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The impact of cardiovascular calcifications in chronic kidney disease: Novel pathomechanistic insights

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ABSTRACT

Vascular calcification including both medial and intimal calcification is the most prominent and a very common manifestation of extraosseous calcification in end stage renal disease (ESRD). It is characteristic of the accelerated course atherosclerosis takes in dialysis patients and associated with arterial stiffness as well as increased mortality. Several risk factors for calcification were identified in the ESRD population including age and time on dialysis, but specifically also an elevated calcium x phosphate product (Ca x P), the dose of calcium-containing phosphate binders and the presence of an inflammatory state. Data from registry studies strongly support the deleterious role of hyperphosphataemia and of an elevated Ca x P product for the survival of ESRD patients. In this context, recent research unravelled that both Ca and P do not just passively precipitate at high extracellular concentrations, but may also induce a phenotypic transformation of vascular smooth muscle cells into osteoblast-like cells. Further, the role endogenous inhibitors of calcification (i.e. matrix Gla protein, osteoprotegerin and fetuin-A) has recently focussed much attention regarding the pathophysiology of unwanted calcifications in uraemia. Decreased serum fetuin-A has been shown to be associated with a reduced capacity to inhibit Ca and P precipitation *in vitro* and is an inflammation-related predictor of mortality in dialysis patients. These new insights into the pathogenetic events

leading to vascular calcification may lead to better prevention and to new treatment options in the cardiovascular high-risk ESRD population.

Key words: calcification; calcimimetics; calcium; end-stage renal disease; phosphorus; sevelamer; fetuin-A; matrix Gla protein; osteoprotegerin

CARDIOVASCULAR MORTALITY AND TYPES OF VESSEL CALCIFICATION

The key problem in the dialysis population is the fact that cardiovascular mortality is dramatically increased and that this phenomenon is already particularly true for young patients[1]. Accelerated calcifying atherosclerosis and valvular calcifications are hallmarks of uraemic cardiovascular disease and the magnitude of both vascular and valvular calcification represent independent risk factors of cardiovascular death[2,3]. Typical metabolic disturbances in uraemia including hyperphosphatemia, an increased Ca x P product, hyperparathyroidism and an increased calcium intake are independent predictors of cardiovascular morbidity and mortality and are all associated with extraosseous calcification in this population[4-7]. Among other reports, the latter observation was shown in two recent studies in young dialysis patients by utilizing electron-beam computed tomography (EBCT) in order to quantify coronary artery calcifications (see example in figure 1)[6,7]. In both studies performed in children and young adults on dialysis (age < 40 years) it was demonstrated that coronary artery calcifications were already highly prevalent especially in the age group of 20 – 40 years and that the calcification burden was related to an increased calcium x phosphate product and an increased calcium intake. In one of the studies, authors reported a strong relationship between the magnitude of calcification and elevated CRP levels, respectively, potentially documenting a cause-and-effect relationship between calcification and inflammation[7].

Vascular calcification may involve particular and different regions within the vessel wall, namely intima or media. Intimal calcification nearly exclusively occurs within atherosclerotic plaques, which are inflammatory lesions per se and may cause ischaemia-related occlusion[8]. Mediasclerosis is characterised by a non-inflammatory circular calcification of muscular arteries[9], which result in the loss of the dampening function of the circulation leading to elevated pulse pressure, left ventricular hypertrophy and dysfunction and to a compromised coronary perfusion[10,11]. While the development of plaque atherosclerosis is associated with the presence of traditional risk factors such as hypercholesterolaemia and smoking, mediasclerosis is found predominantly aggravated in uraemia and diabetes mellitus, conditions in which however both types of intimal and medial calcifications occur[9].

In a recent clinical study performed in uraemic subjects, patients were stratified to groups according to their predominant type of vascular calcification: Dialysis patients with intimal calcifications were older and indeed characterised by the presence of 'traditional' risk factors (e.g. smoking and dyslipidaemia), whereas patients with medial calcifications were younger and characterised by a longer duration of HD treatment and derangements in their Ca x P balance[12]. Adjusting the relative risk of cardiac death for age demonstrated the predominant presence of medial calcification to be associated with a 45-fold increased risk and the presence of intimal calcification to be associated with a 7.5-fold increased risk, respectively, when compared to the non-calcified dialysis patients of this

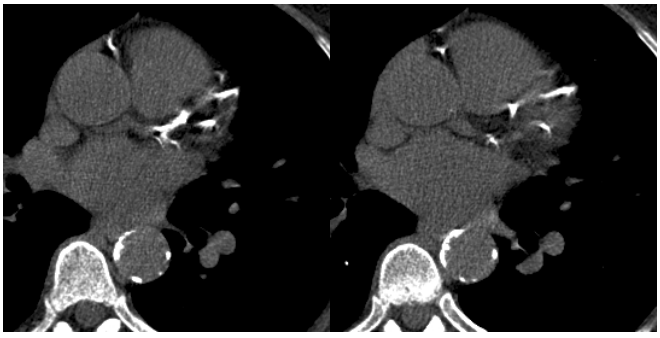

Figure 1– Two serial images (multisclice spiral computed tomography [MSCT]) performed in a 48-year-old haemodialysis patients showing severe coronary and aortic calcifications, resulting in an Agatston score of > 10.000 (> 400: severe coronary artery calcifications).

cohort. Thus, arterial calcification appears to be an extremely strong risk factor in patients on dialysis.

AETIOLOGY OF VASCULAR CALCIFICATION – THE ROLE OF CALCIUM AND PHOSPHATE

As pointed out above, phosphate plays a central role in the calcification process. Experimental *in vitro* studies performed in normal human aortic smooth muscle cells (hVSMC) revealed that phosphate-induced calcification involved intracellular processes including cellular dedifferentiation. When hVSMC were cultured for 9 days in a solution containing a formally physiological level of phosphate (1.4 mmol/l), no calcification developed in the supernatants. However, when the same cell line grew in the presence of a 2.0 mmol/l phosphate solution for the same period, hydroxapatite, the most common form of calciumphosphate precipitate in soft-tissues, accumulated attached to a extracellular matrix similar to that found in the bone[13]. Blocking the neutral sodium–phosphate co-transporter Pit-1 using *in vitro* treatment with phosphonoformic acid and thus preventig a rise in the intracellular phosphate concentration completely abolished hydroxyapatite formation[13,14]. This observation is probably the most convincing evidence available that calcium and phosphate precipitation occurs not just as passive precipitation, but as an active biological process depending on entry of phosphate into hVSMCs.

Another phenomenon observed in such *in vitro* studies in hVSMC is the phosphate-dependent formation of matrix vesicles, which represent membrane-derived structures known to be involved in osteogenesis[14,15]. Further, increasing intracellular phosphate down-regulates hVSMC-specific genes while *de novo* up-regulating core binding factor 1 (Cbfa-1), the central transcription factor in osteoblast differentiation, and inducing expression of bone proteins including alkaline phosphatase, osteocalcin and osteopontin[13,14,16]. Moe *et al*.[17,18] subsequently demonstrated *in vivo* that Cbfa-1, alkaline phosphatase and osteopontin are expressed in human uraemic arteries limited exclusively to areas of calcification confirming and emphasising the relevance of such *in vitro* findings for human disease. Taken together, the appropriate term coined for this transformation of hVSMC into osteoblast-like cells is osteogenic differentiation.

Meanwhile, it became also clear that increasing extracellular calcium concentrations seem to have very similar effects on the development hVSMC-associated calcifications *in vitro* as high phosphate[19,20]. In these recent publications, a synergism of high Ca and high P could be documented affecting the magnitude of calcification, matrix vesicle release, phenotypic changes, and the induction of apoptosis, which may be importantly involved in the progression of vascular calcification as well. In addition, other uraemia-

associated factors appear to affect the phenotypic transformation of hVSMC. Uraemic serum, independently of phosphate levels or blockade of the sodium–phosphate co-transporter, was able to up-regulate the expression of Cbfa-1 in hVSMC[18]. Further, pro-inflammatory cytokines such as TNF-α also induced hVSMC associated calcifications in an *in vitro* setting suggesting an additional role of inflammation in cell-mediated active calcification[21].

Finally, *in vitro* experiments linked hVSCM calcification to several other potential modulators of calcification in ESRD. For example, calcitriol levels, diabetes, advanced glycation end products, leptin and some genetic factors were demonstrated to trigger or increase progressive calcification[22-27]. In contrast, PTH and PTH-related peptide (PTHrP), osteopontin, osteoprotegerin and bone morphogenic protein (BMP)-7 were described to inhibit *in vitro* calcification processes[28-31]. The relevance of most of these factors in uraemia *in vivo* is currently unknown.

AETIOLOGY OF VASCULAR CALCIFICATION – CALCIFICATION INHIBITORS

In order to more completely understand the phenomenon of extraosseous calcifications, it must be imagined that even the physiological serum concentrations of Ca and P are several orders of magnitude above their solubility product in aqueous solutions. Body temperature, pH and the ionic strength (especially the serum NaCl concentration) in the extracellular space are preventive and contribute to keeping Ca and P in solution, however, serum must be regarded as a “metastable” solution regarding Ca and P ions. Thus, precipitation inhibitory mechanisms are clearly necessary to prevent ongoing soft-tissue calcification. Recently, a number of local and systemic calcification inhibitors were identified[reviewed in 32,33], while deficiencies and dysregulations of such factors may contribute to signficant morbidity and perhaps even mortality in some patient populations. In the following paragraphs, three factors with calcification inhibitory properties will be discussed as examples in more detail, while a growing number of emerging Ca-regulatory proteins were meanwhile discovered (table 1)[32,33].

Table 1 – Selected Ca-regulatory factors with direct or indirect inhibitory properties concerning extraosseous calcification (bold-typed factors are discussed in this review in more detail)

Caclification inhibitor	Evidence
Fetuin-A	**in vitro, in vivo, human**
Matrix Gla protein	**in vitro, in vivo, human**
Osteoprotegerin	**in vivo, human[1]**
Pyrophosphates (ANK, ENPP-1)	in vitro, in vivo, human
Bone morphogenetic protein-7 (BMP-7)	in vitro, in vivo
Klotho	in vivo, human[2]
Osteopontin	in vitro, in vivo
Fibrillin-1	in vitro, in vivo, human
Carbonic anhydrase II	in vitro, in vivo, human
Smad6	in vivo

In vitro = cell culture experiments; in vivo = animal models; human = disease phenotype.
[1] The associated human disease phenotype is increased intima media thickness, which may be a surrogate parameter of medial calcification.
[2] Evidence from association studies related to cardiovascular phenotype, but not directly to calcification phenotype
(a reference list relevant to this table can be requested from the corresponding author)

MATRIX GLA PROTEIN (MGP)

MGP is a small Gla protein (MW: 10 kD) expressed in chondrocytes and VSMC requiring activation via vitamin K-dependent g-carboxylation in order to acting locally as a calcification inhibitor[34,35]. MGP-deficient ($MGP^{-/-}$) mice show an impressive and finally lethal phenotype: The aortic media develops most severe calcifications localised to the elastic lamellae leading to "fracture" of the aorta with consecutive internal hemorrhage[35]. Cartilageous metaplasia, similar to the process of VSMC osteogenic differentiation as described above, accompanies calcification of the aorta. Loss-of-function mutations of the MGP gene in humans (Keutel syndrome) cause a phenotype clinically characterised by cartilage and vascular medial calcifications, as known from autopsies of affected individuals[36].

In human atherosclerotic disease, the role of MGP expression is less clear – MGP overexpression especially seems to surround lipid-rich and calcified plaque areas[37]. This pattern may suggest that local MGP up-regulation tries to counteract excessively calcified vascular foci and thus attempts to limit the magnitude of calcifications. However, it is not entirely clear whether or to what part this MGP overexpression is represented by the active γ-carboxylated MGP protein.

Interestingly, recent clinical studies revealed a significant connection between subclinical vitamin K deficiency and coronary artery disease in the normal population[38]. Likewise, it will have to explored in the near future whether vitamin K-antagonist (e.g., phenprocoumon, warfarin) treatment may be associated with aggravated medial calcifications. Such studies may be clinically important in order to better judge the use of vitamin K-antagonists in calcification-prone subjects such as patients with chronic kidney disease. Up to 50% of reported cases of calcific uremic arteriolopathy (CUA, calciphylaxis), a severe disorder usually developing in uraemia and characterised by excessive medial calcification of cutaneous arterioles, occurred associated with vitamin K-antagonist treatment[39,40]. The "Vascular Calcification Work Group" of the National Kidney Foundation consequently agreed on launching registry efforts and clinical studies to evaluate the use of vitamin K-antagonists and the vitamin K-status, respectively, in ESRD patients[41].

FETUIN-A

Fetuin-A is the central circulating calcification inhibitor responsible for up to 50% of the precipitation inhibitory capacity of serum[42,43]. Fetuin-A is produced by hepatocytes, reaches high extracellular concentrations (0.5-1.0 g/l) and reacts as a negative acute-phase protein[44]. In *ex vivo* experiments, fetuin-A was demonstrated to be a potent inhibitor of hydroxyapatite formation[45]. *In vivo*, transgenic fetuin-A-deficient ($Fetuin\text{-}A^{-/-}$) mice develop severe soft-tissue calcifications either spontaneously (DBA2 background) or by additional challenges with high-dose vitamin D and by feeding a mineral-rich diet (C57BL/6 background)[46].

Clinical studies performed in haemodialysis patients recently demonstrated that serum fetuin-A levels were significantly lower in both long- and short-term patients versus control subjects with normal renal function[47]. Serum from dialysis patients was at the same time less efficient to inhibit Ca and P precipitation than normal serum in an *ex vivo* assay and this effect was reversed by adding fetuin-A to the buffer preparation[47]. These findings underscore the functional relevance of fetuin-A as a calcification inhibitor. Since fetuin-A is regulated as a negative acute-phase reactant, serum fetuin-A levels correlated inversely with CRP levels, and forward stepwise regression analysis demons-

trated fetuin-A deficiency being an inflammation-related predictor of all-cause and cardiovascular mortality in this dialysis cohort[47].

THE OSTEOPROTEGERIN(OPG)/RANKL SYSTEM

In the normal population, it appears that osteoporosis and cardiovascular disease may be linked to each other [48]. In case that pronounced vascular calcification may be involved to explain this phenomenon, the OPG/RANK/RANKL (receptor activator of NF-kB-ligand) system, as a central regulator of bone turnover, may be a prime candidate in this context. OPG is a member of the tumor necrosis factor(TNF)-receptor superfamily and serves as am soluble, antagonising decoy receptor for RANKL, which accordingly belongs to the TNF superfamily[49,50]. RANKL-binding to RANK immediately starts a programme of osteoclast activation, differentiation and maturation. Therefore, an excess availability of OPG stops this programme and favors bone build-up, while an OPG deficiency relative to the RANKL leads to high bone turnover with subsequent osteoclast-dependent bone loss.

Experiments in transgenic mice again unravelled the impressive potency of this system: OPG-overexpressing mice develop osteopetrosis, while OPG-deficient ($OPG^{-/-}$) mice develop high-grade osteoporosis, but significant arterial calcifications[49,50]. Until now, it is however not entirely clear, whether OPG deficiency contributes to the development of vascular calcification by the increased Ca and P absorption from the bone or by a direct loss of calcification inhibitory properties within the vessel wall, or perhaps by both mechanisms.

Results from a recent publication by Nitta and colleagues were however somewhat unexpected and confusing, since in this study OPG levels were found increased in haemodialysis patients when compared to a normal population, and elevated OPG levels were positively associated with the severity of aortic calcification[51]. These findings appear counterintuitive at first glance, given that OPG shows properties of a calcification inhibitor, and this is especially true, since this “seeming paradox“ of elevated OPG levels being significantly associated with cardiovascular disease was also observed in previous studies in the normal population[48]. An attractive, but yet unproven hypothesis to explain these observations could be that OPG up-regulation is an active, although perhaps incomplete self-defense mechanism to counteract excessive atherosclerosis and calcification, and elevated OPG levels must therefore be interpreted with caution and perhaps more as a marker of severe cardiovascular disease than as a pathomechanism.

THERAPEUTIC STRATEGIES

Despite these novel pathophysiological insights into calcification mechanisms, treatment and prevention approaches are currently still limited to modifications of the calcium load, treatment of secondary hyperparathyroidism, the use of phosphate binders and performing optimal dialysis procedures. Sevelamer, a phosphate-binding polymer, possesses some significant potential versus calcium-containing phosphate slowing the progression of vascular calcification as shown by the Treat-to-Goal study[52]. Prospective cardiovascular end-point studies, and intervention studies to distinguish between the role of both phosphate and lipids in uraemic vascular calcification, are ongoing to better understand the beneficial properties of sevelamer.

Calcimimetics, new and improved Vitamin D analogues, and bisphosphonates may further prove themselves as effective tools to modify Ca and P levels, i.e. lowering the Ca x P product. Anti-inflammatory strategies including the use of pyrogen-free dialysate and therapies to reduce cytokine levels may offer potential to modulate inflammation-related pathomechaisms of cardiovascular calcification (e.g., fetuin-A deficiency). Finally, the synthesis of endogenous inhibitors of calcification, such as fetuin-A, or alternatively the identification of factors specifically inducing endogenous fetuin-A synthesis in the liver, could also offer significant potential to be pursued for creating novel therapeutic agents.

SUMMARY

Extraosseous and especially cardiovascular calcifications implement a devastating risk to the outcome of patients with chronic kidney disease. In this context, we have learned just recently that extraosseous calcium and phosphate precipitation is not just a process of passive precipitation, but is regulated by a number of local, systemic and cellular factors. Calcification inhibitors (MGP, fetuin-A, osteoprotegerin etc.), together with pH, body temperature and the extracellular ionic strength, are important by keeping Ca and P ions in solution despite a formally supersaturated extracellular environment. Nevertheless, substrate excess (hyperphosphatemia, increased calcium-phosphate product, high calcium load) remains a key factor destroying Ca and P homeostasis and starting extraosseous calcification processes with deleterious cardiovascular consequences, and must thus be well controlled. Inflammation and deficiencies of calcification inhibitory factors, respectively, will however also have to be considered as pathogenic in the progression of accelerated calcifying atherosclerosis in uraemia. Future studies will hopefully tell us how to specifically target dysregulations in calcification inhibition thus becoming an important therapeutic strategy.

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References

1. US Renal Data System. USRDS 1999 annual data report (1999) Am J Kidney Dis 34(2 suppl 1):S87-S94
2. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM (2001) Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. Hypertension 38:938-942
3. Wang AY, Wang M, Woo J, Lam CW, Li PK, Lui SF, Sanderson JE (2003) Cardiac valve calcification as an important predictor for all-cause mortality and cardiovascular mortality in long-term peritoneal dialysis patients: a prospective study. J Am Soc Nephrol 14:159-168
4. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK (2001) Association of elevated serum PO(4), Ca x PO(4) product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. J Am Soc Nephrol 12:2131-2138
5. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM (2004) Mineral Metabolism, Mortality, and Morbidity in Maintenance Hemodialysis. J Am Soc Nephrol 15:2208-2218
6. Goodman WG, Goldin J, Kuizon BD et al. (2000) Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med 342: 1478–1483
7. Oh J, Wunsch R, Turzer M, Bahner M, Raggi P, Querfeld U, Mehls O, Schaefer F (2002) Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure. Circulation 106:100-105
8. Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340: 115–126
9. Proudfoot D, Shanahan CM (2001) Biology of calcification in vascular cells: intima versus media. Herz 26: 245–251
10. London GM (2000) Alterations of arterial function in end-stage renal disease. Nephron 84: 111–118

11. London GM (2003) Cardiovascular disease in chronic renal failure: pathophysiologic aspects. Semin Dial 16: 85–94
12. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H (2003) Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 18:1731-1740
13. Jono S, McKee MD, Murry CE et al. (2000) Phosphate regulation of vascular smooth muscle cell calcification. Circ Res 87: E10–E17
14. Giachelli CM, Jono S, Shioi A, Nishizawa Y, Mori K, Morii H (2001) Vascular calcification and inorganic phosphate. Am J Kidney Dis 38: S34–S37
15. Anderson HC (1995) Molecular biology of matrix vesicles. Clin Orthop 314: 266–280
16. Chen NX, O'Neill KD, Duan D, Moe SM (2002) Phosphorus and uremic serum up-regulate osteopontin expression in vascular smooth muscle cells. Kidney Int 62: 1724–1731
17. Moe SM, O'Neill KD, Duan D et al. (2002) Medial artery calcification in ESRD patients is associated with deposition of bone matrix proteins. Kidney Int 61: 638–647
18. Moe SM, Duan D, Doehle BP, O'Neill KD, Chen NX (2003) Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. Kidney Int 63: 1003–1011
19. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM (2004) Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. J Am Soc Nephrol 15:2857-2867
20. Giachelli CM (2004) Vascular calcification mechanisms. J Am Soc Nephrol 15:2959-2964
21. Shioi A, Katagi M, Okuno Y, Mori K, Jono S, Koyama H, Nishizawa Y (2002) Induction of bone-type alkaline phosphatase in human vascular smooth muscle cells: roles of tumor necrosis factor-alpha and oncostatin M derived from macrophages. Circ Res 91: 9–16
22. Jono S, Nishizawa Y, Shioi A, Morii H (1998) 1,25-Dihydroxyvitamin D3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. Circulation 98: 1302–1306
23. Yamagishi S, Fujimori H, Yonekura H, Tanaka N, Yamamoto H (1999) Advanced glycation endproducts accelerate calcification in microvascular pericytes. Biochem Biophys Res Commun 258: 353–357
24. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL (2001) Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. Circ Res 88: 954–960
25. Lange LA, Lange EM, Bielak LF et al. (2002) Autosomal genome-wide scan for coronary artery calcification loci in sibships at high risk for hypertension. Arterioscler Thromb Vasc Biol 22: 418–423
26. O'Donnell CJ, Chazaro I, Wilson PW et al. (2002) Evidence for heritability of abdominal aortic calcific deposits in the Framingham Heart Study. Circulation 106: 337–34145.
27. Peyser PA, Bielak LF, Chu JS et al. (2002) Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults. Circulation 106: 304–308
28. Jono S, Nishizawa Y, Shioi A, Morii H (1997) Parathyroid hormone-related peptide as a local regulator of vascular calcification. Its inhibitory action on in vitro calcification by bovine vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 17: 1135–1142
29. Steitz SA, Speer MY, McKee MD et al. (2002) Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. Am J Pathol 161: 2035–2046
30. Min H, Morony S, Sarosi I et al. (2000) Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. J Exp Med 192: 463–474
31. Lund RJ, Davies MR, Hruska KA (2002) Bone morphogenetic protein-7: an anti-fibrotic morphogenetic protein with therapeutic importance in renal disease. Curr Opin Nephrol Hypertens 11: 31–36
32. Ketteler M, Westenfeld R, Schlieper G, Brandenburg V, Floege J (2004) "Missing" inhibitors of calcification: general aspects and implications in renal failure. Pediatr Nephrol [Epub ahead of print]
33. Doherty TM, Fitzpatrick LA, Inoue D, Qiao JH, Fishbein MC, Detrano RC, Shah PK, Rajavashisth TB (2004) Molecular, endocrine, and genetic mechanisms of arterial calcification. Endocr Rev 25:629-672.
34. Ketteler M, Wanner C, Metzger T, Bongartz P, Westenfeld R, Gladziwa U, Schurgers LJ, Vermeer C, Jahnen-Dechent W, Floege J (2003) Deficiencies of calcium-regulatory proteins in dialysis patients: a novel concept of cardiovascular calcification in uremia. Kidney Int Suppl 84:S84-S87
35. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G (1997) Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 386:78-81
36. Munroe PB, Olgunturk RO, Fryns JP, Van Maldergem L, Ziereisen F, Yuksel B, Gardiner RM, Chung E (1999) Mutations in the gene encoding the human matrix Gla protein cause Keutel syndrome. Nat Genet 21:142-144
37. Shanahan CM, Cary NR, Metcalfe JC, Weissberg PL (1994) High expression of genes for calcification-regulating proteins in human atherosclerotic plaques. J Clin Invest 93:2393-2402
38. Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, Hofman A, Witteman JC (2004) Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. J Nutr 134: 3100-5.
39. Case Records of the Massachussets General Hospital (2001) New Engl J Med 345:1119-24
40. Coates T, Kirkland GS, Dymock RB, Murphy BF, Brealey JK,

Mathew TH, Disney AP (1998) Cutaneous necrosis from calcific uremic arteriolopathy. Am J Kidney Dis 32:384-91

41. Goodman WG, London G, Amann K, Block GA, Giachelli C, Hruska KA, Ketteler M, Levin A, Massy Z, McCarron DA, Raggi P, Shanahan CM, Yorioka N; Vascular Calcification Work Group (2004) Vascular calcification in chronic kidney disease. Am J Kidney Dis 43:572-579
42. Schinke T, Amendt C, Trindl A, Poschke O, Muller-Esterl W, Jahnen-Dechent W (1996) The serum protein alpha2-HS glycoprotein/fetuin inhibits apatite formation in vitro and in mineralizing calvaria cells. A possible role in mineralization and calcium homeostasis. J Biol Chem 271:20789-20796
43. Jahnen-Dechent W, Schinke T, Trindl A, Muller-Esterl W, Sablitzky F, Kaiser S, Blessing M (1997) Cloning and targeted deletion of the mouse fetuin gene. J Biol Chem 272:31496-31503
44. Lebreton JP, Joisel F, Raoult JP, Lannuzel B, Rogez JP, Humbert G (1979) Serum concentration of human alpha 2 HS glycoprotein during the inflammatory process: evidence that alpha 2 HS glycoprotein is a negative acute-phase reactant. J Clin Invest 64:1118-1129
45. Heiss A, DuChesne A, Denecke B, Grotzinger J, Yamamoto K, Renne T, Jahnen-Dechent W (2003) Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. J Biol Chem 278:13333-13341
46. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W (2003) The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest 112:357-366
47. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Bohm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J (2003) Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 361:827-833
48. Hofbauer LC, Schoppet M (2001) Osteoprotegerin: A link between osteoporosis and arterial calcification? Lancet 358:257-258
49. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309-19
50. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS (1998) Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260-1268
51. Nitta K, Akiba T, Uchida K, Kawashima A, Yumura W, Kabaya T, Nihei H (2003) The progression of vascular calcification and serum osteoprotegerin levels in patients on long-term hemodialysis. Am J Kidney Dis 42:303-309
52. Chertow GM, Burke SK, Raggi P (2002) Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. Kidney Int 62: 245–252