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 or 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. 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, heart failure, and ischemic heart disease. Among the known mutations in mtDNA, 4977bp deletion mutation (mtDNA4977 mut) is one of the most frequently detected genetic alterations, and it has been identified in various human tissues, including skeletal muscle, brain, and heart. Fittingly, the mtDNA4977 mut has been increasingly associated with AF. While a number of studies have been conducted to establish a relationship between mtDNA4977 mut and AF, characterizations of AF patients positive for this mutation are still inadequate and applicability of peripheral mtDNA4977 mut as a biomarker in AF remains untested. Therefore, in this study, we set out to analyze the frequency of somatic mtDNA4977 mut in Korean patients with non-valvular AF and in age-matched controls to outline an association between AF and mtDNA4977 mut in peripheral blood, to correlate the pathophysiological characteristics of AF patients with mtDNA4977 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, 51.1±13.2 years old). All AF patients underwent random 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 mtDNA4977 mutation in whole blood
Total DNA was extracted from the whole blood using the commercially available QIAamp DNA Mini kit (Qiagen, Valencia, CA, 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 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.
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 mtDNA4977mut (Fig. 1). The primer sequences were as follows: mtDNA4977bp-Forward 1:5'-FAM-CAGTGAAAA TGCCCCAACTAAA-3', mtDNA4977bp-Reverse 1:5'-TCGATGATGTTGGCTTTTGGA-3', and mtDNA4977bp-Forward 2:5'-FAM-ATGGCCACCAATTAACC-3', mtDNA4977bp-Reverse 2:5'-GATAGGGCTACGGCG TTTGT-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: 95°C 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 3130xl 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 mtDNA4977mut. 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 mtDNA4977mut 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 mtDNA4977mut in patients with AF vs. control**

The somatic mutation associated with oxidative stress mtDNA4977mut was detectable in the peripheral blood of both patients with AF and their age-matched controls. 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 mtDNA4977mut (Fig. 1). The primer sequences were as follows: mtDNA4977bp-Forward 1:5'-FAM-CAGTGAAAA TGCCCCAACTAAA-3', mtDNA4977bp-Reverse 1:5'-TCGATGATGTTGGCTTTTGGA-3', and mtDNA4977bp-Forward 2:5'-FAM-ATGGCCACCAATTAACC-3', mtDNA4977bp-Reverse 2:5'-GATAGGGCTACGGCG TTTGT-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: 95°C 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 3130xl 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

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**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 mtDNA4977mut, 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 mtDNA4977mut. When AF patients were compared to the control group, the prevalence of peripheral mtDNA4977mut 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 mtDNA4977mut in AF**

While the AF and control groups overall did not show significant differences in the frequency of mtDNA4977mut 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 mtDNA4977mut 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, CHA2DS2-VASc score (Fig. 2A). Within the AF group, AF patients with mtDNA4977mut 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/m²    | 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)         | -         |
| CHADS2 score  | 0.7±0.9    | -               | -         |
| mtDNA4977mut, 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     |

**Fig. 2.** Comparison of the frequency of mtDNA4977mut in AF and control groups, each of which is divided according to the ages of 51 (median value) and 65 years (depending on CHA2DS2-VASc score) (A) and representative color-coded 3D voltage map of LA (B). Mean LA voltage is higher in patients without mtDNA4977mut (upper panel) than in those with mtDNA4977mut (lower panel). AF, atrial fibrillation; LA, left atrium.

AF, atrial fibrillation; PAF, paroxysmal AF; BMI, body mass index; CHF, congestive heart failure; DM, diabetes mellitus; TIA, transient ischemic attack; mtDNA4977mut, 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.
(\(p=0.008\)), 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\(_{4977}\) (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\(_{4977}\). 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 |
| **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.
between peripheral blood mtDNA\textsubscript{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 mtDNA\textsubscript{4977}bp 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.\cite{15}

Leakage of superoxide from the mitochondrial electron transport chain induces oxidative damage and accumulation over time results in mtDNA deletion.\cite{16,17} mtDNA\textsubscript{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.\cite{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).\cite{11,20}

Additionally, peripheral mtDNA\textsubscript{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\textsubscript{4977} mut, AF, and remodeling, suggesting the potential applicability of the mtDNA mutation as a biomarker of cardiac arrhythmia.

**Table 3.** Association between Clinical Parameters and mtDNA\textsubscript{4977} mut in AF Using a Logistic Regression Model

| Parameter                        | Univariate OR (95% CI) | p value  | Multivariate 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\textsuperscript{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\textsubscript{2} score     | 1.193 (0.863-1.650)    | 0.286    |                          |          |
| 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                 | 1.066 (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, mmol/L                  | 1.129 (0.982-1.299)    | 0.089    |                          |          |
| TGF-β, ng/mL                     | 0.995 (0.962-1.030)    | 0.795    |                          |          |

**Fig. 3.** A Kaplan-Meier curve comparing recurrence rates after radiofrequency catheter ablation for AF between patients with and without mtDNA\textsubscript{4977} mut. AF, atrial fibrillation.
veal the pathophysiology of AF have pointed to mitochondrial dysfunction and ROS as important mediators thereof: for example, NADPH oxidase,\textsuperscript{21,22} NOS,\textsuperscript{23,24} and MPO,\textsuperscript{25,26} previously discovered as major sources of ROS in the heart,\textsuperscript{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.\textsuperscript{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.\textsuperscript{10} examined right atrial appendage tissues and found that both aging and AF were independently associated with accumulation of mtDNA\textsuperscript{4977}mut. Lin, et al.,\textsuperscript{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.\textsuperscript{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\textsuperscript{4977}mut.

**Electroanatomical remodeling and diastolic dysfunction in mtDNA\textsuperscript{4977}mut**

Over the course of AF, the presence of mtDNA\textsuperscript{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\textsuperscript{4977}mut. In line with our results, Tsuboi, et al.\textsuperscript{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.\textsuperscript{31} Ultimately, atria were electrically remodeled, beginning a vicious cycle in which AF begets AF.\textsuperscript{32} In addition to electrical remodeling, mtDNA\textsuperscript{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\textsuperscript{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.\textsuperscript{35-35} We previously reported that impaired LV diastolic function significantly contributed to electroanatomical remodeling of LA in patients with PAF.\textsuperscript{42} 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\textsuperscript{4977}mut was present in 24.8% of PAF patients and in 24.1% of PeAF patients ($p=0.867$). This suggests that mtDNA\textsuperscript{4977}mut is more likely to be associated with ageing, metabolic factors, ventricular diastolic dysfunction, or left atrial remodeling, rather than AF burdened 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\textsuperscript{37} or chronicity.\textsuperscript{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\textsuperscript{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\textsuperscript{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,\textsuperscript{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\textsuperscript{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 A4977mut, 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 non-valvular atrial fibrillation.

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