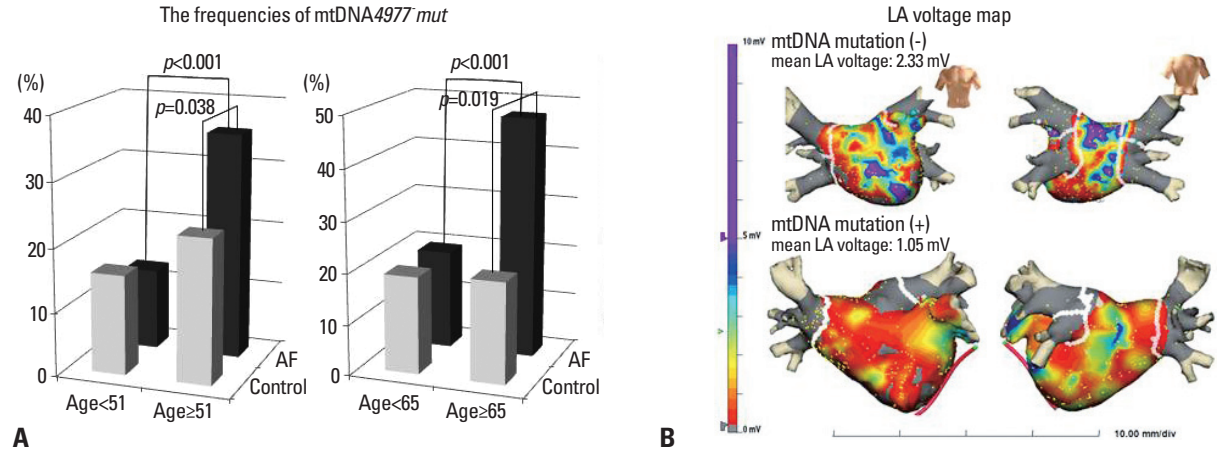

Fig. 2. Comparison of the frequency of mtDNA*4977*-*mut* in AF and control groups, each of which is divided according to the ages of 51 (median value) and 65 years (depending on CHA_2DS_2-VASc score) (A), and representative color-coded 3D voltage map of LA (B). Mean LA voltage is higher in patients without mtDNA*4977*-*mut* (upper panel) than in those with mtDNA*4977*-*mut* (lower panel). AF, atrial fibrillation; LA, left atrium.

(p=0.008), and were more likely to take angiotensin converting enzyme inhibitor/angiotensin II receptor blocker (p=0.007) or statin (p=0.001) than those without mtDNA*4977*-*mut* (Table 2).

mtDNA*4977*-*mut* and electro-anatomical remodeling of the left atrium

Table 2 shows comparisons of electroanatomical characteristics between AF patients with and without mtDNA*4977*-*mut*. Patients with mtDNA*4977*-*mut* had a greater LA size (p=0.014) and higher mitral inflow peak velocity (E)/diastolic mitral annular velocity (Em) ratio (p<0.001) than those without the mutation. Endocardial voltage (p=0.035) as well as conduction velocity (p=0.048) on the posterior LA were lower in patients possessing the somatic mutation (Fig. 2B). Upon multivariate logistic regression analysis, E/Em ratio [odds ratio (OR) 1.113, 95% confidence interval (CI) 1.011–1.225, p=0.029] was found to be independently associated with mtDNA*4977*-*mut* (Table 3). In protein biomarker assay, plasma levels of TIMP-1 (p=0.004) and pro-ANP (p=0.036) were higher in AF patients with mtDNA*4977*-*mut* than those without the mutation (Table 2), and TIMP-1 was independently associated with mtDNA*4977*-*mut* in patients with AF (OR 1.896, 95% CI 1.094–3.284, p=0.023) (Table 3). However, clinical recurrence rates after AF catheter ablation were not significantly different between patients with and without mtDNA*4977*-*mut* (Fig. 3).

DISCUSSION

In the current study, we reported that a somatic mutation, mtDNA*4977*-*mut*, detected in peripheral blood is associated with AF, the presence of which varied depending on age.

Table 2. Comparison of Electroanatomical Phenotypes between AF Patients with and without the mtDNA*4977*- Deletion Mutation

	mtDNA*4977*-*mut* (+) (n=52)	mtDNA*4977*-*mut* (-) (n=160)	p value
Age, yrs	58.1±11.9	48.8±11.9	<0.001
Paroxysmal AF, n (%)	38 (73.1)	115 (71.9)	0.434
BMI, kg/m^2	25.1±5.4	25.3±2.8	0.354
Heart failure, n (%)	2 (3.9)	9 (5.6)	0.309
Hypertension, n (%)	23 (44.2)	54 (33.8)	0.087
Diabetes, n (%)	9 (17.3)	10 (6.3)	0.008
Stroke, n (%)	2 (3.9)	22 (13.8)	0.091
TIA, n (%)	4 (7.7)	5 (3.1)	0.155
$CHADS_2$ score	0.8±1.0	0.6±0.9	0.262
Medication			
ACE inhibitor/ARB, n (%)	22 (42.0)	39 (24.1)	0.007
Statin, n (%)	15 (28.0)	15 (9.4)	0.001
TTE: 2D and Doppler parameters			
LA size, mm	42.2±5.4	40.2±5.6	0.014
LVEF, %	62.5±7.3	62.7±7.3	0.454
E/Em	11.4±5.2	8.9±3.3	<0.001
LA voltage			
Anterior LA, mV	1.1±0.6	1.1±0.6	0.485
Posterior LA, mV	0.8±0.6	1.2±1.0	0.035
LA appendage, mV	2.5±1.5	2.5±1.4	0.473
Conduction velocity			
Anterior LA, m/sec	0.6±0.2	0.7±0.3	0.201
Posterior LA, m/sec	0.6±0.3	0.7±0.3	0.048
Biomarkers			
TIMP-1, ng/mL	1.5±0.8	1.2±0.5	0.004
Pro-ANP, nmol/L	2.7±2.7	2.0±2.1	0.036
TGF-β, ng/mL	13.3±9.9	13.7±9.7	0.404

BMI, body mass index; TIA, transient ischemic attack; LVEF, left ventricular ejection fraction; E, mitral inflow peak velocity; Em, diastolic mitral annular velocity; ACE inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; TIMP-1, tissue inhibitor of metalloproteinase-1; pro-ANP, pro-atrial natriuretic peptide; TGF-β, transforming growth factor-β; AF, atrial fibrillation; LA, left atrium.

Table 3. Association between Clinical Parameters and mtDNA*4977*-*mut* in AF Using a Logistic Regression Model

	Univariate			Multivariate		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Age ≥65, n (%)	3.706	1.770–7.758	0.001	1.767	0.731–4.272	0.206
BMI, kg/m^2	0.974	0.885–1.073	0.598			
Heart failure, n (%)	0.671	0.140–3.210	0.617			
Hypertension, n (%)	1.557	0.823–2.946	0.174			
Diabetes, n (%)	3.119	1.191–8.164	0.021	1.036	0.967–1.110	0.313
Stroke, n (%)	0.266	0.033–2.108	0.210			
TIA, n (%)	2.093	0.340–12.885	0.426			
$CHADS_2$ score	1.193	0.863–1.650	0.286			
TTE: 2D and Doppler parameters						
LA size, mm	1.066	1.006–1.129	0.030	1.036	0.967–1.110	0.313
LVEF, %	0.997	0.955–1.041	0.900			
E/Em	1.154	1.061–1.255	0.001	1.113	1.011–1.225	0.029
LA voltage						
Mean LA, mV	0.772	0.443–1.346	0.362			
LA appendage, mV	1.006	0.780–1.297	0.964			
Conduction velocity						
Anterior, m/sec	1.237	0.534–2.865	0.620			
Posterior, m/sec	0.668	0.314–1.421	0.295			
Biomarkers						
TIMP-1, ng/mL	1.838	1.112–3.039	0.018	1.896	1.094–3.284	0.023
Pro-ANP, nmol/L	1.129	0.982–1.299	0.089			
TGF-β, ng/mL	0.995	0.962–1.030	0.795			

OR, odds ratio; CI, confidence interval; BMI, body mass index; TIA, transient ischemic attack; TTE, trans-thoracic echocardiography; LA, left atrium; LVEF, left ventricular ejection fraction; E, mitral inflow peak velocity; Em, diastolic mitral annular velocity; TIMP-1, tissue inhibitor of metalloproteinase-1; pro-ANP, pro-atrial natriuretic peptide; TGF-β, transforming growth factor-β; AF, atrial fibrillation.

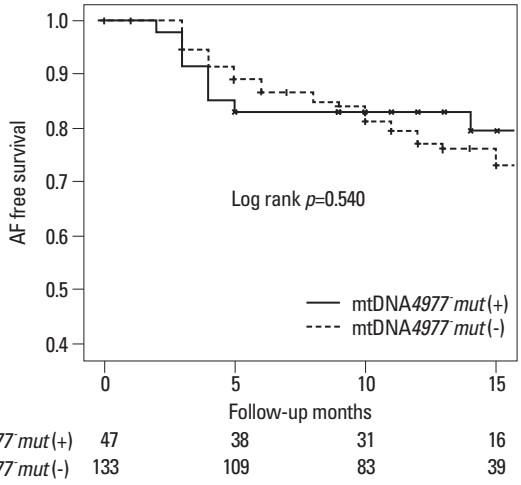

Fig. 3. A Kaplan-Meier curve comparing recurrence rates after radiofrequency catheter ablation for AF between patients with and without mtDNA*4977*-*mut*. AF, atrial fibrillation.

Additionally, peripheral mtDNA*4977*-*mut* was associated with advanced electro-anatomical remodeling of the LA, elevated left ventricular filling pressure estimated by E/Em, and high plasma levels of TIMP-1. To the best of our knowledge, our study is first to demonstrate an association between peripheral blood mtDNA*4977*-*mut*, AF, and remodeling, suggesting the potential applicability of the mtDNA mutation as a biomarker of cardiac arrhythmia.

AF is a degenerative disease and is related to mtDNA4977bp deletion mutation

AF is now commonly recognized as a disease of aging; advanced age increases one's predisposition to this arrhythmia. According to recent studies, the mechanism underlying aging is primarily a progressive decline in mitochondrial function.[15] Leakage of superoxide from the mitochondrial electron transport chain induces oxidative damage and accumulation of damage over time results in mtDNA deletion.[16,17] mtDNA*4977*-*mut* is one of the most common deletion mutations identified in mitochondria. This mutation is frequently found in aging human tissues, especially those vulnerable to increased oxidative stress, like the heart.[18,19] In the mutation, deletion of a sequence that encodes subunits of ATPase and NADH dehydrogenase disrupts aerobic metabolism and ultimately generates increased amounts of radical oxidative stress (ROS).[11,20] Similarly, many studies attempting to re-

veal the pathophysiology of AF have pointed to mitochondrial dysfunction and ROS as important mediators thereof: for example, NADPH oxidase,[21,22] NOS,[23,24] and MPO,[25,26] previously discovered as major sources of ROS in the heart,[27] have now been shown to be critical in arrhythmogenesis. Furthermore, a growing body of evidence supports the idea that mitochondrial dysfunction can directly alter cardiomyocyte excitability and cell-to-cell coupling.[28-30] As aging and AF exhibit surprising similarities, subsequent studies have attempted to investigate whether there indeed exists an association between aging, mitochondrial dysfunction, and AF. Lai, et al.[10] examined right atrial appendage tissues and found that both aging and AF were independently associated with accumulation of mtDNA*4977-mut*. Lin, et al.,[11] also observed increased oxidative damage, including the mtDNA deletion mutation, in atrial muscles from fibrillating hearts in comparison to tissue in sinus rhythm. However, whether ROS and mitochondrial dysfunction as result of the ageing process alone are sufficient to produce an arrhythmogenic atrial substrate remain in question.[15] In our study, we observed that among AF patients, those with the mtDNA mutation were older on average. In comparison to their age-matched controls, elderly AF patients still maintained a higher prevalence of mtDNA*4977-mut*.

Electroanatomical remodeling and diastolic dysfunction in mtDNA*4977-mut*

Over the course of AF, the presence of mtDNA*4977-mut* was associated with more accelerated electroanatomical remodeling. Our current study revealed that parameters reflecting electroanatomical remodeling, such as atrial voltage and conduction velocity, are significantly different between patients with and without the mtDNA*4977-mut*. In line with our results, Tsuboi, et al.[31] postulated that a rapid atrial rate or AF induced hypoxia in the atrium, increasing the generation of oxygen radicals. Further deterioration of mitochondrial function ensued as damage to mtDNA accumulated. The level of ATP in atrial muscle subsequently fell, resulting in impaired calcium handling, increased calcium in the cytoplasm, and reduced L-type calcium current.[31] Ultimately, atria were electrically remodeled, beginning a vicious cycle in which AF begets AF.[32] In addition to electrical remodeling, mtDNA*4977-mut* appears to be also associated with structural remodeling of the LA in AF. E/Em ratio and LA size, according to our observation, were significantly elevated in the mtDNA*4977-mut* positive AF patient group, and E/Em ratio was independently associated with the mutation in AF. From our analysis, we speculated that higher LV filling pressure indicated by higher E/Em evoked more advanced structural remodeling of LA in compensation.[33-35] We previously reported that impaired LV diastolic function significantly contributed to electroanatomical remodeling of LA in patients with PAF.[36] In the current study, this interaction between the two chambers was especially prominent in AF patients with the mtDNA mutation. When we compared AF types, mtDNA*4977-mut* was present in 24.8% of PAF patients and in 24.1% of PeAF patients (p=0.867). This suggests that mtDNA*4977-mut* is more likely to be associated with ageing, metabolic factors, ventricular diastolic dysfunction, or left atrial remodeling, rather than AF burden itself.

Clinical implications

Over time, surrogate markers have gained increasing clinical importance, as detection allows for not only early diagnosis of a disease but also prognosis. This is especially true in chronic degenerative diseases like AF. Several protein biomarkers have been shown to reflect various aspects of AF, such as electro-anatomical remodeling[37] or chronicity.[38] However, multiple confounding factors, such as transient inflammation and associated systemic disease, affect plasma levels of these protein biomarkers and ultimately prevent effective and accurate use in the clinic. In contrast to protein biomarkers, genetic markers are reproducible and stable enough to characterize the state of disease in patients with greater certainty. As our study showed, mtDNA*4977-mut* may serve as a stable indicator of patients with AF or at risk of rapid remodeling of the LA due to AF. Furthermore, the results of the current study suggest the potential utility of peripheral blood for the detection of mtDNA*4977-mut* in cardiac disease or arrhythmias. Previous studies on the association between mtDNA mutation and AF have been conducted by acquiring atrial tissue for analysis,[10,11] which is clinically impractical. As these results have been reproduced in our study with peripheral blood, we demonstrated that mtDNA mutation can be readily assessed as a valuable biomarker of AF; further studies are need to confirm our results in different populations.

Limitations

We analyzed mtDNA*4977-mut* in a Korean population, and this study was a observational study that included a highly-selected group of patients referred from the Yonsei AF Ablation Cohort. The small number of patients in each group

may have affected our analysis.

Conclusion

mtDNA*4977*-*mut*, an oxidative stress-related somatic mutation in mitochondrial DNA detected in peripheral blood, was associated with older age and a greater degree of electro-anatomical remodeling of the LA in patients with nonvalvular atrial fibrillation.

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