Mechanisms of CKD-MBD: New insights in the pathogenesis

Jorge Cannata-Andia
University of Oviedo
Oviedo, Spain
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

- Role of Classic and New Players in the Pathogenesis of Secondary Hyperparathyroidism and CKD-MB
  - Role of
    - * Calcium (Calcimimetics)
    - * Vitamin D Receptor Activators (VDRAs)
    - * Phosphorus and FGF 23
    - * Genomic & Molecular Changes in the Severe and Refractory Secondary Hyperparathyroidism

- The Links Between the Bone and Vascular Axis in CKD-MBD.
  - Role of Phosphate in the Pathogenesis of Vascular Mineralization and Bone Demineralization.
  - Possible Self-defensive Mechanisms Triggered by the Vascular System
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

1943: Renal Osteodystrophy (RO) (Liu et al, Medicine)
- Secondary Hyperparathyroidism
- Osteomalacia
- Osteosclerosis
- Osteoporosis

30 Following Years: Academic Concept With No Chance to be Applied in the Daily Clinical Management of CKD Patients


In 2006 a New Term was Proposed with a Broader Scope
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

Renal Osteodystrophy

Secondary Hyperparathyroidism
the Vessels & Bone
Play Important Role

CKD-MBD
Parathyroid Regulation in Chronic Kidney Disease

- Aluminium
- Estrógenos
- Magnesio
- Acidosis
- Otros……

PTH

FGF23/Klotho

Calcium

Calcitriol

Phosphorus

25(OH)D

Cannata –Andía JBy Rodríguez M.. Nefrología Clínica . Ed L Hernando, 2008,
Parathyroid Regulation in Chronic Kidney Disease

- CaSR Discover and Cloned in 1993
- G Protein-Coupled Receptor (GPCR)
- Cell Surface Receptor
- Able to Recognize and Respond to Extracellular Calcium and Others: Al, La, Sr, Ga, .......

Calcium

Calcitriol

Phosphorus

FGF23/Klotho

Calcium Sensing Receptor (CaSR)

Tissue Distribution:
- Parathyroid and C cells
- Renal proximal tubule
- Nephron segments
- Gastrointestinal tract
- Osteoblast/Osteoclast
- Monocytes/macrophages
- Nervous system
- Bone marrow
- Cardiovascular
Calcium Sensing Receptor (CaSR)

Signaling Pathways Activated by the CaSR

- Phospholipase C (Inositol triphosphate, Ca\(^{2+}\))
- Phospholipase A2 (Arachidonic acid)
- Phospholipase D (Phosphatidic acid) MAP Kinase
- Inhibition of Adenylate Cyclase

How Calcium Influence Parathyroid Hormone Synthesis ?

Low Calcium

Increases the Stability of PTH mRNA

The Stability of PTHmRNA may vary from 5 minutes to 3 hours

Silver et al, 2000-2002
In CKD There is a Reduction of Expression of CaSR (40-60%)

Reduction in Capacity of the Parathyroid Gland to Sense Ca
Changes in the PTH Response to Calcium with the Progression of Secondary Hyperparathyroidism

¿ Is the Decrease of Sensitivity of the Parathyroid Glands to Calcium “Clinically Relevant”? 

Increments in “Non Suppressible” PTH Secretion Due to Gland Growth
Progression of CKD-MBD severely affect the response of the parathyroid glands to calcium.

Serum calcium may influence outcomes in dialysis patients.

**COSMOS Study:** 4600 patients / 20 Countries

**Parathyroid Gland Response to Calcium Changes in CKD 5**

**Serum Calcium (mg/dL)**

95% IC PTH (pg/ml)

COSMOS: Una Fotografía del Escenario Europeo en CKD-MBD

- Multi-centre, Open Cohort and Observational Study.
- **Prospective**: 3-year of follow-up.
- European Focused (20 countries)
- Size of the Sample: 4,500 HD Patients from 227 Centres spread geographically (medium-large hospitals and satellite units)
- Sites and Patients (±20 per centre) Randomly selected

![Map showing Oviedo](image_url)
Parathyroid Gland Response to Calcium Changes in CKD 5

COSMOS Study: 4600 patients / 21 Countries

Calcimimetics Can Improve the Poor Response of the Parathyroid Glands to Calcium Increasing the Sensivity of CaSR to Calcium

JL Fernández et al, ERA-EDTA, 2007
**Calcimimetics**

Reduce PTH Synthesis

**Calcimimetics**

Decrease Cell Proliferation

Long-term treatment with cinacalcet and conventional therapy reduces parathyroid hyperplasia in severe secondary hyperparathyroidism

Mario Meola¹, Ilaria Petrucci² and Giuliano Barsotti²

Nephrol Dial Transplant (2009) 1–8

¹S. Anna School of Advanced Studies, Nephrology and Dialysis Unit, Department of Internal Medicine, University of Pisa, Italy and
²Nephrology and Dialysis Unit, Department of Internal Medicine, University of Pisa, Italy

**Calcimimetics**

Upregulate CaSR and VDR – Interaction and Cooperation –

Consequences of the Action of Calcimimetics

Direct upregulation of parathyroid calcium-sensing receptor and vitamin D receptor by calcimimetics in uremic rats

Francisco J. Mendoza,¹ Ignacio Lopez,¹ Rocío Canalejo,² Yolanda Almudén,² David Martín,³ Escolastico Aguilera-Tejero,¹ and Mariano Rodríguez²

¹Departamento Medicina y Cirugía Animal, Universidad de Córdoba. ²Unidad de Investigación, Servicio de Nefrología, Hospital Universitario Reina Sofía, Córdoba, Spain, and ³Department of Metabolic Disorders, Amgen, Incorporated, Thousand Oaks, California
Effect of Calcimimetics

Improvement in the Parathyroid Response

“Set Point” Shift to the Left

Cinacalcet Reduces the Set Point of the PTH-Calcium Curve

Casimiro Valle, Mariano Rodriguez, Rafael Santamaría, Yolanda Almaden, Maria E. Rodríguez, Sagrario Cañadillas, Alejandro Martín-Malo, and Pedro Aljama

Nephrology Service and Research Unit, Hospital Universitario Reina Sofía, Córdoba, Spain
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Effects of VDR Activation

Rapid Non-Genomic Response

Slow Genomic Response

Cell membrane

Membrane Receptor

2 Messenger

Transcription Factor
Co-activators and co-repressors

Nuclear membrane

VDRE

Protein

ARNm
Effects of VDR Activation

Proteins Regulated by VDR Activation

- **Up Regulated**
  - Osteopontin
  - Osteocalcin
  - RANK-L
  - VDR
  - 24-hydroxilase
  - Calbindin
  - TRPV5-6
  - IL-10, IL-4
  - Insulin
  - p-21, p-27

- **Down Regulated**
  - Cbf1
  - BMP-2
  - PTH
  - Collagen
  - 1α hydroxilase
  - Renin
  - IFN-γ
  - IL-1β, IL-2, -6, -12
  - Cyclin E
  - Gen C-myc

**Multiple Proteins are Regulated by VDR Activation**

Slow Genomic Response

- 1,25-(OH)₂D
  - Cell membrane
  - VDR
  - CITOPLASMA
  - Nuclear membrane
  - NÚCLEUS
  - Transcription
  - VDRE
  - ARNm
  - Protein

- **Up Regulated**
  - 1,25-(OH)₂D
  - Renin
  - IFN-γ
  - IL-Iβ, IL-2, -6, -12
  - Cyclin E
  - Gen C-myc

- **Down Regulated**
  - Osteopontin
  - Osteocalcin
  - RANK-L
  - VDR
  - 24-hydroxilase
  - Calbindin
  - TRPV5-6
  - IL-10, IL-4
  - Insulin
  - p-21, p-27
Effects of VDR Activation

- **Bone and Mineral**
  - Intestine
  - Parathyroid Glands
  - Bone
  - Kidney

- **Cardiovascular System**
  - Myocardial Structure
  - Myocardial Function
  - Vascular System
    - Arterial Pressure
    - Vascular Function

- **Immune System**
  - Infections
  - Inflammatory Response
  - Skin
  - Muscular System
  - Antiproliferative effect
    - Cancer
  - Renoprotection

**Survival**
Oral active vitamin D is associated with improved survival in hemodialysis patients

Manuel Naves-Díaz, Daniel Álvarez-Hernández, Jutta Passlick-Deetjen, Adrian Guinsburg, Cristina Marelli, Diego Rodriguez Puyol and Jorge B. Cannata Andía

The Better Results Were Obtained With Dose < 1 mcg/day
Main Factors Influencing the VDR Response

- Adequate VDR Expression
- Optimal Concentration of VDR Activator
VDR Expression in CKD

No Inhibition of PTH Gene Transcription

Increase mRNA PTH Synthesis

Secretion

Calcitriol Deficit

Decreased Expression of VDR

Transcriptional
Simultaneous changes in the calcium-sensing receptor and the vitamin D receptor under the influence of calcium and calcitriol

Natalia Carrillo-López, Daniel Álvarez-Hernández, Ignacio González-Suárez, Pablo Román-García, Jose M. Valdivielso, José Luis Fernández-Martín and Jorge B. Cannata-Andia

Original Article

doi: 10.1093/ndt/gfn238
Advance Access publication 13 June 2008

VDR Expression in CKD

Normalize Serum Calcitriol Levels

Administration of Calcitriol

Inhibition of PTH Gene Transcription

Cooperation Between VDR & CaSR

Reduce PTH Synthesis

Parathyroid Glands Culture

mRNA VDR/18s (%)

Expression of VDR

mRNA CaR/18s (%)

Expression of CaSR

Control

Calcitriol $10^{-8}$M

$218.7 \pm 42.6$

$212.8 \pm 39.9$

$100.0$

$100.0$

$50.0$

$50.0$

$100.0$

$100.0$

$200.0$

$200.0$

$300.0$

$300.0$

$0.0$

$0.0$

$50.0$

$50.0$

$150.0$

$150.0$

$200.0$

$200.0$

$250.0$

$250.0$

$300.0$

$300.0$
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

Main Factors Influencing the VDR Response

- Adequate Concentration of VDR
- Optimal Concentration of VDR Activator
Why and When the Calcitriol Reduction Start in CKD?

A Levin et al. KI 2007
Serum Calcitriol Levels in Early CKD

Why and When the Calcitriol Reduction Start in CKD?

Possible Effect of Early Increase of FGF23 in CKD
PTH
Urinary P
Calcitriol
Phosphorus
Calcium
FGF 23
1 alpha Hydroxylase
24,25 alpha Hydroxylase
Calcium
Serum Calcitriol Levels in Early CKD

FGF 23 Through its Capacity to Reduce Calcitriol Could Be an Important Indirect Factor “Early” Involved in the Pathogenesis of Secondary Hyperparathyroidism
Indirect Factor

The parathyroid is a target organ for FGF23 in rats

Calcitriol

Phosphorus

Calcium

FGF 23

Calcium

Calcitriol

(+)
FGF23 Do Not Decrease PTH in Advanced SHPT

In Advanced CKD
There is a Resistance to the Effect of FGF 23

<table>
<thead>
<tr>
<th>Grade 4</th>
<th>Grade 3</th>
<th>Grade 2</th>
<th>Grade 1</th>
</tr>
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<tbody>
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<td>FGFR1 score</td>
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<td>P = 0.02</td>
<td>P = NS</td>
<td>P = NS</td>
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<tr>
<td>Klotho score</td>
<td>Klotho score</td>
<td>Klotho score</td>
<td>Klotho score</td>
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<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.055</td>
<td>P = 0.013</td>
</tr>
</tbody>
</table>

\[ P < 0.001 \]

Komaba, Fukagawa: Kidney Int 2010;77 232-238
FGF23 Do Not Decrease PTH in Advanced SHPT

In Advanced CKD
There is a
Resistance to the
Effect of FGF 23
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

Last Decade Phosphorus Have Increased its Importance in PTH Regulation and CKD-MBD

PTH

Calcitriol

Calcium

Phosphorus
Negative Effects of Phosphorus

Outcomes

- Lack of Response to Vit D Metabolites
- Vascular Calcifications
- Increased Mortality
- Decrease in Bone Mass*
- Decrease in Cortical Bone*
- Decrease in Bone Strength*
- Pro-Inflammatory
- Pro-Ageing Element

2012: Combination/Association of Effects of Phosphorus & FGF 23

Makoto Kuro-O. Avances en Metabolismo Óseo y Mineral, Edited by JB Cannata-Andía y col, 2010
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

Proximal Kidney Tubule Cells

- Na-Pi 2a
- Na-Pi 2c
- PTH
- Klotho
- FGF-R
- Calcitriol
- 25(OH)D
- FGF 23
- Phosphorus
- Calcium

- Na⁺/K⁺ ATPase
- FGF-23
PTH

Urinary Phosphate

Calcitriol

25(OH)D

Calcium

FGF 23

Calcium

(+)
¿Cuáles son las sinergias entre fósforo, FGF 23, Klotho y vitamina D?
Effect of Phosphorus on the Parathyroid Glands in CKD

- High Phosphorus Increases PTH Synthesis
- High Phosphorus Increases the Stability of PTH mRNA

Silver et al, 2000-2002
Effect of Phosphorus in Cell Proliferation and CaSR

Decrease CaSR Expression

THE HIGHER THE PHOSPHORUS CONCENTRATION

• THE GREATER THE DIRECT STIMULATION OF PTH SYNTHESIS
• THE GREATER THE CELL PROLIFERATION (Gland Growth)
• AND THE LOWER THE CaSR EXPRESSION

Increase Cell Proliferation

¿ Is the Effect of High Serum Phosphorus on the Parathyroid Glands “Clinically Relevant”?
High Serum Phosphorus and PTH in CKD 5 Patients

JL Fernández et al., ERA-EDTA, 2007
High Serum Phosphorus and PTH in CKD 5 Patients

Serum Phosphorus is the Strongest Factor Associated to PTH Levels in CKD 5

Serum Phosphorus May Influence Outcomes in Dialysis Patients

JL Fernández et al., ERA-EDTA, 2007
Progression of Secondary Hyperparathyroidism

Gland size

Polyclonal:
- VDR
- CaR

Monoclonal:
- VDR
- CaR
- Klotho/FGFR1

Normal
SECRETORY CELLS
DIFFUSE
NODULAR
LATE-SINGLE NODULARITY

Progression of Secondary Hyperparathyroidism

Adapted from Tominaga Y et al. Curr Opin Nephrol Hypertens 1996;5:336–41
Progression of Secondary Hyperparathyroidism

- Severe Molecular Changes
- Genomic Alterations

Adapted from Tominaga Y et al. Curr Opin Nephrol Hypertens 1996;5:336–41
Progression of Secondary Hyperparathyroidism

Parathyroid Tissue from Severe 2ª & 3ª Hyperparathyroidism

Losses & Duplications of Chromosomes

Changes in Gene Expression

Adapted from Tominaga Y et al. Curr Opin Nephrol Hypertens 1996;5:336–41

J Cigudosa, I Santamaría, J Cannata et al
Kidney Int 2003: 63,

Gene Repression

The Parathyroid Glands progressively lose its complex and exquisite regulatory leadership role.

Just produce PTH.

Adapted from Tominaga Y et al. Curr Opin Nephrol Hypertens 1996;5:336–41
Mechanisms of CKD-MBD: New Insights in the Pathogenesis

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  - Possible Self-defensive Mechanisms Triggered by the Vascular System
Patterns of Renal Osteodystrophy

Current Evolution

High PTH

MEDICAL MANAGEMENT

CKD

DIABETES - AGE

HIGH BONE TURNOVER

LOW BONE TURNOVER

PTH (Parathyroid Hormone)
Change in the Prevalence of High and Low Bone Turnover Disease in CKD5

<table>
<thead>
<tr>
<th>Study</th>
<th>High Bone Turnover</th>
<th>Low Bone Turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moriniere et al, 1989 (France)</td>
<td>76 %</td>
<td>24 %</td>
</tr>
<tr>
<td>Lorenzo et al, 1991 (Spain)</td>
<td>71 %</td>
<td>25 %</td>
</tr>
<tr>
<td>Sherrard et al, 1993 (USA)</td>
<td>48 %</td>
<td>37 %</td>
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<td>Herz et al, 1993 (USA)</td>
<td>50 %</td>
<td>50 %</td>
</tr>
<tr>
<td>Torres et al, 1995 (Spain)</td>
<td>52 %</td>
<td>45 %</td>
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<td>Ferreira et al 2008 (Portugal)</td>
<td>32 %</td>
<td>63 %</td>
</tr>
<tr>
<td>Erkan, Gulay et al 2009 (Turkey)</td>
<td>23 %</td>
<td>73 %</td>
</tr>
</tbody>
</table>
Risks of High and Low Bone Turnover

High Bone Turnover

Common Risks

Low Bone Turnover

Vascular Calcifications

Bone Mass Fractures

Mortality
Relationship Between Vascular Calcification & Bone

Bone Turnover

Arterial Calcifications and Bone Histomorphometry in End-Stage Renal Disease

GÉRARD M. LONDON,* CAROLINE MARTY,† SYLVAIN J. MARCAIS,* ALAIN P. GUERIN,* FABIEN METIVIER,* and MARIE-CHRISTINE DE VERNEJOUL†


High Calcification Scores in the Aorta

Low Bone Activity
Coronary Calcification is Associated with Lower Mineralized Bone Volume

Low Bone Volume—A Risk Factor for Coronary Calcifications in Hemodialysis Patients

Coronary Calcification

Low Mineralized Bone

High Pulse Wave Velocity
Relationship Between Vessels & Bone

General Population

CKD

Bone Activity

Vascular Mineralization

Diagram showing the relationship between bone mass and age, with peaks and losses highlighted.

Legend:
- Peak bone mass
- Rapid bone growth
- Postmenopausal bone loss
- Aging bone loss

Graph illustrates changes in bone mass over age, with CKD affecting bone activity and mineralization.
Relationship Between Vessels & Bone

Progression of vascular calcifications is associated with greater bone loss and increased bone fractures

M. Naves · M. Rodríguez-García · J. B. Díaz-López · C. Gómez-Alonso · J. B. Cannata-Andía

Osteoporos Int 19: 1161-1166, 2008

Bone Activity

Vascular Mineralization

4 Years Follow up
Relationship Between Vascular Calcification & Bone

From Patient to Bench

CKD

General Population
Relationship Between Vascular Calcification & Bone

Scheme of the Study

Macroscopic
Histologic
Genomics
Proteomics

Aorta
Bone

High P
High Mortality
50% Vs 10%

Week 0
Normal (n=10)

Week 4
High P (n=10)
Control (n=10)

Week 8
High P (n=10)
Control (n=10)

Week 12
High P (n=10)
Control (n=10)

Week 16
High P (n=10)
Control (n=10)

Week 20
High P (n=10)
Control (n=10)
Normal (n=10)

N 5/6

Normal (n=10)

High P (n=10)
Control (n=10)
Relationship Between Vascular Calcification & Bone

High Phosphorus Diet (+ 50%) 20% NO Aortic Calcification (20 weeks)

High Phosphorus Diet (+ 50%) 80%

Relationship Between Vascular Calcification & Bone

Bone Histology

20% No Vascular Calcification

Vascular Calcification

80%

Relationship Between Vascular Calcification & Bone

Scheme of the Study

Week 0
Normal (n=10)

Week 4
High P (n=10)
Control (n=10)

Week 8
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Control (n=10)

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High P (n=10)
Control (n=10)

Week 16
High P (n=10)
Control (n=10)

Week 20
High P (n=10)
Control (n=10)
Normal (n=10)

Macroscopic
Histologic
Genomics
Proteomics

Aorta
Bone
Gene Expression Profile in Aorta of Rats with Vascular Calcification

Muscle Related Genes

Elastin

<table>
<thead>
<tr>
<th>Fold Change</th>
<th>FC 8NP</th>
<th>FC 16NP</th>
<th>FC 20NP</th>
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Tropomyosin Alpha 1

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</tbody>
</table>

Muscle Related Genes

Red: Overexpression

Calcification

Green: Repression

Gene

No Calcification

-1.5                 +1.5
Signal LogRatio

Román-García et al. Bone 46: 121-128; 2010
Gene Expression Profile in Aorta of Rats with Vascular Calcification

Bone Related Genes

Muscle Related Genes

Román-García et al. Bone 46: 121-128; 2010
Gene Expression Profile in Aorta of Rats with Vascular Calcification

Bone Related Genes

RED: Overexpression

Calcification

No Calcification

Green: Repression

SRFP 4 (Wnt)

Katepsin K

Román-García et al. Bone 46: 121-128; 2010
In the Calcified Aorta: Markers of Active Bone Resorption & Inhibition of Bone Formation

Defensive Mechanism
Relationship Between Vascular Calcification & Bone

Which is Molecular the Link?

Price to Pay?

Vessels: Reduce or Stop Vascular Mineralization

Bone: Decrease Bone Mineralization and Bone Mass

Vascular Mineralization

Bone Mineralization