III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（書籍）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
坂野麻里子, 宮田敏行, 長束一行	4.6.3 循環器疾患	日本ビタミン学会編集	ビタミン総合辞典	朝倉書店		2010	139-141

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Iwashima Y, Horio T, Suzuki Y, Takagi T, Kamide K, Ohishi M, Ogihara T, Yoshikawa J, Kawano Y, Rakugi H.	Impact of concomitant diabetes and chronic kidney disease on preload-induced changes in left ventricular diastolic filling in hypertensive patients.	J Hypertens	29(1)	144-153	2011
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IV.　研究成果の刊行物・別刷り

ビタミン
総合事典

日本ビタミン学会
［編集］

朝倉書店

因の関与も示唆されている．

d. おわりに

ビタミン K_2 はその標的臓器の多様性に加えて，作用機序についても多様性が明らかになってきた．骨粗鬆症の診療においても，新しい生化学的指標が実用化されたところであり，今後ともさらに興味深い知見が得られていく栄養素であろう．

〔細井孝之〕

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4.6.3　循環器疾患

a. はじめに

循環器領域でのビタミンKの重要性は，ビタミンK拮抗薬として抗凝固能を示すワルファリンとの関連で説明されよう．ワルファリンは，1920年代の冬季にカナダで多数の牧畜牛の出血死を引き起こした「スウィート・クローバー病」の原因物質，ジクロマールの誘導体であり，開発に資金を提供した Wisconsin Alumni Research Foundation の頭文字とジクロマールの構造母体（Coumarin）の末尾4文字を使ってワルファリンと命名された．ジクロマールを混ぜた餌を食したネズミは出血死することから，まず殺鼠剤として用いられた．次いで，本剤の凝固能低下活性に着目し，血栓症の予防と治療に用いられるようになった．1955年，米国アイゼンハワー大統領が心臓発作のあとに，ワルファリン治療を受けたことは有名である．

わが国をはじめ欧米先進国では，循環器疾患，とりわけ脳卒中や心筋梗塞，静脈血栓塞栓症などの血栓性疾患が増加の一途をたどっている．血栓性疾患の予防，治療法は，抗血小板療法，抗凝固療法，線溶療法に大別されるが，静脈や心腔内など，比較的血流が遅い環境下に形成される血栓に対しては，抗凝固療法が有効とされている．ワルファリンは，国際的にもっとも広く使用されている経口抗凝固薬であり，心房細動，人工弁置換術後，静脈血栓塞栓症，肺塞栓症後の再発予防に広く使用され，予防効果が確立されている．反面，本剤は治療域を得るのに必要な用量が狭く，かつ個人差が大きい．また，食品中のビタミンKやほかの併用薬剤との相互作用が効果に強く影響するという欠点があり，至適用量の設定が難しいことが使用上の課題として指摘されている．近年，ワルファリン投与量の個人差に関わる遺伝要因の同定を目指す研究が精力的に行われ，遺伝情報を個別のワルファリン用量調節に役立てる試みがなされている[11]．

b. 血液凝固反応におけるビタミンKの役割とワルファリンの作用機序

凝固因子 VII, IX, X, プロトロンビン，および凝固制御因子プロテインC，プロテインSは，分子のN末端側約40残基内に10～12残基の γ-カルボキシグルタミン酸（Gla）残基を含み，この領域はGlaドメインと呼ばれる．このGlaドメインに Ca^{2+} が結合すると，疎水性側鎖が分子表面に露出し，その結果，これらの凝固因子はリン脂質膜

上に結合，濃縮され，凝固反応が効率よく進行する[2]．

凝固因子のリン脂質膜上への結合に必須となるGla残基は，ビタミンK依存性グルタミルカルボキシラーゼの作用により，還元型ビタミンK，O_2，CO_2の存在下，Glu残基のγ位炭素にCO_2が導入されることにより生成される．この過程で酸化されたビタミンKはビタミンKエポキシド還元酵素（VKOR）の作用により，還元型へと変換され再利用される．これをビタミンKサイクルと呼ぶ．

ワルファリンは，VKORの阻害剤であり，ビタミンK-2,3-エポキシドを還元型ビタミンKに変換する過程を阻害する．その結果，ビタミンKの再利用が進まなくなり，Gla残基の生成が阻害され，カルボキシル化されない未成熟で不活性な凝固因子が産生され，凝固能が抑制されることとなる．Glaドメインをもつ凝固因子は生合成にビタミンKを要求することから，ビタミンK依存性凝固因子と総称される．

c. 循環器疾患のワルファリン療法

脳卒中はわが国の死因の第3位を占め，重篤な後遺症を残して高齢者の寝たきりの原因となる．脳卒中は脳梗塞，脳出血，くも膜下出血に分類される．最近では脳卒中の4分の3が脳梗塞である．脳梗塞は血栓による脳動脈の閉塞によって生じるので，その再発予防には抗血栓療法が必要となる．脳梗塞は，その発症機序により心原性脳塞栓症と非心原性脳塞栓症に分類される．心原性塞栓症は，心房細動，左室血栓，急性心筋梗塞，人工弁置換の患者に見られ，心腔内を通過するフィブリン主体の血栓が脳血管を閉塞して発症する．一方，非心原性脳梗塞はアテローム血栓性脳梗塞，ラクナ梗塞，原因不明の脳梗塞を含み，動脈内に形成される血小板主体の血栓が原因となる．したがって，慢性期の再発予防のための抗血栓療法は脳梗塞の病型により異なり，心原性脳塞栓症の再発予防にはワルファリンを用いる抗凝固療法が，非心原性脳塞栓症の再発予防には抗血小板療法が行われる[3,4]．

米国では，約1%のアメリカ人が心房細動をもち，この頻度は加齢とともに急上昇し，80歳以上では約10%に見られるという．平均余命の上昇と心房細動のリスク因子の増加により，心房細動の患者はますます増えると予想される．脳梗塞の再発予防だけでなく，一次予防として心房細動があるだけでも抗凝固療法をすべきであるが，抗血小板療法に比べ抗凝固療法は出血合併症の頻度が高いため，脳梗塞の発症リスクが高い症例を選択して抗凝固療法を施行することが推奨されている．脳梗塞発症リスクを評価する方法としては，最近欧米ではCHADS2スコア[5]が推奨されていて，日本循環器病学会でもこれを取り入れたガイドラインが発表されている．具体的には心不全・高血圧・75歳以上の高齢者・糖尿病があるとそれぞれ1点加算され，脳梗塞/TIAの既往は2点が加算される．総計が2点を超えると抗凝固療法の適応となる．

心原性脳塞栓症の発症予防に用いられるワルファリンの効果は個人差が大きく，食事や併用薬などの影響を受け変動しやすいため，定期的にinternational normalized ratio（INR）を指標とした凝固検査で用量を調節する必要がある．標準的な治療域はINR 2.0～3.0である．心原性脳塞栓症の最大の原因は，非弁膜性心房細動である．高齢の非弁膜性心房細動の患者は出血リスクが大きいのでINR 1.6～2.6が目標値となる[6]．また，人工弁置換患者は塞栓リスクが非常に高いので，INR 2.5～3.5が推奨される．これらの患者では，INRを指標にしてワルファリン量を適切にコントロールする必要がある．ワルファリンは心原性脳塞栓症に加え，肺塞栓症や静脈血栓症患者にも用いられる．こういった患者のワルファリン投与期間は，手術，血管造影検査，外傷，一時的臥床といった危険因子が一過性もしくは可逆的である場合は少なくとも3カ月間，特発性の静脈血栓塞栓症では少なくとも6カ月間，先天性凝固異常症や再発例の場合には，さらに長期に服薬するべきとされている．ワルファリンの服薬を開始すると，INRを治療域に維持するため凝固検査を頻繁に行う必要があり，食事も制限される．また，併用薬との相互作用もある．血栓塞栓症のハイリスク患者は出血のハイリスク患者でもあり，血栓塞栓の予防と

出血合併症の回避という難しい問題が横たわっている．〔坂野麻里子・宮田敏行・長束一行〕

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4.6.4　その他の疾患

a. 消化管疾患

肝疾患以外の消化管疾患に関しては，ビタミンKの吸収障害との関連について考慮する必要がある．ビタミンKの吸収における胆汁酸の必要性については多くの報告があり，例えば胆道閉鎖の新生児では，ビタミンK欠乏による出血をとくに起こしやすい[1]．また成人においても，炎症性腸疾患（IBD；inflammatory bowel disease），とくに小腸における炎症の強いCrohn病においては，吸収障害による脂溶性ビタミン欠乏症は重要な問題である．Kuwabaraらは，IBD患者においては，ビタミンKやDの摂取は一見充足しているにもかかわらず，これらの血液中濃度は非常に低く，これら患者が，治療上の必要性から脂質摂取制限を受けており，それによる吸収障害を指摘している[2]．

従来想定されていなかった疾患と，ビタミンKの関連が，最近報告されているので，以下に述べる．

b. 血液疾患

上記の血栓・止血領域以外に，白血病類縁疾患におけるビタミンKの意義が報告されている．基礎研究において，ビタミンKが，白血病細胞のアポトーシスや分化誘導をするとの報告がなされているが，臨床的にも，骨髄異形成症候群（MDS；myelodysplastic syndrome）の治療におけるビタミンKの有効性を示唆する論文が発表されている．MDSは骨髄の異常により造血障害を起こす症候群であり，前白血病状態ともいわれる．MDSはさらにいくつかの型に分類されるが，MDS-RA（不応性貧血；refractory anemia）患者を，ランダムに2群に分けて，MK-4投与の効果をみたところ，改善率が非投与群では11%に対し，投与群では56%と，MK-4はMDSの改善に有効であった[3]．

c. 糖代謝との関連

近年ビタミンKと糖代謝に関して，重要な報告がなされている．Karsentyらのグループは以前から，脂肪細胞において産生されるホルモンであるレプチンが，交感神経系を介して骨代謝を負に調節することを明らかにしていたが，最近逆に，骨は一種の内分泌臓器であり，脂肪組織の機能を調節している可能性を提唱した．すなわち，ビタミンK依存性の骨基質タンパク質であるオステオカルシン遺伝子欠損（OCN-/-）マウスは，インスリン分泌能低下，インスリン感受性低下，血糖値上昇を認め，脂肪細胞におけるアディポネクチンの遺伝子発現低下を呈した[4]．さらに彼らは，wild-typeのマウスにおいて，オステオカルシンの投与が，インスリン分泌・インスリン感受性改善とともに，インスリン産生細胞である膵臓β細胞の増殖促進，体脂肪量減少，肥満・糖尿病予防などの効果を示したと報告している[5]．しかし問題は，これら一連の研究において，活性を示したとしているのが十分Gla化されていない低カルボキシル化OC（ucOC）であると述べていることである．一般にはむしろ，ucOCは骨におけるビタミンK作用不足の指標であると考えられており，ucOCが生理活性をもつという結果は，従来の概念とはまったく異なるものであり，この点に関してはさらに検討が必要である．

上記のKarsentyらの結果が正しいのなら，ビタミンK作用不足の結果増加するucOCというタンパク質が，生体にとって望ましい作用を示すということになってしまうが，疫学研究の結果は，むしろビタミンKは糖代謝に望ましい影響を与えるものとされている．例えば1971年から1991～5年にかけて，2,719名を追跡したFramingham Offspring Studyの結果，ビタミンK_1摂取によっ

Chronic kidney disease as an independent risk factor for new-onset atrial fibrillation in hypertensive patients

Takeshi Horio[a], Yoshio Iwashima[a], Kei Kamide[a], Takeshi Tokudome[b], Fumiki Yoshihara[a], Satoko Nakamura[a] and Yuhei Kawano[a]

Objective **Chronic kidney disease (CKD) has recently been recognized to be a powerful predictor of cardiovascular morbidity and mortality. Atrial fibrillation (AF), which is a common arrhythmia in hypertensives, is associated with increased risks of cardiovascular events and death. However, the association between CKD and the onset of AF has not been fully elucidated. The present study assessed the hypothesis that CKD may influence the onset of AF in hypertensives.**

Methods **A total of 1118 hypertensive patients (mean age, 63 years) without previous paroxysmal AF, heart failure, myocardial infarction, or valvular disease were enrolled. CKD was defined as decreased glomerular filtration rate (<60 ml/min per 1.73 m^2) and/or the presence of proteinuria (≥1+).**

Results **During follow-up periods (mean, 4.5 years), 57 cases of new-onset AF were found (1.1% per year). Kaplan–Meier curves revealed that the cumulative AF event-free rate was decreased in the CKD group (log-rank test $P<0.001$). By univariate Cox regression analysis, age, smoking, left atrial dimension, left ventricular mass index, and the presence of CKD were significantly associated with the occurrence of AF. Among these possible predictors, CKD (hazard ratio 2.18, $P=0.009$) was an independent determinant for the onset of AF in multivariate analysis. Advanced stages of CKD (stages 4 and 5) were strongly related to the increased occurrence of AF.**

Conclusion **The present study demonstrated that the complication of CKD, especially progressed renal dysfunction, was a powerful predictor of new-onset AF in hypertensive patients, independently of left ventricular hypertrophy and left atrial dilatation.** *J Hypertens* **28:1738–1744 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: atrial fibrillation, hypertension, kidney, proteinuria, renal function

Abbreviations: AF, atrial fibrillation; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IVST, interventricular septal thickness; LA, left atrial; LV, left ventricular; LVDd, left ventricular diameter at end-diastole; LVD, left ventricular diameter at end-systole; PWT, posterior wall thickness; RAS, renin–angiotensin system

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Introduction

Atrial fibrillation (AF) is the most common clinically significant arrhythmia in patients with hypertension, even in the absence of antecedent valvular heart disease or coronary artery disease. AF is a significant risk factor for ischemic stroke and heart failure events, and is also associated with increased risks of total and cardiovascular death, especially due to stroke [1]. Therefore, the occurrence of AF in hypertensive patients not only decreases their quality of life but also has a considerable influence on their prognosis and survival. Older age, blood pressure levels, especially ambulatory systolic blood pressure, increased left ventricular (LV) mass, and increased left atrial (LA) size have been known to be risk factors for the onset of AF in hypertensive patients [2–5]. In particular, a previous study showed that age and LV mass were independent determinants of AF incidence in initially untreated patients with essential hypertension [3].

Renal impairment is a powerful predictor of cardiovascular prognosis. Decreased estimated glomerular filtration rate (eGFR) is clearly associated with the increase in future cardiovascular events [6]. Proteinuria, even microalbuminuria, also increases the risk of cardiovascular events and death [7]. Thus, the involvement of renal impairment in the development of cardiovascular disease has recently been noticed. However, no study has shown the association between the onset of AF and renal impairment in hypertensive patients. To assess the hypothesis that chronic kidney disease (CKD) may affect the incidence of AF, the present study investigated the influence of renal impairment and CKD on the new onset of AF in hypertensives.

Methods

Study participants

From 1263 consecutive hypertensive patients who underwent echocardiography at the Division of Hypertension and Nephrology of our hospital between February 1997 and October 2003, 1118 patients (580 men and 538 women; mean age, 63 years) with normal sinus rhythm

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who had had no history of previous paroxysmal AF and in whom biochemical and urinary data were simultaneously obtained were enrolled in the present study. Patients with various cardiac disorders such congestive heart failure, myocardial infarction, myocardial disease, pericardial disease, valvular heart disease, LV asynergy, or LV systolic dysfunction (fractional shortening <0.25) were excluded from this study. Individuals after permanent pacemaker implantation or patients receiving dialysis were also excluded. Hypertension was defined as a systolic blood pressure of 140 mmHg or more and/or a diastolic blood pressure of 90 mmHg or more by repeated measurements or when medication was taken for treatment of hypertension. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria, such as a fasting plasma glucose of 7.0 mmol/l or more and/or a plasma glucose level at 2 h after a 75-g oral glucose load of 11.1 mmol/l or more, or when medication was taken for treatment of hyperglycemia.

All procedures of the present study were carried out in accordance with institutional and national ethical guidelines for human studies. All participants enrolled in this study were Japanese, and all gave informed consent to participate in this study.

Echocardiography

A comprehensive two-dimensional echocardiography was performed using a cardiac ultrasound unit (Sonos 5500; Philips Medical Systems, Andover, Massachusetts, USA) as previously described [8]. Echocardiographic parameters were measured by the consensus of two experienced investigators who were blinded to the clinical data of the patients. Measurements included interventricular septal thickness (IVST), posterior wall thickness (PWT), LV diameter at end-diastole (LVDd), LV diameter at end-systole (LVDs), and LA diameter. LV fractional shortening was calculated as (LVDd-LVDs)/LVDd. LV mass was estimated using the formula validated by Devereux and Reichek [9]: LV mass $(g) = 1.04 \times \{(IVST + PWT + LVDd)^3 - LVDd^3\} - 13.6$. LV mass was normalized for body surface area and expressed as the LV mass index.

Clinical parameters

At the time of the echocardiographic examination, blood pressure, heart rate, and body mass were determined. Blood pressure was measured by a physician in a hospital outpatient clinic with the patient in a sitting position after over 10 min of rest, using an appropriate-size arm cuff and mercury sphygmomanometer. The first and fifth Korotkoff sounds were used to identify systolic and diastolic values, respectively, and measurements were taken to the nearest 2 mmHg.

Peripheral blood and urine samples were obtained in the morning after an overnight fast. The serum creatinine level was determined by the enzymatic method and eGFR was calculated by the formula of the Modification of Diet in Renal Disease Study with a modified equation for Japanese [10]: eGFR (ml/min per $1.73\,m^2$) = $194 \times age^{-0.287} \times serum\ creatinin^{-1.094} \times 0.739$ (if woman). Urinary protein excretion was assessed by the dipstick test from spot urine samples.

CKD was defined as decreased eGFR less than 60 ml/min per $1.73\,m^2$ and/or the presence of proteinuria (≥1+). The classification of CKD stages was performed according to the guidelines of the National Kidney Foundation classification of CKD [11] as follows; eGFR 90 ml/min per $1.73\,m^2$ or more with proteinuria (stage 1), eGFR 60–89 ml/min per $1.73\,m^2$ with proteinuria (stage 2), and stages 3, 4, and 5 were classified by the levels of eGFR (30–59, 15–29, and <15 ml/min per $1.73\,m^2$, respectively), regardless of the presence of proteinuria.

Follow-up

After the initial assessment, all patients visited our hospital periodically (every 1–2 months) for the treatment of hypertension and concurrent diseases. The pulse and heart beat were checked at every examination. Individuals with irregular pulse or cardiac rhythm and/or patients with complaint of palpitation or chest discomfort received 12-lead electrocardiogram and 24-h Holter recordings. In addition, all patients received standard 12-lead electrocardiogram at least once a year. AF was defined as absence of P waves before each QRS complex, irregular atrial electrical activity with fibrillatory waves varying in size, shape and timing, and completely irregular RR intervals. New-onset AF as the study endpoint was defined as the first presentation of AF during follow-up. Transient postoperative AF, occurring as an isolated episode within one month after surgery, was not counted as an outcome event. Because newly documented AF, not the duration or persistence of the arrhythmia, was the outcome event of interest, no distinction was made between paroxysmal and persistent AF. For patients without any AF event, the date of censor was that of the last contact with the patient.

Statistical analysis

Statistical analysis was performed using StatView Version 5 Software (Abacus Concepts Inc., Berkeley, California, USA). Values were expressed as mean ± SD. An unpaired Student's *t*-test was used for comparison between the two groups. AF event-free curves were derived by means of the Kaplan–Meier method and were compared by log-rank test. Possible predictors of new-onset AF were tested by univariate Cox proportional hazards regression analysis. Then, a multivariate analysis was applied to identify independent predictors and their predictive power. Independent predictors of AF incidence were also evaluated by using a stepwise regression analysis. A value of $P < 0.05$ was accepted as statistically significant.

Table 1 Clinical characteristics of total participants ($n=1118$)

Variable	
Age (years)	63±11
Sex (men) (%)	52
Body mass index (kg/m^2)	24.3±3.4
Duration of hypertension (years)	16±11
Diabetes mellitus (%)	24
Smokers (current or past) (%)	48
Systolic blood pressure (mmHg)	146±16
Diastolic blood pressure (mmHg)	82±11
Heart rate (bpm)	67±8
eGFR (ml/min per 1.73 m^2)	68±32
Urinary protein	
(−) to (±) (%)	74
(1+) to (2+) (%)	16
≥(3+) (%)	10
Antihypertensive treatment	
Ca channel blockers (%)	69
RAS inhibitors (%)	35
β-blockers (%)	29
Diuretics (%)	17
Others (%)	13
Statin use (%)	29

Values are mean ± SD or percentage. eGFR, estimated glomerular filtration rate; RAS, rennin–angiotensin system.

Results

Patient characteristics

The clinical characteristics of all patients are summarized in Table 1. The average duration of hypertension of the patients was 16±11 years, and they had had history of antihypertensive treatment of 11±9 years as average. At baseline, 83% of the study patients were receiving antihypertensive drugs, and 17% were treated with diet and/or exercise therapy only. Ca channel blockers, rennin–angiotensin system (RAS) inhibitors (i.e., angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors), β-blockers, diuretics, and other classes of agents were used alone or in various combinations in 69, 35, 29, 17, and 13% of the study patients, respectively.

The mean duration of follow-up was 4.5 years (range, 0.1–9.1 years), for a total of 5079 patient-years of observation. During follow-up, 57 cases of new-onset AF were found, indicating the incidence was 1.1% per year. Of these 57 AF cases, 39 (68%) were symptomatic and the other 18 (32%) were asymptomatic at the time of the first documented event.

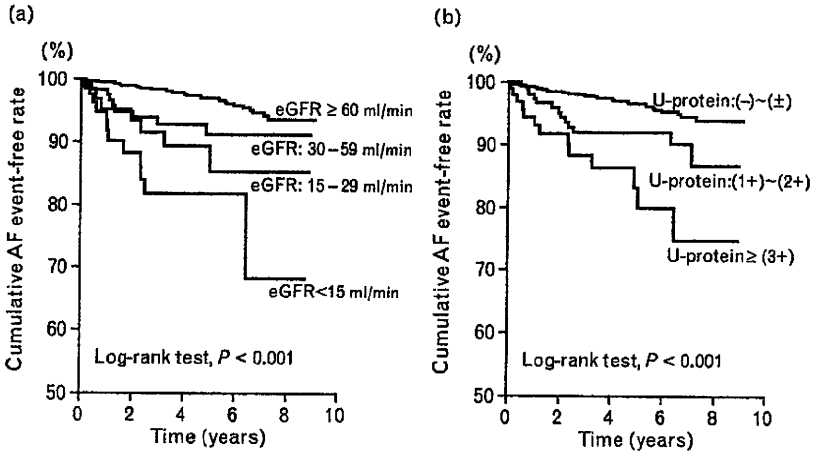
Relations of estimated glomerular filtration rate and proteinuria to the incidence of atrial fibrillation

The effect of eGFR and proteinuria on the incidence of new-onset AF was evaluated. The cumulative AF event-free rate was significantly decreased according to the reduction of basal eGFR (Fig. 1a). Likewise, AF event-free rate was clearly decreased according to the increase in urinary protein levels (Fig. 1b). In the Cox regression analysis, both eGFR (hazard ratio 0.82 per 10 ml/min per 1.73 m^2, $P<0.001$) and proteinuria [(1+) to (2+): hazard ratio 2.31, $P=0.012$; ≥(3+): hazard ratio 5.07, $P<0.001$ vs. (−) to (±)] were significantly related to the incidence of AF.

Effect of chronic kidney disease on the incidence of atrial fibrillation

We divided the present patients into two groups by the absence or presence of CKD, which was defined as decreased eGFR less than 60 ml/min per 1.73 m^2 and/or the presence of proteinuria (≥1+). The participant group with CKD was associated with older age, higher

Fig. 1



Atrial fibrillation (AF) event-free curves obtained with the Kaplan–Meier method in the respective groups divided by basal estimated glomerular filtration rate (eGFR, a) or urinary protein levels (U-protein, b). (a) All participants were divided into four groups according to basal eGFR levels. Cumulative AF event-free rates in the groups with basal eGFR of ≥60 ($n=818$), 30–59 ($n=128$), 15–29 ($n=73$), and <15 ml/min per 1.73 m^2 ($n=99$) were 93.6, 91.2, 85.3, and 68.2%, respectively (log-rank test, $P<0.001$). (b) All participants were divided into three groups according to basal U-protein levels. Cumulative AF event-free rates in the groups with basal levels of U-protein of (−) to (±) ($n=827$), (1+) to (2+) ($n=183$), and ≥ (3+) ($n=108$) were 93.9, 86.7, and 74.7, respectively (log-rank test, $P<0.001$).

Table 2 Comparison of basal characteristics between the two groups without and with CKD

	CKD (−) ($n=732$)	CKD (+) ($n=386$)
Age (years)	62 ± 11	65 ± 11*
Sex (men) (%)	47	61*
Body mass index (kg/m^2)	24.5 ± 3.4	23.8 ± 3.4*
Duration of hypertension (years)	15 ± 10	18 ± 11*
Diabetes mellitus (%)	18	35*
Smokers (current or past) (%)	44	55*
Systolic blood pressure (mmHg)	144 ± 15	150 ± 17*
Diastolic blood pressure (mmHg)	82 ± 11	81 ± 11*
Heart rate (beats/min)	67 ± 8	67 ± 8
eGFR (ml/min per 1.73 m^2)	83 ± 20	40 ± 30*
Urinary protein		
(−) to (±) (%)	100	25*
(1+) to (2+) (%)	0	47*
≥(3+) (%)	0	28*
Antihypertensive treatment		
Ca channel blockers (%)	61	83*
RAS inhibitors (%)	32	41*
β-Blockers (%)	26	35*
Diuretics (%)	10	30*
Statin use (%)	26	33*
LA diameter (mm)	36 ± 5	37 ± 5*
LV mass index (g/m^2)	121 ± 31	145 ± 44*
LV fractional shortening	0.42 ± 0.07	0.40 ± 0.07*

Values are mean ± SD or percentage. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; LA, left atrial; LV, left ventricular; RAS, rennin–angiotensin system. *$P<0.05$ compared with CKD (−).

proportion of men, smaller body mass index, and higher rate of diabetes mellitus and smokers (Table 2). In addition, the patients with CKD had longer duration of hypertension, higher systolic blood pressure, and more use of antihypertensive drugs. As for echocardiographic parameters, LA diameter and LV mass index were significantly greater, and LV fractional shortening was slightly lower in patients with CKD than in those without CKD.

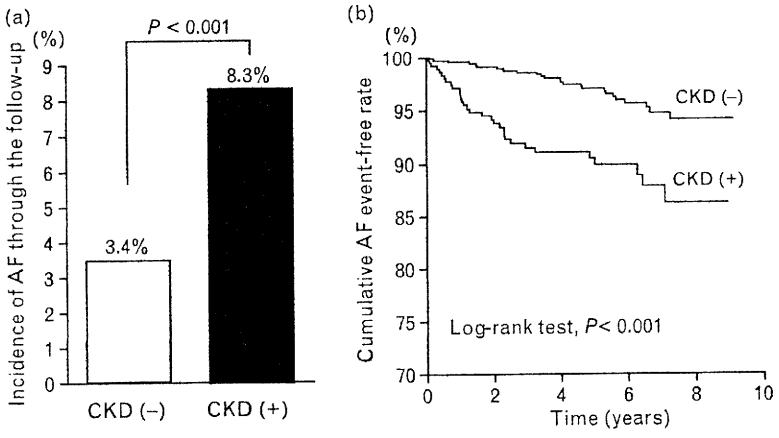
When comparing the incidence of new-onset AF between the two groups, the total incidence of AF through the follow-up periods was markedly higher in the patient group with CKD, compared to that without CKD (Fig. 2a). The cumulative AF event-free rate was also significantly decreased in the CKD group, compared to the non-CKD group (Fig. 2b).

As several confounding factors might be involved in the association between CKD and the incidence of AF in the present participants, we examined the independent predictors of new-onset AF by Cox regression analysis. In the univariate analysis, age, smoking, use of diuretic, LA diameter, LV mass index, and the presence of CKD were significantly related to the incidence of AF (Table 3). Among these possible predictive factors, age, smoking, and the presence of CKD were independent predictors of new-onset AF by the multivariate analysis. The adjusted hazard ratio of having CKD for new-onset AF during follow-up was 2.18 (95% confidence interval 1.21–3.90, $P=0.009$). Independent predictors of AF incidence were re-examined by stepwise regression analysis including all clinical and echocardiographic variables as possible independent factors. The presence of CKD as well as age, smoking, and LA diameter was an independent predictor of new-onset AF (age, hazard ratio 1.48 per 10 years, $P=0.008$; smoking, hazard ratio 1.92, $P=0.037$; LA diameter, hazard ratio 1.43 per 5 mm, $P=0.015$; CKD, hazard ratio 2.36, $P=0.004$).

Chronic kidney disease stages and the incidence of atrial fibrillation

The association of CKD stages with the incidence of AF was finally examined. In the univariate Cox analysis, the occurrence of new-onset AF was significantly increased in

Fig. 2



(a) Incidence of atrial fibrillation (AF) through the follow-up periods in the two groups without and with chronic kidney disease (CKD). The total rates of new-onset AF in the patients without and with CKD were 3.4% (0.7% per year) and 8.3% (2.1% per year), respectively ($P<0.001$). (b) AF event-free Kaplan–Meier curves in the two groups without and with CKD. Cumulative AF event-free rates in the non-CKD group and CKD group were 94.1 and 86.3%, respectively (log–rank test, $P<0.001$).

Table 3 Predictors of new-onset AF by univariate and multivariate Cox regression analysis

	Hazard ratio (95% CI)	P
Univariate analysis		
Age, 10 years	1.65 (1.24–2.19)	<0.001
Sex, men	1.51 (0.89–2.55)	0.128
Body mass index, 1 kg/m^2	1.01 (0.93–1.09)	0.839
Duration of hypertension, 1 year	1.02 (1.00–1.05)	0.100
Diabetes mellitus, yes	1.34 (0.75–2.40)	0.318
Smoking (current or past), yes	2.23 (1.29–3.84)	0.004
Systolic blood pressure, 10 mmHg	1.06 (0.90–1.25)	0.480
Diastolic blood pressure, 10 mmHg	0.88 (0.69–1.13)	0.316
Heart rate, 1 bpm	0.98 (0.94–1.01)	0.165
Ca channel blocker, yes	1.56 (0.84–2.89)	0.162
RAS inhibitor, yes	0.82 (0.47–1.44)	0.492
β-Blocker, yes	1.38 (0.81–2.35)	0.236
Diuretic, yes	2.23 (1.23–4.03)	0.008
Statin, yes	1.00 (0.57–1.76)	0.990
LA diameter, 5 mm	1.43 (1.10–1.87)	0.008
LV mass index, 10 g/m^2	1.09 (1.03–1.15)	0.004
LV fractional shortening, 0.01	0.98 (0.94–1.02)	0.250
CKD, yes	2.99 (1.77–5.05)	<0.001
Multivariate analysis		
Age, 10 years	1.54 (1.16–2.04)	0.003
Smoking (current or past), yes	1.78 (1.01–3.15)	0.047
Diuretic, yes	1.23 (0.65–2.32)	0.533
LA diameter, 5 mm	1.26 (0.94–1.68)	0.118
LV mass index, 10 g/m^2	1.03 (0.96–1.10)	0.457
CKD, yes	2.18 (1.21–3.90)	0.009

In the multivariate analysis, all variables that had a significant association in the univariate analysis were included as possible independent factors. AF, atrial fibrillation; CI, confidence interval; CKD, chronic kidney disease; LA, left atrial; LV, left ventricular; RAS, rennin–angiotensin system.

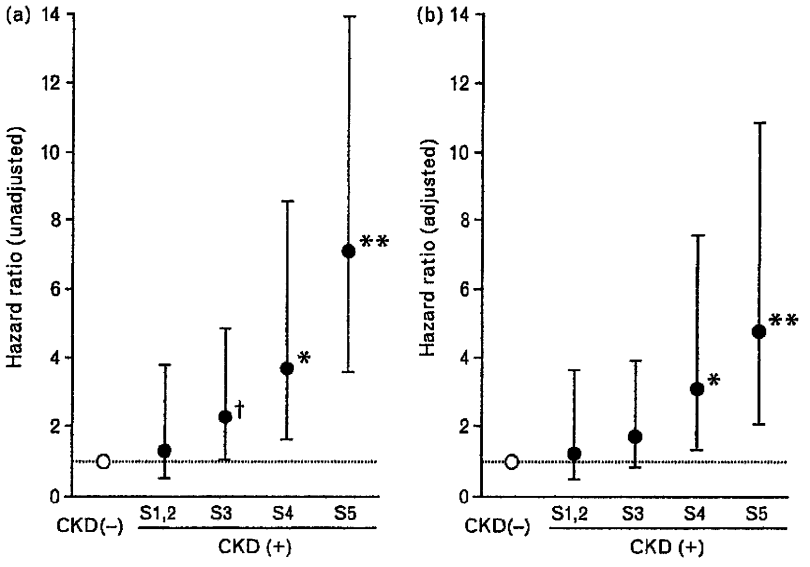
the participant groups with CKD stage 3 and more advanced stages (Fig. 3a). After adjustment for confounding factors (i.e., age, smoking, use of diuretic, LA diameter, and LV mass index) by the multivariate analysis, CKD stages 4 and 5 were still significantly associated with the increased incidence of AF (Fig. 3b).

Discussion

The present study has shown that CKD defined as decreased eGFR and/or the presence of proteinuria is longitudinally associated with the incidence of new-onset AF in hypertensive patients. Our results indicate that antecedent existing CKD has a significant influence on new-onset AF in hypertensives.

Several clinical and population-based studies showed that the prevalence of AF was independently associated with decreased eGFR and increased levels of urinary albumin [12–14], although these cross-sectional investigations did not elucidate whether antecedent renal dysfunction affects the incidence of AF. Prospective observational studies examining postoperative AF showed that renal impairment (decreased eGFR or renal failure) was associated with an increased risk of AF after cardiac surgery [15,16]. A recent study reported that decreased baseline eGFR was associated with an increased risk of subsequent new onset AF in a large scale of community-based cohort [17]. The findings of our study are fundamentally consistent with these observations. However, previous

Fig. 3



Relation of chronic kidney disease (CKD) stages to the incidence of atrial fibrillation (AF) evaluated by univariate (a) and multivariate (b) Cox regression analysis. Respective data present hazard ratios (open or solid circles) and the 95% confidence intervals (vertical lines) in the groups without CKD ($n=732$) and with CKD stages 1–2 (S1,2, $n=86$), 3 (S3, $n=128$), 4 (S4, $n=73$), and 5 (S5, $n=99$). In the multivariate analysis, all variables that had a significant association in the univariate analysis (i.e., age, smoking, use of diuretic, left atrial diameter, and left ventricular mass index) were included as confounding factors. $^{\dagger}P<0.05$, $^{*}P<0.01$, $^{**}P<0.001$ vs. CKD (–).

studies have shown that many factors are involved in the development of AF in a general population and patients with cardiovascular disorders [18–20]. As for hypertensive patients, it has been revealed that age, systolic blood pressure, LV mass, and LA size are related to the incidence of AF [2–5,21]. Thus, there was the possibility that these factors might mediate the association between CKD and AF incidence observed in the present and other studies, because GFR generally decreases with age, and pressure and volume load augmented by renal dysfunction directly increases LV mass and LA size. In fact, the present patients with CKD had older age, higher systolic blood pressure, and greater LV mass index and LA diameter, compared with those without CKD. In addition, age, LV mass index, and LA diameter as well as CKD were relating factors to the incidence of AF in the univariate Cox regression analysis of this study. By the multivariate analysis, however, the association of CKD with new-onset AF was warranted to be still significant independently of these confounders, although the adjusted hazard ratio of CKD for AF incidence was diminished compared to the crude risk ratio before adjustment. Therefore, the present study has demonstrated for the first time that the existence of CKD in hypertensive patients is an independent predictor of new-onset AF, apart from the effects of aging, LV hypertrophy, and LA dilatation.

Verdecchia *et al.* [3] showed that age and LV mass were the sole independent predictors of new-onset AF in a large cohort of initially untreated patients with essential hypertension. In our patients with chronically treated hypertension, LV mass index was not an independent determinant of the incidence of AF. The exact reason for the discrepant findings is unclear, but there was a possibility that antihypertensive treatment before the enrollment might have modified LV mass in our study.

In the univariate analysis of our study, basal systolic or diastolic blood pressure was not significantly related to the incidence of AF. Previous studies showed that systolic blood pressure and pulse pressure were good predictors of incident AF in large cohorts of the general population [22,23]. In hypertensive patients, however, there have been discrepant findings concerning the significant influence of blood pressure levels on the incident of AF [2–4,21]. Antihypertensive treatment and changes in blood pressure during follow-up might have modified the outcome and have spoiled the possible relation between systolic blood pressure and incident AF in our retrospective observational study. Since the present patients with CKD had a significantly higher systolic blood pressure than those without CKD, there might be a possibility that elevated blood pressure in the CKD group promoted renal dysfunction further, resulting in contribution of new-onset AF partly.

In the present study, the incidence of new-onset AF was clearly associated with the decrease in eGFR. In fact, CKD stages 4 and 5 were a significant predictor of incident AF after adjustment for confounding factors by the multivariate analysis. The incidence of AF was also increased according to the severity of proteinuria. Therefore, our findings suggested that advanced renal dysfunction including massive proteinuria chiefly contributed to the incidence of new-onset AF in the present hypertensive patients.

The causal mechanism by which renal impairment has a great and partly cardiac overload-independent influence on the occurrence of AF in hypertensive patients could not be drawn from our observational study, but there are some possible speculations. The increased risk of developing AF in CKD may be related in part to activation of signaling pathways of inflammation, because previous studies have shown that renal insufficiency is associated with elevations of inflammatory markers such as C-reactive protein [24] and that C-reactive protein predicts increased risk for developing future AF [25]. Possible involvement of oxidative stress and endothelial dysfunction in the development of AF has also been shown [26,27]. Since the patients with chronic renal failure have increased levels of oxidative stress markers and impaired endothelial function [28], oxidative stress and endothelial dysfunction caused by renal impairment may be involved in the increased risk of new-onset AF in patients with CKD. In addition, these mechanisms might be also involved in the association between smoking habit and incident AF observed in the present study, because smoking is known to increase oxidative stress and deteriorate endothelial function.

Limitations

Screening 24-h electrocardiographic recordings were not performed in our study, although standard 12-lead electrocardiograms were periodically done for all the present patients. Therefore, it is possible that asymptomatic cases of AF may have gone undetected. In fact, 68% of 57 cases of newly documented AF were accompanied by some symptom such as palpitation and chest discomfort, and the other 32% were asymptomatic cases in the present study. However, all patients visited our hospital periodically (every 1–2 months) and the pulse and heart beat were checked at every examination. Individuals with irregular pulse or cardiac rhythm received 12-lead electrocardiogram and 24-h Holter recordings, even they had no cardiac symptom. In addition, the incidence of new-onset AF in our study (1.1% per year) was similar to the incidence rates in other studies for patients with essential hypertension (0.5–1.7% per year) [3–5,21] and higher than those in middle-aged and elderly adults from population-based studies (0.2–1.1% per year) [17,18,22,23,29,30]. Thus, it is less likely that there were a considerable number of missed AF cases in the present study. Furthermore, since any misclassification or underdetection of incident AF is

expected to occur at random and independent of renal function, such misclassification would not overestimate the true risk of new-onset AF associated with CKD. The small number of new-onset AF during follow-up, however, must be considered as a limitation of the study, especially in comparing AF incidence rates among more than three groups.

Several studies have revealed that RAS inhibitor treatment and hydroxymethylglutaryl coenzyme A reductase inhibitor (statin) use are associated with reduced incidence of AF in patients with cardiovascular disease [21,31,32]. As another study limitation, therefore, we must consider the possibility that these treatments might bias the outcome of the present study.

Moreover, there was a possibility that the obtained findings in this study might be limited to the Japanese population. Further studies are needed to validate our results in Western and other racial populations.

In conclusion, the present study demonstrated that CKD defined as decreased eGFR and/or the presence of proteinuria was associated with an increased risk of new-onset AF in hypertensive patients, and that the impact of CKD on the incidence of AF was independent of LV hypertrophy and LA dilatation. In particular, advanced stages of CKD were strongly related to the increasing occurrence of AF. In managing hypertensive patients, therefore, it may be important to prevent the progression of renal dysfunction in prevention of the occurrence of new-onset AF.

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There are no conflicts of interest.

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ORIGINAL ARTICLE: BIOLOGY

Association of carotid atherosclerosis with genetic polymorphisms of the klotho gene in patients with hypertension

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Aim: Previous studies suggest that klotho gene polymorphisms may be associated with atherosclerosis, but did not assess the relationship between klotho gene polymorphisms and atherosclerosis parameters such as carotid artery intima-media thickness (IMT). Here, we studied whether *klotho* single nucleotide polymorphisms (SNP) were associated with carotid atherosclerosis.

Methods: All subjects were Japanese. Eight-hundred and fifty-three patients with hypertension (465 men and 388 women) in the outpatient clinic and 1783 subjects from the general population (821 men and 962 women) attending health check-ups were analyzed in the present study. We measured mean IMT of the common carotid artery to evaluate carotid atherosclerosis. Four single nucleotide polymorphisms (SNP) (rs7323281; intron1, rs5644481; exon4, rs3752472; exon3, rs650439; intron4) of *klotho* were selected as representative SNP in haplotype blocks.

Results: Multivariate logistic regression analysis adjusted by confounding factors showed a significant association of rs650439 with carotid atherosclerosis in hypertensive patients (TT vs TA vs AA, $P < 0.01$; TT + TA vs AA, $P < 0.01$). By ANCOVA considering confounding factors, rs650439 was also significantly associated with mean IMT (TT + TA vs AA, $P = 0.04$) in the hypertensive population. However, there was no significant association between *klotho* SNP and carotid IMT in the general population. Compared to the general population, the subject group with hypertensive patients clearly had more atherosclerosis risk factors.

Conclusion: Only in hypertensive patients was *klotho* rs650439 strongly associated with mean IMT thickening of the common carotid artery. Therefore, *klotho* SNP (rs650439) may

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influence on the progression of carotid atherosclerosis in patients with hypertension. **Geriatr Gerontol Int 2010; 10: 311–318.**

Keywords: carotid atherosclerosis, klotho, single nucleotide polymorphism.

Introduction

Myocardial infarction and stroke originating from atherosclerosis are the main causes of death in middle-aged and elderly people. The prevention of atherosclerosis is important to maintain the health of these groups of people. Defects in klotho gene expression in mice result in a syndrome that is similar to human aging, including arteriosclerosis, osteoporosis, infertility, emphysema and ectopic calcification.[1] In *klotho*-deficient mice, Mönckeberg-type arteriosclerosis, which is seen in aged humans, was observed from the aorta to small arterioles,[1] and impairments in angiogenesis and vasculogenesis were additionally observed.[2] Endothelial cell dysfunction has been suggested as the initiating process in the development and progression of atherosclerosis. Endothelium-dependent vasodilation in response to acetylcholine is attenuated in the aorta and arterioles from *klotho*-deficient mice, and this endothelial dysfunction was prevented by parabiosis between wild-type mice and heterozygously *klotho*-deficient mice.[3] Subsequent studies in humans have identified a functional variant of *klotho*, dubbed KL-VS, that is associated with longevity[4] and early-onset occult coronary artery disease (CAD).[5] Furthermore, a recent study by Arking *et al.*[6] reported the association of KL-VS with high-density lipoprotein (HDL) levels, systolic blood pressure (SBP) and an increased risk of stroke, which suggests an association of *klotho* with atherosclerosis in white subjects. In the Japanese population, the G-395A polymorphism in the promoter region of human *klotho* was reported to be associated with bone density,[7] cognitive impairment[8] and CAD.[9]

We have previously reported that klotho gene delivery suppresses oxidative stress in mice,[10] and that klotho protein reduced H_2O_2-induced apoptosis and senescence in vascular cells.[11] Furthermore, we recently reported that klotho protein suppresses tumor necrosis factor-α-induced expression of adhesion molecules in the endothelium and monocyte adhesion to endothelium cells.[12] Atherosclerosis is thought to be a chronic inflammatory disease initiated and perpetuated by a variety of cardiovascular risk factors.[13] Thus, our experimental findings suggest that klotho protein plays a protective role in the pathogenesis of atherosclerosis.

Previous studies suggest that klotho gene polymorphisms may be associated with atherosclerosis. However, previous studies did not assess the relationship between klotho gene polymorphisms and atherosclerosis parameters such as carotid artery intima-media thickness (IMT). Here, we studied whether *klotho* single nucleotide polymorphisms (SNP) were associated with carotid atherosclerosis in Japanese using IMT measured by ultrasonography.

Methods

Subjects and DNA samples

Study subjects consisted of patients with hypertension and a general population participating in the so-called "Suita Study", which is a cohort study for cardiovascular diseases at the National Cardiovascular Center. The characteristics of patients with hypertension were as follows. Subjects with hypertension included 953 patients with hypertension (522 men and 431 women, average age 65.1 ± 10.5 years) recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center. Ninety-two percent of subjects (880 subjects) had essential hypertension, and the remaining 8% had secondary hypertension. The criteria for hypertension were a SBP greater than 140 mmHg or a diastolic blood pressure (DBP) greater than 90 mmHg or both, or the use of antihypertensive agents. Subjects from the general population were people who had visited the National Cardiovascular Center every 2 years for general health checkups. Age, SBP, DBP, body mass index (BMI), percentage of current smokers, percentage of current drinkers, and prevalence of hypertension and diabetes mellitus were significantly higher in men than in women. Total cholesterol, HDL cholesterol, and percentage of hyperlipidemia were significantly higher in women than in men. For both populations, in addition to performing a routine blood examination that included lipid profiles, glucose levels, blood pressure, anthropometric measurements, a physician or nurse administered questionnaires covering personal history of cardiovascular diseases, including angina pectoris, myocardial infarction and stroke. Smoking was defined as current smoking, past smoking and never. All subjects were Japanese. Because of a lack of clinical data or unsuccessful sequencing and genotyping, 853 patients with hypertension (465 men and 388 women) and 1783 subjects from the general population (821 men and 962 women) were analyzed in the present study.

DNA samples were obtained with written informed consent and the protocol was approved by the Ethical Review Committee of the National Cardiovascular