Original Article

Association Of Klotho Gene With The Renal Stone Disease- A Meta-Analysis

Neha Martin Honnalli[1], Chaitra.D[2], Divya N Pai[3*], Dr. Deepika Kamath M[4]

[1]Research Scholar, Department of Biochemistry, KS Hegde Medical Academy, Nitte-Deemed to be University.
[2]Tutor, Department of Anatomy, KS Hegde Medical Academy, Nitte (Deemed to be University), Mangalore
[3*]Tutor, Department of Anatomy, KS Hegde Medical Academy, Nitte (Deemed to be University), Mangalore
[4]Lecturer, University of Sharjah, College of Medicine, Dept. of Basic Medical Science, Sharjah, UAE

***Corresponding author:** Divya N Pai

[*]Tutor, Department of Anatomy, KS Hegde Medical Academy, Nitte (Deemed to be University), Mangalore
Email.id: divyas@nitte.edu.in
DOI: 10.47750/pnr.2022.13. S05.302

Abstract

Introduction: Nephrolithiasis is a multifactorial disease. Genetic polymorphisms have been proposed as risk factors for kidney stone incidence and recurrence. The Klotho gene is one of the important proteins involved in calcium homeostases such as para thyroid gland, kidney, and the epithelium of the choroid plexus in the brain.

Objectives: To assess the association of G395A Single Nucleotide polymorphisms (SNPs) of the klotho gene with renal stone disease.

Materials and methods: PubMed, and Google scholar were searched between November 2020 and April 2021 to identify eligible studies with the keywords G395A, klotho, polymorphism, and Nephrolithiasis or renal calculi. Total of three articles were included in the study. Random effect models as well as fixed effect models were used to find out the association of G395A SNP with kidney stone patients. Forest plot as well as funnel plot is plotted to assess the heterogeneity and publication bias respectively.

Results: The present study results were carried out using medcalc. Our pooled calculation found that GG genotype has more risk effect against kidney stone disease with OR of 2.383 with a 95% confidence interval of 1.700 to 3.340 , $p<0.001$. No publication bias was observed.

Conclusion: GG genotype of G395A SNP are significantly associated with the risk of kidney stone disease. However, the GA & AA genotype of Klotho gene G395A were not associated with the kidney stone disease.

Keywords: Klotho gene polymorphism, G395A, Kidney stone

INTRODUCTION

Renal stone disease is caused by over-availability of stone-forming ions like calcium oxalate and calcium phosphate, as well as supersaturated urine with high amounts of uric acid and cysteine. Over the last four decades, the frequency and prevalence of kidney stones has grown. Renal stones range in severity from asymptomatic to extremely painful, resulting in kidney failure. (2) A number of factors have been recognised as contributing to the rise in kidney stone disease. The most prevalent and high risk factors include a lack of water consumption, environmental changes, and genetic variants. Kidney stone disease is thought to be linked to the interactions of numerous genes, as well as lifestyle and environmental factors.

Although there is no specific gene evidence to be the primary cause of kidney stones(3) various genes such as VDR, CasR, KLOTHO, ASHG, SIRT1, CLDN14, TRPV5, VEGF, OPN,IL1R1,SLC2 etc have been verified to be related to nephrolithiasis. The present study is emphasize on exploring the role of klotho gene in causing the kidney stone disease.

Klotho gene and kidney stone

Klotho, a glucuronidase, entraps TRPV5 in the plasma membrane by hydrolyzing extracellular sugar residues on the channel. This keeps calcium channel function and membrane calcium permeability in the kidneys stable. According to a study (9,10), idiopathic renal calcium stone is linked to decreased bone mineral density. In the current meta-analysis, we have chosen a strong linked KLOTHO SNP G935A with bone mineral density (11).

Aim of the meta-analysis was to assess the association of G395A SNP with the urinary calcium oxalate stones formation.

MATERIALS AND METHODS

The present meta-analysis was carried out between November 2020 and April 2021 at the department of Biochemistry, KS Hegde Medical Academy, NITTE (Deemed to be university), Mangalore, Karnataka, India.

Publication search:

Suitable studies were identified by searching the database PubMed and Google Scholar for the publications on Klotho gene in renal calculi using the search index terms ''G395A, klotho, polymorphism and Nephrolithiasis or renal calculi''. The timeline we chose for the published articles were between the year 2010-2020. Articles being the original research work and written in English language were the primary criteria for the selection of articles.
Study design: Case- control study

Inclusion criteria

The Articles which examined the associations between klotho gene polymorphism and urinary calcium oxalate risk.
The original articles including human subjects, comprising extractable data on gene frequencies of Klotho gene polymorphisms.

Exclusion criteria

1)Patients with uric acid/cysteine and other stones
2) Review articles
3) Duplicated or overlapping population studies, studies with incomplete information, studies with different locus of Klotho gene were excluded from the study.
Ethical Approval: central Ethical approval was not taken as it is not required in meta-analysis

Data extraction

As per our eligibility criteria, relevant data were extracted from the all three studies in this meta-analysis. The information obtained were expressed as follows: The first author's name, the year of publication, country of origin, ethnicity, genotyping methods, subjects, numbers of case and controls, frequency of GG genotype of klotho polymorphisms in case and controls.

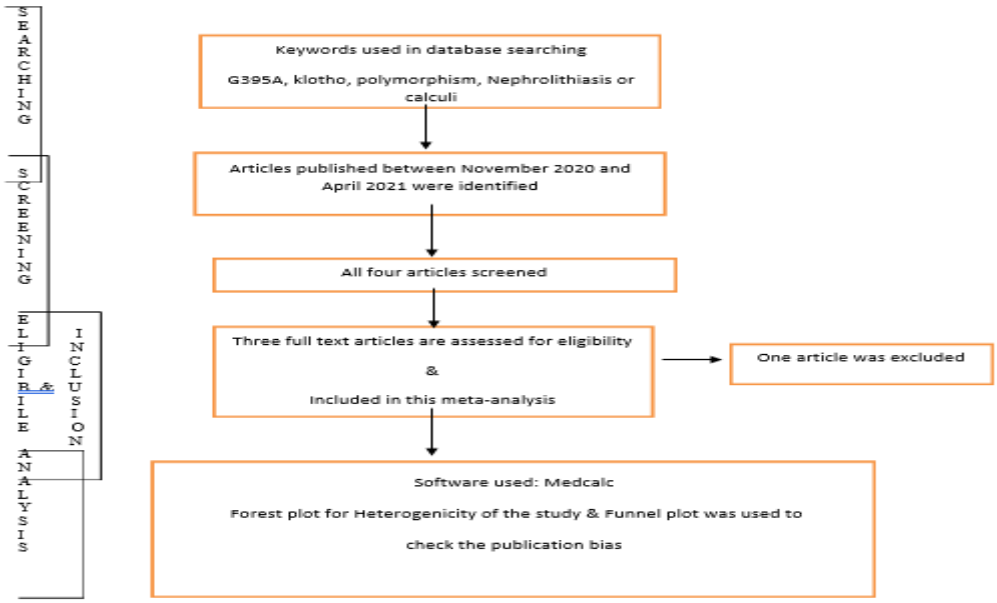
Statistical analysis:

The statistical analysis for present study was carried out using MedCalc for windows. A frequency of GG genotype in cases versus controls were estimated and their association in patients were noted. Pooled Odds ratios (OR) were calculated using Random effect model. Egger's test and Begg's test were carried out to assess the publication biases. Effect size of GG allele on risk of developing kidney stones for each study was expressed in terms of Odds Ratio with 95% confidence interval (CI). Heterogeneity assumption was checked by chi square based Q-test. A p value less than 0.05 was considered significant.

RESULT:

Study selection and characteristics
Fig 1. Represents the selection criteria for the eligible studies present in this study A total of 4 articles were identified with electronic database search.Data from three articles met our inclusion criteria. So we have included three articles for the association between klotho gene polymorphism and Nephrolithiasis[11,12,13].

Figure 1: Flow chart showing the process of selection of articles of meta-analysis



One article was excluded for different allele distribution of Klotho gene's polymorphism as we considered the G395A polymorphism in this meta-analysis. Of the three studies, 1 was from India [12] and 2 were from Turkey [11,13]. Present study

Table1: List of studies and their characteristics

Study	Year	Country Ethnicity	Genotyping Method	Number of cases	Number of control	Total subjects
Telci	2011	Turkey	PCR	108	51	159
Gurel	2015	Turkey	PCR	103	102	205
Lanka	2020	India	PCR	150	100	250
Total				361	253	614

recruited a total of 614 subjects. Among the 361cases, 225 were GG carriers. There were 103 GG carriers among 253 controls. Data from the 3 articles are summarized in the table below (Table no. 2).

Table 2: Meta analysis of GG genotype of Klotho gene polymorphism in Kidney stone patients.

First author., year of publication	GG carriers		Odds ratio	95% CI	z	P	Weight (%)	
	Cases	Controls					Fixed	Random
Telci et al., 2011	63/108	19/51	2.358	1.189 to 4.675			24.32	24.32
Gürel 2015	54/103	32/102	2.411	1.364 to 4.261			35.12	35.12
Lanka 2020	108/150	52/100	2.374	1.397 to 4.033			40.56	40.56
Total (fixed effects)	225/361	103/253	2.383	1.700 to 3.340	5.041	<0.001	100.00	100.00
Total (random effects)	225/361	103/253	2.383	1.700 to 3.339	5.041	<0.001	100.00	100.00

Table 3: Meta analysis of GA and AA genotype of Klotho gene polymorphism in Kidney stone patients.

First authors., year of publication	Intervention	Controls	Odds ratio	95% CI	z	P	Weight (%)	
							Fixed	Random
Telci et al., 2011	45/108	32/51	0.424	0.214 to 0.841			24.45	24.45
Gürel 2015	49/103	70/102	0.415	0.235 to 0.733			35.31	35.31
Lanka 2020	42/150	58/100	0.282	0.165 to 0.480			40.24	40.24
Total (fixed effects)	136/361	160/253	0.357	0.255 to 0.501	-5.964	<0.001	100.00	100.00
Total (random effects)	136/361	160/253	0.357	0.254 to 0.501	-5.966	<0.001	100.00	100.00

We have considered both random as well as fixed effect model to assess the association between the SNP and outcome. Both models showed same result conveying that GG carriers were more prone to kidney stone diseases.
The forest plot represents three studies (Figure 2). It is divided by the line of no effect into right and left side. All the studies are lying on right side of the line suggests that GG carriers were the risk factor for kidney stone diseases. Each study result has two representations. The square box represents the point estimate of the study results and it also represents the size of the study in terms of number of participants in the particular study. The study by Telci et al., shows smaller size representing a smaller study population compared to other two studies.

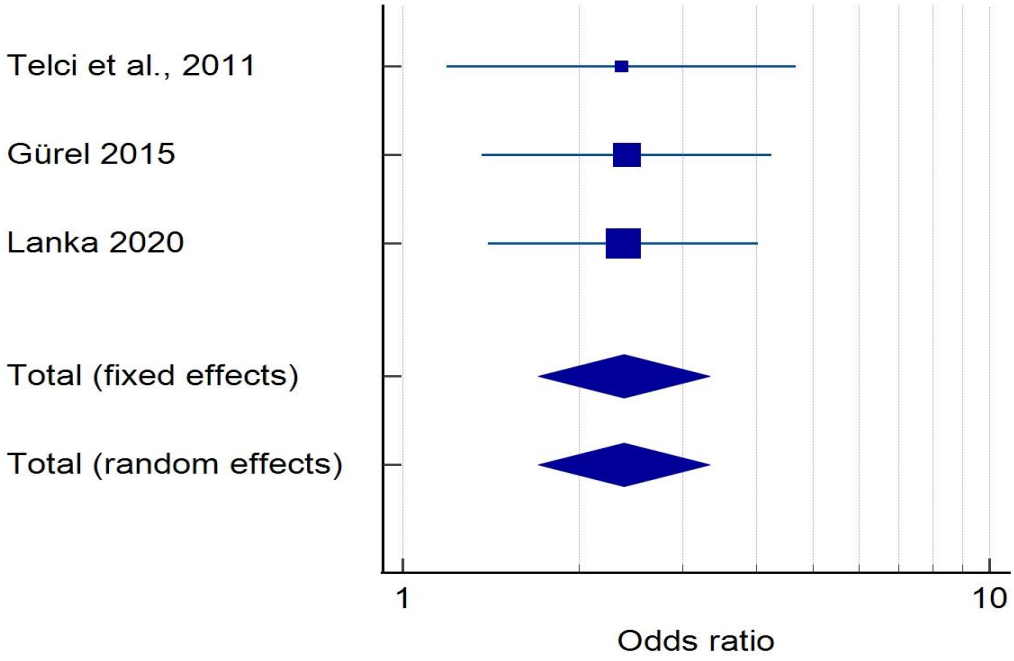


Figure 2: Forest plot depicting association of GG genotype of klotho gene and kidney stone formation

The horizontal line passing through the boxes depict the 95% CI of the study results. Longer the line, wider the confidence interval which intern convey that the study results are less reliable. The forest plot observation shows that there is no much difference in CI among all the studies (CI= 1.7 to 3.33) (Table 2).
As we have considered Odds ratio as the measure of comparison among the chosen studies, the value of line of no effect becomes '1'. None of the study passes through the line of no effect conveys that, the presence of GG carriers plays a significant role in kidney stone formation (p value <0.001). Figure 3 shows there is no association with alleles GA+AA and kidney stone disease.

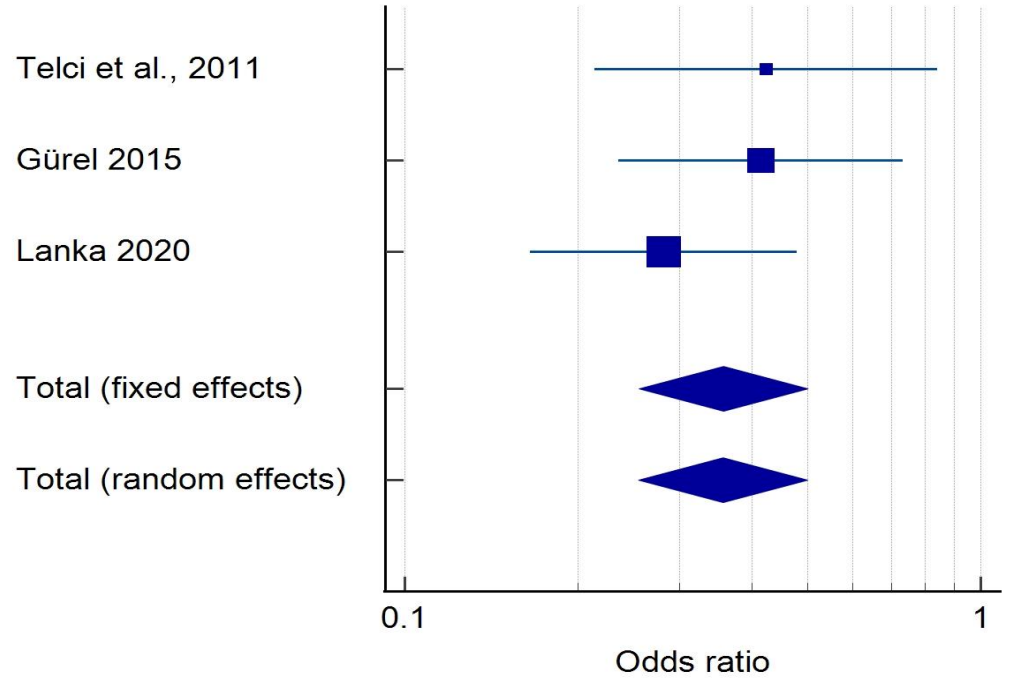


Figure 3: Forest plot depicting association of GA+AA genotype of klotho gene and kidney stone formation.

The diamond indicates the pooled odds ratios with its 95% confidence interval. The diamonds of both fixed and random effects are situated on the right side of the line of no effect, which shows that the GG Carriers may be a predictor biomarker for kidney stone disease. 5.041 was the 'z' value for both the random and fixed effect models and CI (1.700 to 3.339) p value being <0.001.

Table 4: Analysis of Publication bias

Egger's test	
Intercept	0.4640
95% CI	-5.3290 to 6.2570
Significance level	P = 0.4944
Begg's test	
Kendall's Tau	0.3333
Significance level	P = 0.6015

Egger's test and begg's funnel plot were applied for comparison to assess the publication bias of the literature, and no possible bias for this test was observed in both. Egger's intercept 0.4640, 95% CI -5.3290 to 6.2570, and P = 0.4944, Begg p=0.6015(Table 4)

TEST FOR HETEROGENEITY:

Studies did not show any heterogeneity. Q test for heterogeneity was 0.002715, the degree of freedom, DF=2 and I^2 = 0.00% marks zero percent inconsistency with CI = 0.00 to 0.00 and CI= 0.00 to 94.71. A 'p' value of 0.9986 represents almost no heterogeneity between the studies for GG carriers. The Q value was 1.2793 for GA+AA carriers with p value 0.531 showing minimum heterogeneity between the studies.

Fig 4 & 5 represents the distribution of the risk effect from individual studies in relation to their respective standard error in funnel plot. Funnel plot for publication bias was symmetrical, inverted funnel shaped, boundaries being straight lines and studies scattered within the funnel.

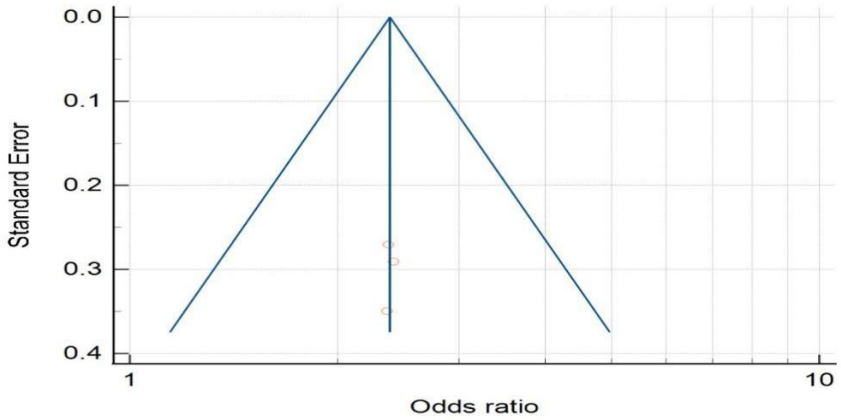


Figure 4: Funnel plot of Standard Error of comparison of GG carriers among cases and controls to determine publication bias.

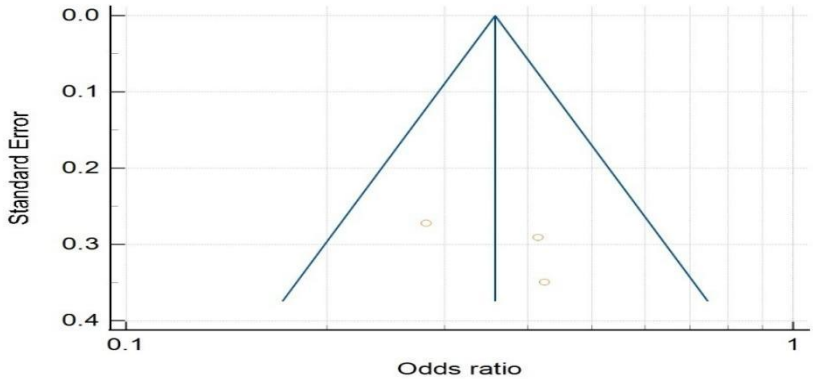


Figure 5: Funnel plot of Standard Error of comparison of GA+AA carriers among cases and controls to determine publication bias.

The graphical representation of Funnel plot did not provide evidence for selection and publication.

DISCUSSION

There is no meta-analysis exploring the association between G395A SNP and renal stones available. Present study seems to be the first meta-analysis on association of G395A in kidney stone diseases. This comprehensive meta-analysis suggests that GG genotypes are associated with kidney stone patients. Multiple comparisons showed that the carriers of GG genotype have high risk of developing kidney stones compared to GA and AA genotype carriers of the same gene.

The study by Telci et al., published in 2011 was a prospective study carried out on 108 calcium kidney stone patients and 51 controls. The study reported a significant association between GG genotypes and kidney stone disease[11]. For GG genotypes the serum calcium and urine phosphate excretion were positively correlates with risk of kidney stones. Klotho gene is believed to contribute in calcium- phosphorus homeostasis by enhancing the calcium channel receptors on the plasma membrane via β-glucuronidase activity, resulting in enhanced calcium absorption from the kidneys [14,15,16]. At first it was Gurel et al., in his study aimed at investigating the role of klotho gene polymorphism and its effects on β-glucuronidase activity and also compared the same in kidney stone patients and healthy controls [13]. They found that mean 24-h urinary calcium level was higher in patients compared to controls though, it was not statistically significant. This study gave some hints on the possibility of Klotho gene polymorphism (GG genotype) having the ability to alter the β-glucuronidase activity leading to decreased calcium absorption from the renal tubules. Accumulation of excess calcium due to alteration in β-glucuronidase activity is hypothesized to initiate the stone formation in Kidney among GG carriers. This is supported by the fact that the Klotho gene is dominantly expressed in Kidney tubules [17].

The study by P. Lanka et al., recruited 150 patients of renal stone disease and 100 healthy controls. The risk of kidney stone was significantly higher patients carrying G allele. This study concluded that G allele carriers were twice at risk of developing renal stone. serum calcium levels was high in cases with GG genotype as compared to those with GA and AA genotypes [12].

Currently, routine genetic testing of Klotho gene (GG carriers) is not in clinical practice. As G395A SNP determines risk and critical functions in development of kidney Stone Disease [11,12,13], it might open a new door into therapeutics as a specific genotype is associated with the bad outcome.

CONCLUSION:

The present study concluded that there is a significant association between G395A SNP in cases and controls. Patients with GG genotype were found to be risk factor for kidney stone formation.

CONFLICT OF INTEREST: No

REFERENCES:

1. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. *Nature reviews Disease primers*. 2016;2(1):1-23.
2. Curhan GC. Epidemiology of stone disease. *Urologic Clinics of North America*. 2007;34(3):287-93.
3. Alelign T, Petros B. Kidney stone disease: an update on current concepts. *Advances in urology*. 2018;2018.
4. Kuro-o M. Klotho. Pflügers Archiv-European J*ournal of Physiology*. 2010;459(2):333-43.
5. Mengel-From J, Soerensen M, Nygaard M, McGue M, Christensen K, Christiansen L. Genetic variants in KLOTHO associate with cognitive function in the oldest old group. Journals of Gerontology Series A: *Biomedical Sciences and Medical Sciences*. 2016;71(9):1151-9.
6. Ding HY, Ma HX. Significant roles of anti-aging protein klotho and fibroblast growth factor23 in cardiovascular disease. *Journal of geriatric cardiology*: JGC. 2015;12(4):439.
7. Sopjani M, Dermaku-Sopjani M. Klotho-dependent cellular transport regulation. *Vitamins & Hormones*. 2016;101:59-84..
8. Agarwal R. Chronic kidney disease is associated with oxidative stress independent of hypertension. *Clinical Nephrology*. 2004;61(6):377-83.
9. Olejnik A, Franczak A, Krzywonos-Zawadzka A, Kałużna-Oleksy M, Bil-Lula I. The biological role of klotho protein in the development of cardiovascular diseases. *BioMed research international*. 2018;2018.
10. Chang Q, Hoefs S, Van Der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG. The ß-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science*. 2005;310(5747):490-3.
11. Xu Y, Sun Z. Molecular basis of Klotho: from gene to function in aging. *Endocrine reviews*. 2015;36(2):174-93.
12. Boonla C. Oxidative Stress in Urolithiasis. Reactive Oxygen Species (ROS) in Living Cells. 2018:129-59.
13. Telci D, Dogan AU, Ozbek E, Polat EC, Simsek A, Cakir SS,et al. KLOTHO gene polymorphism of G395A is associated with kidney stones. *American journal of nephrology*. 2011;33(4):337-43.
14. Lanka P, Devana SK, Singh SK, Sapehia D, Kaur J. Klotho gene polymorphism in renal stone formers from Northwestern India. *Urolithiasis*. 2021;49(3):195-9.
15. Gürel A, Üre İ, Temel HE, Çilingir O, Uslu S, Celayir MF, Aslan S, Başeskioğlu AB. The impact of klotho gene polymorphisms on urinary tract stone disease. *World journal of urology*. 2016;34(7):1045-50.
16. Nabeshima YI. The discovery of α-Klotho and FGF23 unveiled new insight into calcium and phosphate homeostasis. *Cellular and molecular life sciences*. 2008;65(20):3218-30.
17. Civitelli R, Ziambaras K. Calcium and phosphate homeostasis: concerted interplay of new regulators. *J Endocrinol Invest*. 2011;34(7 Suppl):3-7.
18. Chang Q, Hoefs S, Van Der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG. The b-Glucuronidase Klotho Hydrolyzes and Activates the TRPV5 Channel.
19. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *nature*. 1997;390(6655):45-51.