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# The predictive value of Klotho polymorphism, in addition to classical markers of CKD-MBD, for left ventricular hypertrophy in haemodialysis patients

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## Abstract

**Purpose** Cardiovascular events are the major reasons for mortality in haemodialysis patients. Fibroblast growth factor 23 (FGF23), Klotho protein and G-395A Klotho gene polymorphism have been associated with effects on the cardiovascular system. Our study investigates the interrelationship between Klotho protein gene variations, mineral–bone metabolism and left ventricular hypertrophy in patients undergoing chronic haemodialysis programme.

**Materials and methods** Patients ($n = 142$) were genotyped for G-395A Klotho gene. Components of mineral–bone metabolism, classical and non-classical (FGF23, Klotho and vitamin D) as well as echocardiographic examination were determined. Predictive models were designed to determine the significance of Klotho gene variations and mineral–bone metabolism components for left ventricle hypertrophy (LVH).

**Results** A-allele carriers were longer on haemodialysis ($p = 0.033$), and had higher phosphorus levels ($p = 0.016$) while the level of Klotho protein was significantly lower ($p = 0.001$) compared to non-A-allele carriers. The best gains were achieved upon addition of allele A, and all three new markers; the AUC made significant improvement from 0.596 to 0.806 ($p < 0.001$), and improved net reclassification for 82.1% (95% CI 42.9–121.3%).

**Conclusions** The genetic background of A-allele carriers of the G-395A Klotho gene polymorphism increases the susceptibility patients to haemodialysis. A-allele carriers are at a higher risk for the development of cardiovascular complications. The addition of non-classical to classical mineral metabolism components improves prediction power to LVH.

**Keywords** Klotho gene polymorphism · FGF23 · Klotho · LVH · Haemodialysis · Predictive models

## Introduction

Chronic kidney disease (CKD) is a major medical and socio-economic problem in the world, with the majority of patients in terminal CKD, being treated with haemodialysis. This population records a high mortality rate, with cardiovascular complications as the most common cause [1, 2]. Profound disturbances in mineral–bone metabolism associated with cardiovascular complications conditioned calcium and phosphorus accumulation in soft tissues. Therefore, vascular calcification associated with micro-inflammation and oxidative stress leads to the degradation of vascular smooth muscle cells, osteoblastic transformation and blood vessel stiffness [3, 4]. There is increasing evidence that fibroblast growth factor 23 (FGF23) and Klotho protein, have an essential role in this complex pathophysiological mechanism which participates in cardiovascular morbidity [5, 6]. FGF23 alongside with the transmembrane Klotho, has a phosphaturic effect in proximal tubules in early CKD [7], while in haemodialysis patients elevated values of FGF23 and hyperphosphatemia are present. Primarily in the kidneys, brain, and parathyroid

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glands, a soluble Klotho protein is separated from the transmembrane Klotho protein and as such affect distant organs [8]. It has been established that Klotho has a cardioprotective effect due to blocking calcium influx in cardiomyocytes, therefore prevents the development of left ventricular hypertrophy (LVH) [9].

Polymorphism in the Klotho gene G-395A in the promoter region has been associated with effects on the cardiovascular system [10, 11]. A-allele is assumed as a mutant, while G-allele is wild type for the G-395A polymorphism [12].

Our study investigates the interrelationship between Klotho protein gene variations, mineral–bone metabolism and LVH in patients undergoing chronic haemodialysis programme.

## Patients and methods

One hundred and forty-two patients with terminal CKD undergoing haemodialysis treatment on the Clinic of Nephrology were included in this cohort study. The inclusion criteria for study participation were patients above the age of 18, with at last 3 months duration on haemodialysis for three times weekly. The study was approved by the Ethics Committee of Faculty of Medicine, under the number 12-12123-1, and all participants signed informed consent. The study was performed respecting the principles of evidence-based medicine and by the Declaration of Helsinki.

## Biochemical analysis

Routine laboratory analyses were performed on the automatic biochemistry analyser Erba XL-600 (Erba Diagnostics Mannheim, GmbH, Germany), and haematology analyser Cell Counter, MEK-4100, Nihon Kohden, Japan. C-reactive protein serum levels were determined using the immunoturbidimetric method, on Olympus AU-600 auto-analyzer (Olympus Diagnostic, GmbH, Germany). Determination of intact PTH was performed by immuno-radiometric analysis (IRMA).

Determination of the concentration of fibroblast growth factor 23 and Klotho protein was performed from serum ELISA method using commercial kits from producer Cusabio Biotech, Wuhan, China. The results were expressed as pg/mL. The concentration of vitamin D (25-hydroxy vitamin D3) is determined by the HPLC method [13].

Dialysis adequacy was evaluated by *Kt*/*V* index, calculated according to the following formula: $K_t/V_{sp} = -\ln(C2/C1 - 0008 \times T) + (4 - 3.5 \times C2/C1) \times UF/W$, where C1 is the predialysis urea value (mmol/L), C2 the post-dialysis urea value (mmol/L), *T* haemodialysis duration (h), *UF* interdialysis yield (L), and *W* body weight after haemodialysis session (kg).

## Genotyping

Of the whole blood sample with EDTA as an anticoagulant, 200 µl was taken for DNA isolation with the help of Genomic DNA Purification Mini Kit (Fermentas, Thermo Scientific, Lithuania) according to the manufacturer's instructions. The modified method of Shimoyama and associates PCR-CTPP (confronting 2-pair primers) was used to determine the G-395A polymorphism in the Klotho protein gene [14]. This method is characterised by a reaction mixture to which 4 primers are added, two inferior (Fwd1, Fwd2, Forward Example 1, Forward Example 2) and two origins (Rev1, Rev2, Reverse Example 1, Reverse Example 2). The reaction mixture (for each subject) in a total volume of 25 µl contain 12.5 µl of the commercial mix of KAPA2G ReadyMix (KAPA2G Fast HotStart ReadyMix PCR Kit; Cap BioSystems, Boston, Massachusetts, United States) containing a Hot Start DNA polymerase, dNTPs, MgCl2 and stabilisers. In addition to the commercial KAPA2G ReadyMix, to each mixture 0.5 µl of each primer (Fwd1: 5′-GAT CCC GCC CCC AAG TCG GGA-3′ and Fwd2: 5′-GTC CCT CTA GGA TTT CGG CCAG-3′ and rev1: 5′-GTT TCG TGG ACG CTC AGG TTC ATT CTC-3′ and Rev2: 5′-GAG AAA AGG CGC CGA CCA ACT TTC-3′), 9.5 µl of deionised water and 1 µl of isolated DNA (average concentrations of 50 ng/µl) was added. For the amplification of PCR products G-395A SNP of Klotho gene, the following programme was used: initial denaturation for 2 min at 95 °C, followed by 35 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 60 °C and elongation for 15 s at 72 °C with a final elongation for 30 s at 72 °C. PCR products were detected on 2% agarose gel with ethidium bromide staining. Genotyping was performed as follows; 252, 175, 121 bp for GA genotype, 252, 175 bp for GG genotype and 252, 121 bp for AA genotype.

## Echocardiographic examination

A complete two-dimensional (2D) transthoracic echocardiographic examination was performed in a non-invasive diagnostic cabinet at Toshiba Power vision 6000, Toshiba Co, Tokyo, Japan. Respondents were examined by one experienced echo sonographer who was not previously familiar with their clinical status. The parameters of morphology, geometry and function of left ventricular were determined. All measurements and calculations were made according to the latest recommendations of the American

Society of Echocardiography and the European Association of Cardiovascular Imaging [15].

### Statistical analysis

Data were analysed using R software [16]. Qualitative data were expressed as frequencies and percentages while quantitative data are presented as mean ± standard deviations. Normally distributed data were compared by Student's *t* test, while the Mann–Whitney test was used for abnormally distributed data. For the comparison of frequencies, the Chi-square test was used.

As measures of discrimination, respecting the ability of a model to discriminate those who will develop heart hypertrophy from those who will not, we calculated: the areas under the curves (AUC), which is equivalent to the concordance (c) statistic; the net reclassification improvement (NRI); and the integrated discrimination index (IDI) [17, 18]. The differences between areas under the curve for two models were tested using Delong's method [19]. For calibration estimation, i.e. the agreement between observed and predicted outcomes, we used the Hosmer–Lemeshow goodness-of-fit test [20]. Statistical significance was accepted for $p < 0.05$.

## Results

Genotype distribution of Klotho G-395A: GG, GA, AA, were 97(68.3%), 42 (29.6%) and 3 (2.1%), respectively, and fulfil Hardy–Weinberg equilibrium ($\chi^2 = 0.398$; $p = 0.528$). Distribution of alleles is as follows: G-allele 236 (83.1%) and A-allele 48 (16.9%) (Table 1). In a further analysis all patients are divided into A-allele carriers (45 (31.7%)) and non-carriers (GA and AA versus GG) (97 (68.3%)).

Baseline demographic and clinical data are shown in Table 2. The average age of the whole population was 59.00 ± 12.33 years (min 23, max 84 years), with mean haemodialysis duration of 74.68 ± 78.40 months (min 3, max 360 months). In addition to basic biochemical parameters, classical parameters of mineral–bone metabolism as well as new markers, such as vitamin D, Klotho, and FGF23, were determined.

Clinical and biochemical parameters between carriers and non-carriers of A-allele are shown in Table 2. Statistically, significant difference exists among these two groups in HD duration, which is longer among A-allele carriers ($p = 0.033$), as well as years at starting renal replacement therapy, where A-allele carriers were younger ($p = 0.044$). Phosphorus concentration was statistically higher among A-allele carriers ($p = 0.016$) while the level of Klotho protein was significantly lower ($p = 0.001$).

Cardiovascular events (coronary artery disease, stroke, heart failure, hypertensive heart disease, cardiomyopathy, heart arrhythmia, peripheral artery disease) were more frequent among A-allele carriers ($p = 0.014$).

Echocardiography parameters are presented in Table 3. There was no statistical difference among all examined parameters between A-allele carriers and non-carriers. Also, all patients are divided according to the heart geometry into the normal, remodelling heart, concentric and eccentric hypertrophy, but without establishing any difference in distribution among examined groups. Also, when grouping normally and remodelling heart into the normal heart group, and both hypertrophies into one group, we did not find a significant difference between the carriers and non-carriers of A-allele.

In the 'basic' model, left heart hypertrophy was the outcome variable and covariates included basic mineral–bone metabolism parameters such as Ca, P, ALP and PTH. We added allele A genotype and new clinical biomarkers (Vitamin D, Klotho protein and FGF23 protein) separately to the basic model to evaluate their contribution to risk prediction. Using Delong's method we compared each model with the basic model (Fig. 1). The best gains were achieved upon addition of allele A, and all three new markers; the AUC made significant improvement from 0.596 to 0.806 ($p < 0.001$), and improved net reclassification for 82.1% (95% CI 42.9–121.3%) (Table 4).

## Discussion

Despite the overall progress of medicine, technical improvement of haemodialysis machines and the advancement of the pharmacological approach, the patient mortality rate in the chronic haemodialysis programme is extremely high. This high mortality rate is mainly due to cardiovascular events where aetiopathogenesis of bone and mineral disorder has a significant role. The knowledge of population genetics is necessary, especially in the domain of mineral–bone disorder and cardiovascular complications in haemodialysis patients.

**Table 1** The frequency distribution of G-395A Klotho genotype and alleles

| Genotype | *n* (%) | Alleles | *n* (%) | A-allele | *n* (%) |
|---|---|---|---|---|---|
| GG | 97 (68.3) | G (GG + GA) | 236 (83.1) | Non-carriers | 97 (68.3) |
| GA | 42 (29.6) | A (GA + AA) | 48 (16.9) | Carriers | 45 (31.7) |
| AA | 3 (2.1) | | | | |

**Table 2** Comparison of clinical and biochemical characteristics between carriers and the non-carriers of A-allele

| | All | Alleles | | *p* value |
|---|---|---|---|---|
| | | Non-A (GG) | A (GA + AA) | |
| Sex (male) | 88 (62.0) | 55 (62.5) | 33 (37.5) | 0.057 |
| Age (years) | 59.00 ± 12.33 | 59.11 ± 12.32 | 58.76 ± 12.49 | 0.873 |
| HD start (years) | 51.72 ± 14.80 | 53.42 ± 13.84 | 48.06 ± 16.25 | 0.044 |
| HD duration (months) | 74.68 ± 78.40 | 69.02 ± 78.84 | 86.73 ± 77.04 | 0.033 |
| BUN (mmol/L) | 25.03 ± 5.67 | 25.65 ± 5.84 | 24.06 ± 5.39 | 0.125 |
| Serum creatinine (μmol/L) | 771.28 ± 139.67 | 767.89 ± 138.92 | 778.65 ± 142.64 | 0.674 |
| CRP (mg/dL) | 6.81 ± 11.37 | 51.89 ± 10.86 | 8.71 ± 12.23 | 0.212 |
| Haemoglobin (g/L) | 106.01 ± 15.33 | 104.62 ± 13.87 | 108.96 ± 17.59 | 0.067 |
| Leukocytes ($\times 10^9$/L) | 6.81 ± 1.93 | 6.80 ± 2.08 | 6.86 ± 1.59 | 0.827 |
| Albumin (mg/dL) | 37.99 ± 2.69 | 38.12 ± 2.71 | 37.67 ± 2.63 | 0.337 |
| Total cholesterol (mmol/L) | 4.68 ± 1.22 | 4.74 ± 1.24 | 4.56 ± 1.17 | 0.416 |
| Kt/V | 1.36 ± 0.33 | 1.34 ± 0.31 | 1.41 ± 0.35 | 0.203 |
| Calcium (mmol/L) | 2.25 ± 0.26 | 2.24 ± 0.25 | 2.26 ± 0.27 | 0.578 |
| Phosphorus (mmol/L) | 1.59 ± 0.47 | 1.46 ± 0.45 | 1.66 ± 0.4 | 0.016 |
| PTH (pg/mL) | 289.83 ± 455.33 | 268.52 ± 347.73 | 334.74 ± 623.29 | 0.835 |
| ALP (U/L) | 81.31 ± 64.59 | 78.24 ± 45.12 | 87.80 ± 93.38 | 0.820 |
| Vitamin D (ng/mL) | 28.38 ± 12.32 | 27.94 ± 13.29 | 30.08 ± 11.09 | 0.209 |
| Klotho (pg/mL) | 421.18 ± 727.27 | 490.94 ± 787.67 | 264.94 ± 449.73 | 0.001 |
| FGF23 (pg/mL) | 3.86 ± 4.94 | 3.75 ± 3.92 | 3.96 ± 5.83 | 0.734 |
| HTA | 126 (88.7) | 89 (91.8) | 37 (82.2) | 0.095 |
| DM | 21 (14.8) | 16 (16.5) | 5 (11.1) | 0.400 |
| CVE | 117 (14.8) | 65 (46.4) | 52 (63.4) | 0.014 |
| Antihypertensive drugs | | | | |
| Beta blockers | 74 (52.1) | 50 (51.5) | 24 (53.3) | 0.843 |
| Ca antagonists | 60 (42.3) | 45 (46.4) | 15 (33.3) | 0.143 |
| ACEi | 151 (68.0) | 55 (67.1) | 96 (68.6) | 0.817 |
| Vitamin D therapy | 35 (24.6) | 24 (24.7) | 11 (24.4) | 0.969 |

*HD start* years at beginning with renal replacement therapy, *CRP* C-reactive protein, *PTH* parathyroid hormone, *ALP* alkaline phosphatase, *FGF23* fibroblast growth factor 23, *HTA* hypertension, *DM* diabetes mellitus, *CVE* cardiovascular events (coronary artery disease, stroke, heart failure, hypertensive heart disease, myocardiopathy, heart arrhythmia, peripheral artery disease), *ACEi* angiotensin-converting enzyme inhibitor), *Vitamin D therapy* alfacalcidol, calcitriol, paricalcitol

Bearing in mind the small distribution of the AA genotype in our population, we divided all patients into two groups, the A-alleles-carriers (represented by 31.7%) and the non-A-allele carriers. In a recent study of Zeng et al. in end-stage renal disease population distribution of A-allele carriers was 63.5% compared to 40% in healthy volunteers [21]. In a study of Shimoyama distribution of A-allele carriers in healthy subjects was 31.9% [14].

There was no difference in age between carriers and non-carriers. However, A-allele carriers were significantly longer on haemodialysis, and significantly younger have started renal replacement therapy, which supports the hypothesis that they had a faster progression of CKD compared to non-A-allele carriers. In accordance with our results, Korean study presented that A-allele carriers showed a higher probability of terminal stage in CKD and mortality than non-A-allele carriers [22].

Klotho gene variations lead to changes in the activity of the Klotho protein [23]; consequently, the polymorphic gene carriers synthesise a slightly structurally different target proteins, which have a different protein half-life and different affinity to the receptor binding and protein levels. In our study, A-allele carriers had significantly lower values of Klotho protein compared to non-A-allele carriers. Experimental animal studies suggest that the lack of a Klotho gene leads to degenerative changes in many organs, reduces life expectancy, therefore occurs in a syndrome similar to ageing, and thus the gene can be characterised as an anti-ageing gene [24]. Chronic kidney disease is considered as a natural model of accelerated ageing, as the loss of renal tissue results in decreased expression of Klotho protein, FGF23 binds to FGF receptors with reduced affinity, decreasing of phosphorus excretion. Hyperphosphatemia further triggers the cascade of adverse effects on some systems and organs

**Table 3** Comparison of echocardiology parameters between carriers and the non-carriers of A-allele

| | All | Alleles | | *p* value |
|---|---|---|---|---|
| | | Non-A (GG) | A (GA + AA) | |
| | | $\bar{X} \pm$ SD; *n* (%) | $\bar{X} \pm$ SD; *n* (%) | |
| EDD (mm) | 51.75 ± 6.78 | 51.87 ± 6.91 | 51.49 ± 6.60 | 0.754 |
| ESD (mm) | 32.51 ± 7.02 | 32.59 ± 7.07 | 32.33 ± 6.97 | 0.840 |
| IVS (mm) | 12.63 ± 2.21 | 12.68 ± 2.39 | 12.50 ± 1.81 | 0.698 |
| LVPW (mm) | 12.22 ± 1.98 | 12.31 ± 20.06 | 12.03 ± 1.79 | 0.569 |
| FS LV (%) | 37.51 ± 8.37 | 37.63 ± 8.06 | 37.48 ± 9.09 | 0.973 |
| EF LV (%) | 64.09 ± 9.34 | 63.73 ± 9.80 | 64.87 ± 8.31 | 0.890 |
| LA (mm) | 43.65 ± 6.91 | 43.73 ± 9.80 | 44.53 ± 6.94 | 0.273 |
| LVM (g) | 263.75 ± 82.28 | 258.07 ± 76.02 | 266.38 ± 85.287 | 0.609 |
| LVMi (g/m$^2$) | 152.34 ± 39.41 | 140.33 ± 38.34 | 149.46 ± 43.12 | 0.188 |
| RWT | 0.49 ± 0.09 | 0.47 ± 0.09 | 0.48 ± 0.10 | 0.734 |
| Left ventricular geometry | | | | |
| Normal LV | 7 (4.9) | 3 (3.1) | 4 (8.9) | 0.401 |
| Remodelling | 21 (14.8) | 13 (13.4) | 8 (17.8) | |
| Concentric LVH | 88 (62.0) | 63 (64.9) | 25 (55.6) | |
| Eccentric LVH | 26 (18.3) | 18 (18.6) | 8 (17.8) | |
| Left ventricular hypertrophy | | | | 0.079 |
| No | 28 (19.7) | 16 (16.5) | 12 (26.7) | |
| Yes | 114 (80.3) | 81 (83.5) | 33 (73.3) | |

*EDD* end-diastolic dimension, *ESD* end-systolic dimension, *IVS* interventricular septum, *LVPW* left ventricle posterior wall thickness, *FS* fraction shortening, *LVEF* left ventricle ejection fraction, *LA* left atrium, *LVM* left ventricular mass, *LVMi* left ventricular mass index, *RWT* relative wall thickness, *LV* left ventricular, *LVH* left ventricular hypertrophy

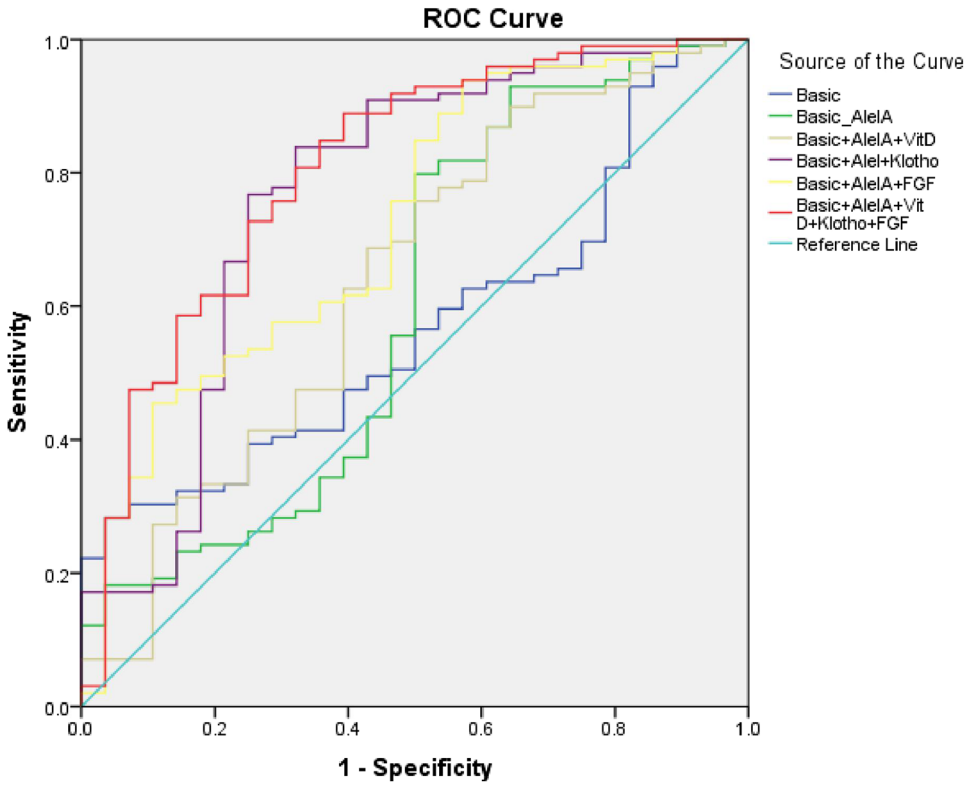


**Fig. 1** Receiver-operating characteristic (ROC) analysis of risk predictors for left ventricular hypertrophy in haemodialysis patients

**Table 4** Performance of predictive models (AUC, NRI, IDI)

| Model | Covariates | AUC | 95% CI | Co | *p* value* | NRI | 95% CI | *p* | IDI | 95% CI | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Heart hypertrophy | | | | | | | | | | | |
| 1 | Basic (Ca + P + AF + PTH) | 0.569 | 0.459–0.679 | – | – | | – | – | | – | – |
| 2 | Basic + allele A | 0.601 | 0.469–0.733 | 1 vs. 2 | 0.566 | 0.343 | − 0.068–0.753 | 0.102 | 0.025 | − 0.003–0.053 | 0.081 |
| 3 | Basic + allele A + VitD | 0.640 | 0.517–0.764 | 1 vs. 3 | 0.260 | 0.344 | − 0.071–0.759 | 0.104 | 0.027 | − 0.002–0.056 | 0.069 |
| 4 | Basic + allele A + Klotho | 0.766 | 0.652–0.880 | 1 vs. 4 | 0.005 | 0.605 | 0.208–1.002 | 0.003 | 0.143 | 0.053–0.233 | 0.002 |
| 5 | Basic + allele A + FGF23 | 0.724 | 0.615–0.833 | 1 vs. 5 | 0.008 | 0.548 | 0.141–0.954 | 0.008 | 0.118 | 0.059–0.177 | < 0.001 |
| 6 | Basic + allele A + VitD + Klotho + FGF23 | 0.806 | 0.708–0.903 | 1 vs. 6 | < 0.001 | 0.821 | 0.429–1.213 | < 0.001 | 0.220 | 0.112–0.328 | < 0.001 |

Full model (basic model + allele A + Vit D + Klotho + FGF23)

*AUC* area under the receiver operating characteristic curve, *NRI* net reclassification improvement, *IDI* integrated discrimination improvement

Models were established with basic biochemical parameters (calcium, phosphorus, alkaline phosphatase and PTH) and addition of allele A, vitamin D, Klotho protein and FGF23 constructed full mode

*De Long test

[25]. This genetic background of being A carrier may lead to a decrease of Klotho expression resulting in mineral–bone disorder and consequently kidney damage. A-allele carriers in our population, in addition to the reduced concentration of Klotho protein, had increased levels of phosphorus, suggesting they were at increased risk of adverse events on the heart and blood vessels. Vascular calcifications which occur on blood vessels in patients with CKD differ from patients without renal failure because calcium deposits precipitate in the intima of arteriosclerotic plaques while in patients with CKD, this precipitation occurs in tunica media [26, 27].

In the 1980s, echocardiographic studies identified adverse structural changes in the heart of haemodialysis patients, called uremic cardiomyopathy. Nowadays, new echocardiographic techniques have enabled the analysis of subtle subclinical changes in hearts geometry and function [28]. Left ventricular hypertrophy is the most frequent cardiovascular manifestation, found in approximately 60–80% of patients starting renal replacement therapy [29]. The most characteristic morphologic changes seen in the heart are the enlargement of the heart cavity and the progressive thickening of the left ventricle walls, which becomes less reversible the longer the patient stays on haemodialysis [30]. LVH is an adaptive remodelling process aimed at minimising the stresses applied to the ventricular wall [31]. Most of our respondents, 80.3% of them, had LVH, with the majority of concentric hypertrophy (77.2%). However, we have not shown statistically significant differences between the A-allele carriers and the non-carriers by echocardiographic characteristics. We should point out that LVH is an extremely multifactorial process and hypertension to be a leading cause. However, in our population group, there was no significant difference in the distribution of hypertension and diabetes mellitus among A-allele carriers and the non-carriers. Despite that, the cardiovascular events were significantly more prevalent among the carriers of A-allele, which suggests that they were at greater risk for cardiovascular mortality.

The literature review indicates that Klotho has a cardioprotective role due to its down-regulatory impact on the transient receptor TRPC6 channel (transient receptor potential cation channel). In stress conditions, over expression of the TRPC6 gene leads to the increased synthesis of TRPC6 channels and hypertrophy. Phosphatidylinositol 3 kinase, stimulates the exocytose of the TRPC6 channel. Soluble Klotho via insulin-like growth factor I blocks phosphatidylinositol 3 kinase and reduces the exocytose of the TRPC6 channel, thereby it prevents the development of left ventricular hypertrophy [9]. FGF23 has a direct effect, independent of Klotho, on the cardiomyocytes in aetiopathogenesis of LVH. FGF23 causes pathological hypertrophy of isolated cardiomyocytes via FGF receptor-dependent activation of the calcineurin–NFAT (nuclear factor of activated T cells) signalling pathway. Intramyocardial and intravenous injection of FGF23 in Klotho-deficient mice develops LVH, while blocking FGF receptors signalling can prevent LVH independently [29, 32, 33].

Haemodialysis patients suffer from the deficiency of vitamin D [34]. Literary data show that the lack of active form of vitamin D (calcitriol) affects the vascular smooth muscle cells via the cytosolic vitamin D receptor (VDR), leading to cardiomyocyte hypertrophy and hyperplasia. Cardiomyocyte-specific deletion of the vitamin D receptor gene results in LVH [35]. Vitamin D therapy has a cardioprotective effect through the modulation of the renin–angiotensin system due to direct suppression of the renin gene expression by calcitriol, regardless of the effect of vitamin D on calcium metabolism [36]. However, the effects of vitamin D on the outcomes of patients with CKD are controversial; some studies indicate that vitamin D excess contributes to

the risk of hypercalcaemia and vascular calcification which increased morbidity [37].

To determine the significance of Klotho gene variations and the new mineral–bone metabolism components for LVH in haemodialysis patients, we designed predictive models. The predictive capabilities of the various models, measured with AUC and IDI increased by adding more variables to the models. By separately adding Klotho protein and FGF23, we increased the discriminatory power of the basic model. By adding both together, along with vitamin D, we achieved the best predictor for LVH with net reclassification for 82%. In a recent study of Hu et al., similar results have been described identifying the joint effects of low Klotho and high phosphate on cardiac hypertrophy and fibrosis. By statistical modelling of all experimental data, Hu et al. conclude that Klotho and phosphate are independent promoters of heart remodelling and that FGF23 is a third contributor, but only if Klotho levels are decreased [5]. The better understanding of pathophysiological mechanisms, the interrelationship of standard and non-standard mineral–bone metabolism components as well as their correlation with cardiovascular morbidity and mortality will lead to additional knowledge and allow better stratification of risk factors. The study of Klotho polymorphisms contributes to the personalised management of the patient, which would affect the quality of life and the more prolonged survival of the patients in the chronic haemodialysis programme [38].

### Limitations of the study

This was a single-centre study and had a relatively small sample size which could lead to an overfitted predictive model. However, according to our knowledge, this is the first study among haemodialysis patients where the reclassification of patients with and without heart hypertrophy was determined. Another limitation of the study was the lack of external validation of the predictive model. For the development of the applicable predictive model, there is a need to conduct a more comprehensive multicentric research with a larger population, and subsequently, external validation. External validation brings with itself the possibility of generalisability. In this case, the generalisability may also differ by genotype distribution and country.

## Conclusion

The genetic background of A-allele carriers of the G-395A Klotho gene polymorphism increases the susceptibility patients to haemodialysis. A-allele carriers have decreased Klotho protein and increased phosphorus level; therefore, they are at higher risk for the development of cardiovascular complications. Our results confirmed the prognostic value of Vitamin D, FGF23 and Klotho protein. The addition of non-classical to classical mineral metabolism components improves prediction power to left heart hypertrophy. Thus, those parameters should be recorded to give supplementary information to the clinician regarding the prognosis of a patient as well as in the judgment of appropriate treatment.

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### Compliance with ethical standards

**Conflict of interest** The authors report no declarations of interest.

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