Klotho: renal and extra-renal effects

Juan F. Navarro-González, MD, PhD, FASN
Nephrology Service and Research Division
University Hospital Nuestra Señora de Candelaria
Santa Cruz de Tenerife. Spain
Klotho: the beginning.

Mutation of the mouse *klotho* gene leads to a syndrome resembling ageing.

*nature* 1997, Nov 6;390:45-51.

Kuro-o M, et al.

- Short lifespan (Av. 60 days)
- Growth retardation
- Skin atrophy
- Arteriosclerosis
- Osteoporosis
- Atrophy of genital organs and thymus
- Ectopic calcification
- Hypokinesis, gait abnormality
- Dysfunction of pituitary grand (growth-hormone deficiency)
- Pulmonary emphysema
The 2 K connection

Kidney <-- Phosphorus --> Klotho
The discovery of phosphorus

Joseph Wright

Henning Brand, 1669

“The discovery of phosphorus”
Joseph Wright
ALCHEMY, THE KIDNEY AND PHOSPHORUS

• Urine is boiled to obtain a thick syrup.

• Heating until it distils a reddish oil.

• Cooling the remainder material: a black spongy top and a bottom saline part.

• Discard the salt and mix the reddish oil with the black part.

• Heating the mixture vigorously for nearly a day.

• White smoke first and then an oily fluid.

• Finally you obtain a waxy white material that emits light in the darkness.

Phosphorus → “that produces light”
Phosphorus balance in normal physiology

The kidney is the main regulator of human phosphate homeostasis.
Renal tubular phosphate reabsorption occurs through two families of sodium-phosphate co-transporters: NaPi-II and PIT-2.

70% of P reabsorption
Classical view of phosphate regulation
Parathyroid hormone and Vitamin D

Normal S Pi

↑ S Pi   ↓ S Pi

↑ S PTH  ↓ S PTH

↑ U Pi excretion  ↓ U Pi excretion

1α-Hydroxylase  1α-Hydroxylase

↓ 1α,25(OH)₂D and intestinal Pi absorption  ↑ 1α,25(OH)₂D and intestinal Pi absorption

Normal S Pi
Classical view of phosphate regulation
Parathyroid hormone and Vitamin D

- Tumor induced osteomalacia
- X-linked hypoP rickets
- Autosomal-dominant hypoP rickets

Variations in renal P reabsorption
Without changes in vitamin D and PTH

Altered P, normal PTH & Vit. D

Phosphate regulation needed a specific hormone
Fibroblast growth factor 23
Implant of cells genetically modified to produce FGF23 resulted in:

- Reduction of serum P
- Increase in urinary P excretion
Fibroblast Growth Factor-23 Relationship to Dietary Phosphate and Renal Phosphate Handling in Healthy Young Men

Serge L. Ferrari, Jean-Philippe Bonjour, and René Rizzoli

The Journal of Clinical Endocrinology & Metabolism 90(3):1519–1524
Copyright © 2005 by The Endocrine Society
doi: 10.1210/jc.2004-1030
How FGF-23 works?
How FGF-23 works?

Reduction of renal tubular P reabsorption
Increased renal phosphate excretion

\[
\begin{align*}
\text{NaPT2a} & : 3\text{Na}^+ \cdot \text{HPO}_4^{2-} \\
\text{NaPT2c} & : 2\text{Na}^+ \cdot \text{HPO}_4^{2-}
\end{align*}
\]

Tubular lumen
Renal tubular cell

FGF-23
FGF23 needs a co-receptor

Intracellular pathways
Klotho converts canonical FGF receptor into a specific receptor for FGF23

Itaru Urakawa¹, Yuji Yamazaki¹, Takashi Shimada¹, Kousuke Iijima¹, Hisashi Hasegawa¹, Katsuya Okawa¹, Toshiro Fujita³, Seiji Fukumoto² & Takeyoshi Yamashita¹

NPT2a
3Na⁺ HPO₄²⁻

NPT2c
2Na⁺ HPO₄²⁻

Renal tubular cell

FGF-23
Klotho

FGFR
Klotho

Klotho gene (13q12)
Renal actions of Klotho:

- Phosphaturic effect (FGF23-dependent).

- Phosphaturic effect (independent of FGF23).
Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule

Plasma levels

Fractional excretion

Renal expression of NaPi-2a

Chang et al. FASEB J 2010
Renal actions of Klotho:

- Phosphaturic effect (FGF23-dependent).

- Phosphaturic effect (independent of FGF23).

- Regulation of ion channels (calcium and potassium).
The β-glucuronidase klotho exclusively activates the epithelial Ca\textsuperscript{2+} channels TRPV5 and TRPV6

Calbindin and Transient Receptor Potential Vanilloid 5 & 6

Chang et al. Science 2005

Lu et al. NDT 2008
Regulation of Renal Outer Medullary Potassium Channel and Renal $K^+$ Excretion by Klotho

Seung-Kuy Cha, Ming-Chang Hu, Hiroshi Kurosu, Makoto Kuro-o, Orson Moe, and Chou-Long Huang

Mol Pharmacol 76:38–46, 2009
Renal actions of Klotho:

- Phosphaturic effect (FGF23-dependent).

- Phosphaturic effect (independent of FGF23).

- Regulation of ion channels (calcium and potassium).

- Regulation of Vitamin D.
Mediation of Unusually High Concentrations of 1,25-Dihydroxyvitamin D in Homozygous klotho Mutant Mice by Increased Expression of Renal 1α-Hydroxylase Gene

TORU YOSHIDA, TOSHIHIKO FUJIMORI, AND YO-ICHI NABESHIMA

Endocrinology 143(2):683–689
Copyright © 2002 by The Endocrine Society

Renal expression of 1α-hydroxylase

Serum levels

[Graph showing relative serum levels of 1,25(OH)2D at 2 wk, 5 wk, 7 wk, and 9 wk for +/+ and kl/kl genotypes, with significant differences indicated by stars.]

[Image of gel showing relative signal intensity for samples 1 and 2, with kl/kl showing higher intensity than +/+.]

[Arrow pointing to 18S RNA loading control on gel.]
Renal actions of Klotho:

- Phosphaturic effect (FGF23-dependent).
- Phosphaturic effect (independent of FGF23).
- Regulation of ion channels (calcium and potassium).
- Regulation of Vitamin D.
- Renoprotective properties.
Renoprotective effects of Klotho:

- Inhibition of cell senescence.
- Inhibition of cell apoptosis.
- Antioxidation.
- Inhibition of fibrosis.
- Activation of kidney regeneration.
- Preservation of stem cells.
- Protection of endothelial function.
- Restoration of renal angiogenesis.

Hu et al. Nat Rev Nephrol 2012
CKD is a state of Klotho deficiency
Klotho beyond the kidney

Cardiovascular dysfunction

Klotho deficiency

Secondary hyperparathyroidism

Klotho

Vitamin D

CKD

Donate-Correa et al. Cytokine Growth Factor Rev 2012
Parathyroid gland
Parathyroid gland in CKD

Klotho expression

Secondary hyperparathyroidism
Klotho Deficiency Causes Vascular Calcification in Chronic Kidney Disease

Hu et al. JASN 2011
What about the expression of the Klotho gene in the human vascular wall?
Expression of FGF23/KLOTHO system in human vascular tissue

Fig. 1. RT-PCR products of the four FGFRs mRNA tested and KLOTHO (KL) mRNA showing expression of FGFR1, 2, 3 and KLOTHO in human aorta. M, 100-bp marker. Control, RT-PCR without reverse transcriptase.
A Decreased Level of Serum Soluble Klotho Is an Independent Biomarker Associated with Arterial Stiffness in Patients with Chronic Kidney Disease

Kitagawa et al. PlosOne 2013
CKD is a state of Klotho deficiency

Moreno et al. JASN 2011
Figure 1 | Potential mechanisms of uremic toxin-induced silencing of the Klotho gene in chronic kidney disease involve DNA methyltransferase-mediated regulation.
Is there any relationship between Klotho and atherosclerotic disease?
Atherosclerosis: an inflammatory disease
Klotho gene polymorphism and ischemic heart disease

**KL-VS polymorphism**

- P = 0.002

<table>
<thead>
<tr>
<th>Allele</th>
<th>Occult CAD</th>
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<tbody>
<tr>
<td>+/+</td>
<td>15%</td>
</tr>
<tr>
<td>+/VS</td>
<td>25%</td>
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<tr>
<td>VS/VS</td>
<td>40%</td>
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**G-395A polymorphism**

<table>
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<tr>
<th>Allele</th>
<th>Occult CAD</th>
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<tbody>
<tr>
<td>-395A</td>
<td>30%</td>
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<tr>
<td>-395G</td>
<td>13%</td>
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Choi et al. Int Heart J 2009

p = 0.03
Plasma Klotho and Cardiovascular Disease in Adults

% of subjects with Cardiovascular Disease

<table>
<thead>
<tr>
<th>Tertil of plasma klotho</th>
<th>% of subjects</th>
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</thead>
<tbody>
<tr>
<td>T1 (N=340)</td>
<td>32.7*</td>
</tr>
<tr>
<td>T2 (N=342)</td>
<td>23.1</td>
</tr>
<tr>
<td>T3 (N=341)</td>
<td>20.2</td>
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* p<0.001
CVD: coronary heart disease, heart failure, stroke, peripheral vascular disease
371 patients underwent coronary angiography

Serum samples

468 patients

97 patients under elective cardiac surgery

Thoracic aorta samples

Klotho and Coronary Artery Disease

Navarro-González et al. Heart 2014
Serum Klotho and coronary heart disease

![Bar chart showing serum Klotho levels in patients with and without coronary heart disease.](Navarro-Gonzalez et al. Heart 2014)
Klotho expression and coronary heart disease

No (N=27)  Yes (N=50)

mRNA (u.a.)

p<0.01

Navarro-González et al. Heart 2014
Serum Klotho and coronary heart disease

LOGISTIC REGRESSION
Dependent variable: Presence / Absence significant CAD

MODEL - Age, sex tobacco, Hypertension, diabetes, dyslipidemia, BP, BMI, Uric acid, Ca, P, CRP, FGF23, Klotho

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<tr>
<th></th>
<th>OR</th>
<th>IC 95%</th>
<th>P</th>
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<tbody>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.01 – 1.07</td>
<td>&lt;0.05</td>
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<tr>
<td>Hypertension</td>
<td>2.50</td>
<td>1.01 – 6.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.83</td>
<td>1.18 – 6.81</td>
<td>&lt;0.05</td>
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<tr>
<td>Tobacco</td>
<td>3.47</td>
<td>1.52 – 7.90</td>
<td>&lt;0.01</td>
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<tr>
<td>CRP</td>
<td>11.29</td>
<td>4.41 – 28.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Klotho</td>
<td>0.991</td>
<td>0.90 – 0.99</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Navarro-González et al. Heart 2014
Klotho Functions

- Energy metabolism
- Anti-inflammatory
- Antioxidation
- Anti-aging
- Anti-renin-angiotensin system
- Wnt signal transduction
- Glucose metabolism
- Anti-apoptosis
Klotho: An Elixir of Youth for the Vasculature?

Rukshana Shroff* and Catherine M. Shanahan†


Klotho

A Master Regulator of Cardiovascular Disease?

Sharon M. Moe, MD

Circulation. 2012;125:2181-2183
Ageing/DM/HTN
Inflammation

↓Klotho

CKD (Klotho deficiency state)
(chronic stress factors: TNF-α, disordered mineral levels/uremia)

PTH/vitD
↑Klotho

↑FGF-23
FGF Resistance

+Klotho

Phosphaturia
LVH

↓Longevity

Ageing Syndrome
(Osteoporosis / arteriosclerosis, ectopic calcifications / skin atrophy)

↑ROS
↓NO
↑Inflammation

Endothelial dysfunction

Vascular calcification

Atherosclerosis

Reno-Cardio-Vascular Disease
“Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning”

Sir Winston Churchill