

Matrix Gla protein: the inhibitor of vascular and osteoarticular calcifications

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Abstract. Ectopic vascular calcification is a cardiovascular risk factor. This phenomenon is no longer regarded as a simple precipitation of calcium phosphate in the extracellular matrix, but it is considered to be the result of an active process similar to bone mineralization. Also, calcium is deposited at osteoarticular level as calcium phosphate or hydroxy-apatite crystals, initially leading to the formation of articular and periarticular asymptomatic deposits. Ectopic calcification inhibitors play a central role in the association of vascular calcification with reduced bone mineral density, a phenomenon called "calcification paradox". Matrix Gla-protein (MGP) is a local inhibitor of ectopic calcifications. This paper reviews up-to-date information about the fundamental aspects, the determination methods and the clinical implications of MGP in vascular and osteoarticular pathology.

Key words: Matrix Gla-protein, vascular calcification, osteoarticular calcification.

Rezumat. Calcificările ectopice vasculare reprezintă un factor de risc cardiovascular. Acest fenomen nu mai este privit ca o simplă precipitare a fosfatului de calciu în spațiul extracelular, ci este rezultatul unui proces activ similar mineralizării osoase. De asemenea, calciul se depune la nivel osteoarticular sub formă de cristale de fosfat de calciu sau hidroxi-apatită care conduc inițial la formarea unor depozite asimptomatice articulare sau periarticulare. Inhibitorii calcificărilor ectopice au un rol central în cadrul asocierii calcificărilor vasculare cu scăderea densității minerale osoase, fenomen denumit "paradoxul calcificării". Matrix Gla-proteina (MGP) este un inhibitor local al calcificărilor ectopice. Acest articol aduce la zi aspectele fundamentale, metodele de determinare precum și implicațiile clinice ale MGP în patologia vasculară și osteoarticulară.

Cuvinte cheie: Matrix Gla-proteina, calcificări vasculare, calcificări osteoarticulare.

Introduction. The matrix Gla protein (MGP) represents a key factor in the process of physiological and pathological calcification of the extracellular matrix, being considered an important inhibitor of ectopic calcifications. Physiological calcification occurs in bones and epiphyseal cartilages during growth, while the pathological ectopic calcifications can be found in blood vessels, cartilages and other soft tissues. Although it was believed to be a passive degenerative process linked to aging, the ectopic calcification of the extracellular matrix is now recognized to be an active cell-mediated process similar to osteogenesis (Demer & Tintut 2008).

In a meta-analysis on vascular calcification, Rennenberg et al (2009) observed a positive correlation between the presence of calcifications, strokes, coronary events and mortality. Also, Budoff & Gul (2008) concluded that the presence of calcium in coronary arteries has a predictive value for future coronary events both in symptomatic and asymptomatic populations.

Calcium can be deposited in tissues as calcium phosphate or hydroxyapatite crystals. These crystals can form asymptomatic joint or periarticular deposits, being involved in the pathogenesis of osteoarticular diseases such peri-arthritis, Milwaukee shoulder syndrome, osteoarthritis, tendinitis, bursitis etc.

The paradox of coexistence of the two age-related diseases, atherosclerosis and osteoporosis, is supported by presence of vascular calcifications accompanied by decrease in bone mineral density (BMD). An increase in the coronary calcification score

and the presence of complicated atherosclerotic plaque, diagnosed by computer tomography (CT), were associated with decreased BMD in both post- and premenopausal women (Choi et al 2009).

Vitamin K and Gla proteins. Vitamin K is a γ -glutamyl carboxylase cofactor and the resulting γ -carboxylation products are known as K-dependent proteins. Gla term comes from the γ -carboxy glutamic acid, an amino acid formed during the post-translational carboxylation of glutamic acid (Glu).

For K-dependent proteins, the presence of Gla residues is a prerequisite to become biologically active. Thus, they may chelate Ca^{+2} ions due to the electronegativity of the Gla groups (Stafford 2005).

K-dependent proteins are represented by coagulation factors II, VII, IX and X, by proteins C, S and Z, by osteocalcin (OC), MGP, growth arrest-specific protein 6 (Gas6) and also by proline-rich Gla proteins 1 and 2 (PRGP1 and PRGP2) and transmembrane Gla proteins 3 and 4 (TMG3 and TMG4).

Gla proteins have various physiological or pathological functions, such as:

a) K-dependent coagulation factors II, VII, IX and X (synthesized largely by liver and also by macrophages or vascular endothelium) have a pro-coagulant activity; proteins C and S are inhibitors of coagulation; protein Z has no enzymatic activity, but inhibits coagulation by forming a complex with the protein Z-dependent protease inhibitor (Castoldi & Hackeng 2008).

b) at osteoarticular level, protein S and OC are regulators of bone mineralization, and MGP inhibits the mineralization of the growth cartilage (Luo et al 1997).

c) in vessel walls, protein S is a local inhibitor of thrombosis; Gas6 is involved in protection against apoptosis and in regulation of chemotaxis and movement of vascular smooth muscle cells; MGP is an inhibitor of vascular calcifications (Wallin et al 1999; Cario-Toumaniantz et al 2007).

MGP: Structure, localization and role. MGP is a protein secreted in the extracellular matrix, first isolated by Price et al (1983) from bovine bone. The mature protein comprises 84 amino acid residues and has a molecular weight of 14 kD. MGP has 9 Glu residues, 5 of which are γ -carboxylated (Gla) in positions 2, 37, 41, 48, 52 and 5 serine (Ser) residues, 3 of which are phosphorylated (pSer) in positions 3, 6 and 9. Also MGP has a single disulfide bridge between cysteine (Cys) residues in positions 54 and 60 (see Figure 1).

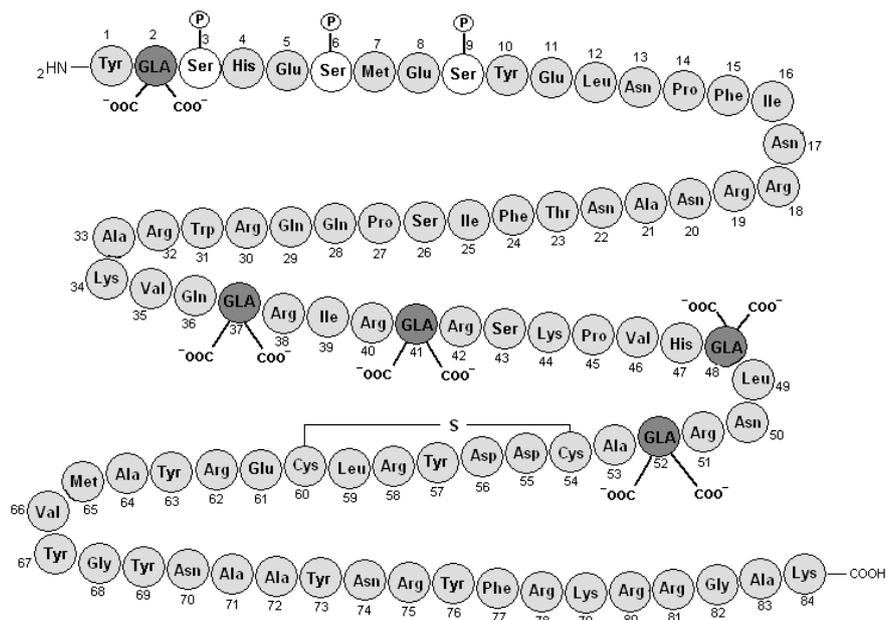


Figure 1. MGP structure (fully carboxylated and phosphorylated conformation)

In spite of its small size and low percentage (32%) of hydrophobic amino acids, MGP is insoluble in water (Price & Williamson 1985). MGP mRNA expression was found in different tissues (cartilages, bones, lungs, heart, kidney, uterus, spleen, arterial and venous wall) (Hale et al 1988; Luo et al 1995; Frazer & Price 1988; Wallin et al 1999; Cario-Toumaniantz et al 2007). Although there was an increased expression of MGP mRNA in the heart, kidneys, spleen and lungs, the concentration of MGP was decreased in these tissues (Frazer & Price 1988).

MGP is synthesized and secreted in the extracellular matrix especially by:

- vascular smooth muscle cells (VSMCs) (Wallin et al 1999; Cario-Toumaniantz et al 2007)
- resting proliferative and also mature, hypertrophied chondrocytes of the epiphyseal growth plate (Luo et al 1995).

MGP inhibition of the extracellular matrix calcification might have several possible explanations:

1. MGP binds Ca^{+2} ions with the -COOH groups of Gla residues, clearing the excess of calcium through the bloodstream. In rats (but not in humans), the calcium clearance is achieved by a circulating complex consisting of MGP, fetuin-A, hydroxyapatite and other proteins (Price & Faus 1998; Price et al 2002). The Ca^{+2} binding capacity of MGP was confirmed by reduced MGP retention time in the presence of CaCl_2 during high performance liquid chromatography (HPLC) (Hackeng et al 2001). Also, MGP strongly binds hydroxyapatite crystals, thus inhibiting crystal growth (Price & Faus 1998).
2. MGP binds to extracellular matrix components. For instance, MGP binds through its C-terminal end to vitronectin, a glycoprotein found in the extracellular matrix of arteries (Nishimoto & Nishimoto 2005). This interaction is not dependent on either Ca^{+2} ions or MGP carboxylation status. The same study showed that MGP does not bind to collagen, fibromodulin, heparin, osteocalcin, chondroitin sulfate, laminin, ovalbumin or albumin. However, MGP binds to elastin fibers in the arterial wall, these fibers being substrate for the nucleation of calcium phosphate crystals (Price et al 2006). Presumably, the MGP molecules create a network covering the extracellular elastic fibers of the vascular walls, thus preventing nidation of calcium crystals.
3. MGP binds to cellular structures. It is known that matrix vesicles (MV) and apoptotic bodies (AB) play an important role in the calcification process, because they could initiate nucleation of hydroxyapatite crystals in cartilage or VSMCs. MGP was detected in the MV and AB produced by VSMCs. It is not yet clear how the MGP and the cell surface or expelled cells vesicles interact, but it is hypothesized that MGP may bind to vesicles via phosphatidyl serine (a component of AB and MV) (Proudfoot et al 2000). In addition, MGP may bind directly to calcium phosphate crystals associated with MV. The connection between MGP and apoptosis, as an initiation point for calcification, was also studied. High levels of MGP mRNA were found when apoptosis was induced in cell cultures derived from rat glioma (Baudet et al 1998). Newman et al (2001) reported an over or under expression of MGP at different stages of chondrocyte maturation, leading to chondrocyte apoptosis in mice cell cultures. It still remains to be established whether increased or decreased levels of MGP are protective against apoptosis in VSMCs.

Schurgers¹ et al (2007) concluded that both the Gla and pSer domains, and also the C-terminal end of MGP, contribute to its calcification inhibitory function by mediating the MGP binding to cellular (MV and AB) or extracellular structures (Ca^{2+} , apatite crystals, elastin, vitronectin).

Luo et al (1997) were the first to demonstrate the chondrogenic metaplasia of the VSMCs using histochemical techniques. This function of MGP, as inhibitor of VSMCs differentiation into chondrocyte/osteoblast-like cells and protector of the VSMCs contractile phenotype, appears to be promoted by MGP binding to bone morphogenetic protein-2 (BMP-2). Wajih, Borrás et al (2004) hypothesized that N- and C-terminal

regions of MGP are attached to the extracellular matrix, leaving the Gla domain free for binding to BMP-2.

Post-translational modifications. Regulatory factors of MGP expression and activity. MGP is subject to two post-translational modifications: γ -carboxylation of glutamate (five Glu residues from positions 2, 37, 41, 48 and 52 will be converted into Gla residues) and serine phosphorylation (three Ser residues will turn into pSer in positions 3, 6 and 9).

At cellular level, γ -carboxylation occurs in the rough endoplasmic reticulum (RER). The main organs where this process takes place are the liver (especially for vitamin K-dependent coagulation factors), the bones, the cartilages, the blood vessels and other soft tissues.

The importance of extrahepatic carboxylation was partly elucidated by Schurgers² et al (2005). They have demonstrated that MGP is present both in healthy or calcified blood vessels (intima calcification of the atherosclerotic vessel wall or media calcification in diabetes patients with Mönckeberg sclerosis). Also, in healthy blood vessels the prevailing conformation is the active, carboxylated MGP (GlaMGP), while the inactive, uncarboxylated fraction (GluMGP) is dominant in calcified vessels where it is associated with calcification areas. Thus the GluMGP/GlaMGP ratio could show the extent of protein biological activity. VSMCs will continuously synthesize the carboxylated, active MGP. Thus, the GlaMGP prevents local saturation with Ca^{2+} crystals, as a defense mechanism (Danziger 2008). Nutritional deficiency of vitamin K or warfarin therapy might block the hepatic and extrahepatic carboxylation, producing severe anticoagulation effects and vascular injury.

The second post-translational MGP modification is the phosphorylation of Ser residues in positions 3, 6 and 9. This process is controlled by casein kinase and takes place in the Golgi apparatus. The sequential motif recognized for phosphorylation is Ser-X-Glu/Ser (P) (Price et al 1994). Although the exact role of Ser phosphorylation is not elucidated, two theories exist to date. The first suggests that phosphorylation interferes with protein secretion in the extracellular matrix: the pSerMGP fraction leaves VSMCs via the secretory pathway, while the SerMGP fraction is not secreted but released as cytoplasmic vesicles from the Golgi apparatus (Wajih, Borrás et al 2004). The second theory sustains that Ser phosphorylation, along with γ -carboxylation, contributes to the inhibition of calcification through MGP binding to VSMCs-derived MV (Schurgers¹ et al 2007).

There are factors regulating the MGP activity and also factors regulating the expression of MGP mRNA (see Table 1).

MGP assays. Local or circulating MGP levels are determined by using enzyme immunoassays. Competitive ELISA technique uses a single type of monoclonal antibody directed against a specific epitope of MGP and the sandwich-ELISA assay uses two antibodies directed against two different epitopes, corresponding to the modified post-translational sequences of MGP. The four monoclonal antibodies used against MGP (mAcMGP) are:

- mAcSerMGP: monoclonal antibodies against the 3-15 amino acid sequence of the dephosphorylated MGP conformation;
- mAcPserMGP: monoclonal antibodies against the 3-15 amino acid sequence of the phosphorylated MGP conformation;
- mAcGluMGP: monoclonal antibodies against the 35-54 amino acid sequence of the uncarboxylated MGP conformation;
- mAcGlaMGP: monoclonal antibodies against the 35-54 amino acid sequence of the carboxylated MGP conformation.

Antibodies for native MGP determination are not yet available. Possible conformational combinations of MGP for the sandwich-ELISA assay are shown in Figure 2.

Table 1

A. Regulating factors of MGP mRNA expression

<i>A. Regulating factors of MGP mRNA expression</i>	<i>Action</i>	<i>Observations</i>	<i>References</i>
Vitamin D ₃	+	↑ Vit. D3 levels → vascular calcifications in vivo Vit. D3 → vascular calcifications of VSMCs cultures	Farzaneh-Far et al 2001 Jono et al 1998 Owen et al 1991
Retinoic acid	+/-	Dichotomous action depending on cell type Modulator of chondrocyte mineralization/maturation ↑ retinoids consumption → vascular calcification in vivo	Farzaneh-Far et al 2001 Cancela & Price 1992
Extracellular Ca ²⁺	+	In vivo in rats: ↑ Ca ²⁺ → ↑ plasma MGP VSMCs respond to increased extracellular Ca ²⁺ levels by stimulating the expression of MGP mRNA and the MGP secretion via a mechanism mediated by cation-sensitive G protein	Price et al 2002 Farzaneh-Far et al 2000
Transforming growth factor-β (TGF-β)	+/-	Contradictory effects TGF-β will induce MGP gene expression in embryonic lung cells cultures	Farzaneh-Far et al 2001 Zhao & Warburton 1997
Epidermal growth factor (EGF)	-	↑ Inhibition of MGP transcript	Cancela et al 1997
Insulin-like growth factor 1 (IGF-1)	-	Stimulates chondrocytes differentiation Potent anti-apoptotic factor	Stheneur et al 2003
Fibroblast growth factor 2 (FGF2)	+	Inhibits chondrocytes differentiation	Stheneur et al 2003
Triiodothyronine (T3)	+	In hypothyroid rat model, MGP was reduced and aortic calcification exacerbated.	Sato et al 2005

(+ stimulation; - inhibition; ↑ increase; → result; VSMCs vascular smooth muscle cells)

Table 1

B. Regulating factors of MGP activity

<i>B. Regulating factors of MGP activity</i>	<i>Action</i>	<i>Observations</i>	<i>References</i>
Vitamin K	+	Acts as a γ -carboxylation cofactor of MGP Glu residues Because of vitamin K recycling, daily demand is decreased Vit. K deficiency causes vascular calcification, and \uparrow consumption of vitamin K has regressive effects on calcification	Stafford 2005 Price & Faus 1998 Schurgers ² et al 2007
Warfarin	-	Anticoagulant, vitamin K antagonist Treatment in the first semester of pregnancy \rightarrow risk of warfarin embryopathy Blocks vitamin K metabolism \rightarrow \downarrow activity and local MGP expression \rightarrow promotes arterial and valvular calcifications	Price & Faus 1998 Schurgers ² et al 2007 Menger et al 1997
Calumenin	-	Binds to vitamin K-dependent epoxide reductase; inhibits γ -carboxylation; contributes to the accumulation of GluMGP in atherosclerotic lesions	Wajih, Sane et al 2004
Lanthanum acetate	+	Increases GlaMGP synthesis in vessel walls	Zhou et al 2009

(+ stimulation; - inhibition; \uparrow increase; \rightarrow result; VSMCs vascular smooth muscle cells)

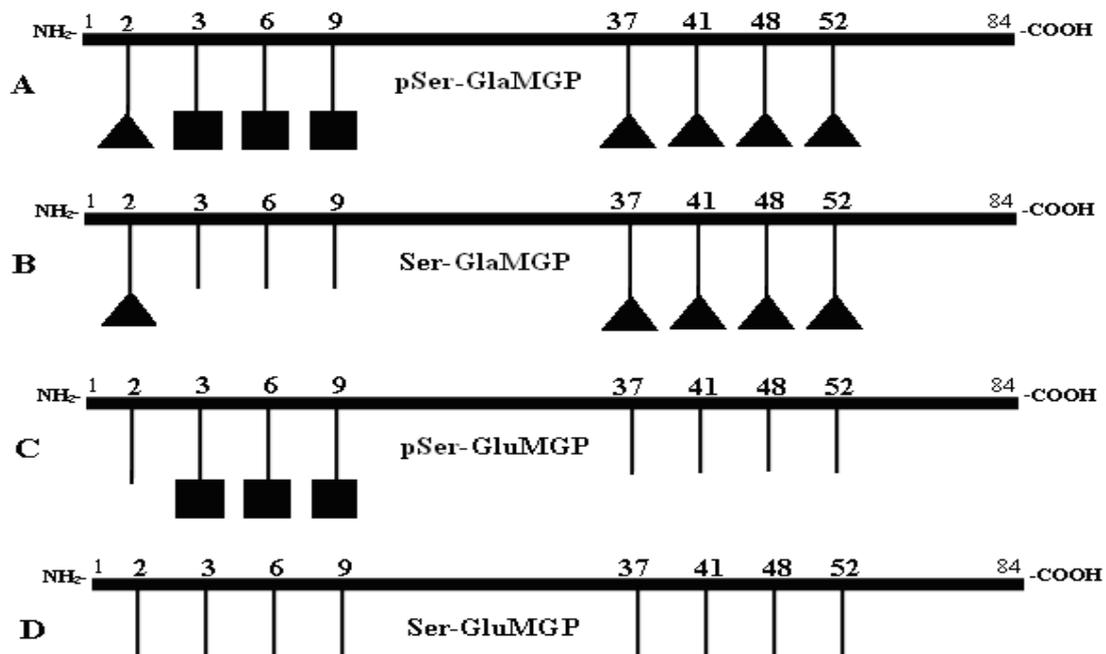


Figure 2. Possible conformational combinations of MGP for the sandwich-ELISA assay
 A - Phosphorylated and carboxylated MGP (*pSer-GlaMGP*); B - dephosphorylated and carboxylated MGP (*Ser-GlaMGP*); C - phosphorylated and uncarboxylated MGP (*pSer-GluMGP*); D - dephosphorylated and uncarboxylated MGP (*Ser-GluMGP*). The horizontal line represents the protein chain with the 84 amino acid residues; triangles are Gla residues and squares are pSer residues.

Determination of local MGP. Clinical implications. Using immunohistochemical techniques (IHC) and *in situ* hybridization in uremic and hemodialysis patients, iliac crest bone biopsy showed MGP localization and the tissue distribution of its different conformational forms (Coen et al 2009):

1. Total MGP is barely perceptible in the calcified bone matrix (monoclonal antibodies were used against the 3-15 amino acid sequence). Also, the calcified extracellular matrix of the bone biopsies showed a weak and diffuse immunostaining for GlaMGP. Using monoclonal antibodies directed against the C-and N-terminus of MGP, Spronk et al (2001) reported a weak immunostaining for MGP in the uncalcified extracellular matrix of human fetal bone.
2. Osteoblasts, osteoclasts and osteocytes (usually the youngest) had a positive reaction for total MGP and GlaMGP, and they also expressed mRNA for MGP.
3. Instead, IHC staining was negative for GluMGP in almost all bone biopsies.

We can conclude that in bone, MGP is present in its active form (GlaMGP) and, apparently, there is no deficiency of vitamin K necessary for carboxylation.

MGP synthesis by VSMCs is relatively decreased in healthy artery walls, compared to elevated levels found in intima and media of the calcified arteries, where the need in calcification inhibitors is increased (Schurgers et al 2005; Dhore et al 2001). However, in patients with diabetes, the MGP found in arteries has a lower level than in healthy vessels, thus favoring calcification (for identification, MGP polyclonal antibodies from rabbits were used against a 19 amino acid sequence from bovine MGP) (Shanahan et al 1999).

With the advent of conformational antibodies, which discriminate between GlaMGP and GluMGP, subsequent studies have shown that GluMGP accumulates in atherosclerotic plaques (at the edge of calcification areas of the intimal elastin fibers), in the media of the peripheral arteries in patients with Mönckeberg sclerosis, or in calcified walls of the aorta, while GlaMGP is almost absent at these levels (Schurgers² et al 2005; Sweatt et al 2003). Schurgers² et al (2005) offered the explanation of this phenomenon, stating that GluMGP accumulation is due to incomplete MGP carboxylation from the calcification areas of the vascular wall, due to vitamin K deficiency at this level, thus resulting in a suboptimal inhibition of arterial calcification.

An increased MGP mRNA expression and a more abundant presence of the protein was found in the varicose veins compared to non-varicose veins (Cario-Toumaniantz et al 2007). The study shows that GlaMGP is more abundant in the media of non-varicose veins than the varicose veins, and GluMGP was detected abundantly in the media of the varicose veins. Because MGP inhibits the proliferation and mineralization of VSMCs from the vein wall, the authors concluded that MGP contributes to vascular wall remodeling in varicose veins probably by altering the protein carboxylation status.

Due to development of specific conformational antibodies, numerous *in vitro* and *in vivo* studies (Sweatt et al 2003; Wajih, Borrás et al 2004; Schurgers² et al 2007) have brought new data on the processing and secretion of MGP in human VSMCs cultures, stating that GlaMGP binds to BMP-2 and GluMGP accumulates in areas of arterial calcifications in rats treated with warfarin and that vascular elasticity increases and calcifications regress in rats treated with vitamin K, due to a remediation in MGP carboxylation status.

Also, an important aspect is that circulating MGP does not induce calcification inhibition, only local MGP has an anticalcificant effect (Murshed et al 2004).

Determination of circulating MGP. Clinical implications. Because certain aspects about MGP kinetics are yet unknown, various studies present conflicting results. For instance, there is no knowledge on how MGP circulates; the only thing known is that MGP is insoluble in water and its free form was not identified in bovine serum (Wallin et al 2000). Although GluMGP was purified from human plasma by immunoprecipitation (Cranenburg et al 2008), we do not know if MGP uses a soluble protein carrier or it is transported by a lipoprotein fraction. As mentioned before, in rat serum a complex of MGP, fetuin-A, calcium and phosphate was found (Price et al 2002), but its presence was not confirmed in humans. Furthermore, it is unclear which of the possible conformations

of MGP predominates in circulation, as well as which circulating MGP conformation could be a specific marker for certain cardiovascular or osteoarticular disease.

Contradictory results in different studies are caused by the type of assay used. So far there have been described the following four assay types for circulating MGP determination:

a) Immunoassay determination of SerMGP. The first non-commercial immunoassay method for circulating MGP measurement was published by Braam et al (2000). They found significantly elevated serum SerMGP levels in patients with severe atherosclerosis or type I diabetes, while serum SerMGP in patients with low BMD and osteoporosis was normal. Using a commercial kit (Biomedica, Austria), Schurgers¹ et al (2005) have validated the competitive ELISA for SerMGP determination (slightly different from the method mentioned above). The results were as follows: diabetic/nondiabetic patients with angina pectoris showed the lowest levels of serum SerMGP, while patients with stroke and the post-myocardial infarction had normal serum levels. In patients with osteoarticular diseases (osteoarthritis, chondropathy and ankylosing spondylitis), serum SerMGP levels were decreased (not as low as in patients with angina pectoris). In patients with post-menopausal osteoporosis, SerMGP levels were close to those of reference subjects (apparently healthy), and in patients with severe kidney disease, SerMGP serum was increased, as well as osteocalcin and creatinine levels, probably because of the impaired glomerular filtration. Serum MGP levels depend on the protein synthesis and secretion rate by VSMCs and the amount of MGP present in vascular calcification areas. Part of MGP found in bone and cartilage (which is an avascular tissue), is retained at this level and therefore its participation in the circulating pool is lower. This could be caused by MGP processing at this level: in bone, a proteolytic cleavage of 7 amino acids with acid character of the C-terminal end was demonstrated, thus MGP becoming insoluble (Hale et al 1991). Probably due to the interactions of MGP with different components of bone extracellular matrix and because of the bone and cartilage structure, only a small amount of MGP may escape into circulation.

A study by Jono et al (2004) confirms the inverse association between serum SerMGP levels and the extent of coronary artery calcifications (CAC) measured by CT scan. This inverse correlation could be explained by the osteoblastic metaplasia of VSMCs in response to local calcification, thus inhibiting the synthesis of MGP. Therefore, SerMGP may predict the local stress of VSMCs (Schurgers et al 2008).

Although the mechanisms are not yet elucidated, cardiovascular diseases are the leading cause of mortality in patients with end-stage renal disease (Wang 2009). The association between serum SerMGP and CAC in patients with chronic kidney disease is often conflicting: one study reported no correlation between serum SerMGP levels and CAC in patients with chronic kidney disease (Moe et al 2005), another study found elevated serum SerMGP levels in these patients (Schurgers¹ et al 2005).

The SerMGP immunoassay uses specific monoclonal antibodies for the 3-15 amino acid sequence, the SerMGP conformation, therefore pSerMGP probably remains undetected. The method does not discriminate between native MGP and MGP fragments (both being detected), neither between GluMGP and GlaMGP conformations.

b) Radioimmunoassay (RIA) determination of MGP. O'Donnell et al (2006) have shown no consistent associations between serum MGP and CAC after adjustment for coronary heart disease risk score. They actually demonstrated that MGP is associated with risk factors for atherosclerosis, namely the decrease of HDL-cholesterol and increased Framingham risk score. Crosier et al (2009) identified an association between certain polymorphisms of MGP and CAC in men, but did not notice a correlation between serum MGP levels and CAC. Both studies used polyclonal antibodies against MGP purified from human bones. Another study concluded that dietary supplementation with vitamin K1 (phylloquinone) slows the progression of CAC determined with Agatston score, independent of serum MGP changes (Shea et al 2009).

RIA for MGP determination does not discriminate between native MGP and MGP fragments (both being detected), neither between GluMGP and GlaMGP conformations.

c) Immunoassay determination of GluMGP. The competitive ELISA (developed by a group of Dutch researchers from Maastricht), uses monoclonal antibodies directed

against the 35-53 amino acid sequence (GluMGP conformation) (Hermans et al 2007; Cranenburg et al 2008). The assay does not discriminate between native MGP and MGP fragments, neither between pSerMGP and SerMGP. Cranenburg et al (2008) reported a significant decrease in circulating GluMGP in patients with end stage renal disease (ESRD) and in calciphylaxis patients, compared with age-sex matched control subjects. Also, low GluMGP levels were found in patients with aortic stenosis and in those who underwent angioplasty. The conclusion was that GluMGP can be used as a marker of cardiovascular calcifications. Hermans et al (2007) have demonstrated a negative correlation between circulating GluMGP levels and aortic augmentation index and a positive correlation between low serum GluMGP levels and the fetuin-A. Fetuin-A, produced in the liver, is a systemic inhibitor of calcification and contributes about 50% to the calcification inhibitory capacity of plasma (Price & Lim 2003).

Association between circulating GluMGP levels and renal function and also with CAC score in dialysis patients was addressed in several studies by the Dutch research group (Cranenburg et al 2009, Parker et al 2009). They confirmed the negative correlation between circulating GluMGP levels and CAC score quantified by multi-slice computer tomography (MSCT) in hemodialysis patients. Significantly lower GluMGP levels were found in these patients (193 +/- 65nM) compared to age matched controls (441 +/- 97nM) and in patients with rheumatoid arthritis without CAC (560 +/- 140nM) (Cranenburg et al 2009). The explanation of the negative correlation between serum GluMGP levels and CAC score may be that local carboxylation status is exceeded, therefore GluMGP will accumulate at calcification sites, not being able to be released into bloodstream. The lack of correlation between circulating GluMGP and SerMGP levels (Cranenburg et al 2008), could give rise to other explanation: most of GluMGP is found in pSer-GluMGP conformation (Schurgers et al 2008) and therefore, only MGP phosphorylation itself is insufficient for MGP to bind to the sites of vascular calcification. Due to the association of low GluMGP levels with the moderate decreases of glomerular filtration rate in patients with cardiovascular disease, Dutch researchers concluded that GluMGP may be a marker of the presence and severity of vascular calcification in patients with chronic kidney disease (Parker et al 2009). The study also demonstrated a positive correlation between GluMGP levels and Ca^{2+} , phosphorus or fetuin-A, the strongest correlation being with fetuin-A.

In dialysis, Caucasian patients without diabetes, Schlieper et al (2009) have estimated the cardiovascular calcifications by a semi-quantitative Adragao calcification score (using pelvis and hand X-rays) and by a composite score of calcification, including the Adragao score, calcifications detected by x-rays of the arms fistulas, carotid ultrasound and cardiac valves echocardiography. Serum GluMGP levels were not associated with the presence of cardiovascular calcifications quantified by the methods mentioned above.

d) Immunoassay determination of Ser-GluMGP and Ser-GlaMGP. Sandwich ELISA for Ser-GluMGP and Ser-GlaMGP determination uses monoclonal antibodies directed against the 3-15 amino acid sequence (SerMGP conformation), together with monoclonal antibodies against the 35-49 sequence (for GluMGP conformation), or 35 - 54 (for GlaMGP conformation). Either of these conformations does not discriminate between native MGP and MGP fragments.

In hemodialysis patients, increased circulating levels of Ser-GluMGP and Ser-GlaMGP were observed (Schlieper et al 2011). However, low levels of Ser-GlaMGP represent a mortality predictor in dialysis patients. Ueland et al (2010) found increased plasma Ser-GlaMGP and particularly Ser-GluMGP levels in patients with symptomatic aortic stenosis. Also increased plasma Ser-GluMGP levels correlated with the severity of aortic calcifications were determined in patients with chronic kidney disease (Schurgers et al 2010). The study suggests that Ser-GluMGP could be a surrogate marker for vascular calcifications in chronic kidney disease.

Conclusions. As noted before, depending on the antibodies used, MGP can evaluate different aspects of ectopic calcifications: SerMGP could predict the local stress of VSMCs, GluMGP can be used as a marker of cardiovascular calcification, and Ser-GluMGP as a

surrogate marker for vascular calcifications in chronic kidney diseases. By altering the physiological function of MGP, vitamin K deficiency is presumed to contribute to bone demineralization and vascular calcification (the so-called calcification paradox). Therefore, prolonged use of vitamin K antagonists for long-term anticoagulation therapy can be a risk, especially in patients susceptible to vascular calcification often associated with renal osteodystrophy (manifested by alterations in bone remodeling processes).

Diversification of commercial kits required for different MGP conformations and the development of antibodies for native MGP determination, will bring new information on the role of MGP and its usefulness in monitoring vascular carboxylation status and in diagnosis or prognosis of different cardiovascular or osteoarticular diseases. The molecular mechanism of this local inhibitor of ectopic calcifications could be elucidated only if we corroborate the information on the local expression of MGP mRNA with the protein activity and with the corresponding MGP conformation found in circulation.

References

- Baudet C., Perret E., Delpech B., Kaghad M., Brachet P., Wion D., Caput D., 1998 Differentially expressed genes in C6.9 glioma cells during vitamin D-induced cell death program. *Cell Death Diff* **5**:116-25.
- Braam L. A., Dissel P., Gijssbers B. L., Spronk H. M., Hamulyák K., Soute B. A., et al, 2000 Assay for Human Matrix Gla Protein in Serum: Potential Applications in the Cardiovascular Field. *Arterioscler Thromb Vasc Biol* **20**:1257-61.
- Budoff M. J., Gul K. M., 2008 Expert review on coronary calcium. *Vasc Health Risk Manag* **4**:315-24.
- Cancela M. L., Price P. A., 1992 Retinoic acid induces matrix Gla protein gene expression in human cells. *Endocrinology* **130**:102-8.
- Cancela M. L., Hu B., Price P. A., 1997 Effect of cell density and growth factors on matrix GLA protein expression by normal rat kidney cells. *J. Cell. Physiol* **171**:125-34.
- Cario-Toumaniantz C., Boularan C., Schurgers L. J., Heymann M. F., Le Cunff M., Léger J., et al, 2007 Identification of differentially expressed genes in human varicose veins: involvement of matrix gla protein in extracellular matrix remodeling. *J Vasc Res* **44**:444-59.
- Castoldi E., Hackeng T. M., 2008 Regulation of coagulation by protein S. *Curr Opin Hematol* **15**:529-36.
- Choi S. H., An J. H., Lim S., Koo B. K., Park S. E., Chang H. J., et al, 2009 Lower bone mineral density is associated with higher coronary calcification and coronary plaque burdens by multidetector row coronary computed tomography in pre- and postmenopausal women. *Clin Endocrinol (Oxf)* **71**:644-51.
- Coen G., Ballanti P., Silvestrini G., Mantella D., Manni M., Di Giulio S., et al, 2009 Immunohistochemical localization and mRNA expression of matrix Gla protein and fetuin-A in bone biopsies of hemodialysis patients. *Virchows Arch* **454**:263-71.
- Cranenburg E. C., Vermeer C., Koos R., Boumans M. L., Hackeng T. M., Bouwman F. G., et al, 2008 The circulating inactive form of matrix Gla Protein (ucMGP) as a biomarker for cardiovascular calcification. *J Vasc Res* **45**:427-36.
- Cranenburg E. C., Brandenburg V. M., Vermeer C., Stenger M., Mühlenbruch G., Mahnken A. H., et al, 2009 Uncarboxylated matrix Gla protein (ucMGP) is associated with coronary artery calcification in haemodialysis patients. *Thromb Haemost* **101**:359-66.
- Crosier M. D., Booth S. L., Peter I., Dawson-Hughes B., Price P. A., O'Donnell C. J., et al, 2009 Matrix Gla protein polymorphisms are associated with coronary artery calcification in men. *J Nutr Sci Vitaminol (Tokyo)* **55**:59-65.
- Danziger J., 2008 Vitamin K-dependent Proteins, Warfarin, and Vascular Calcification. *Clin J Am Soc Nephrol* **3**:1504-10.
- Demer L. L., Tintut Y., 2008 Vascular calcification: pathobiology of a multifaceted disease. *Circulation* **117**:2938-48.

- Dhore C. R., Cleutjens J. P., Lutgens E., Cleutjens K. B., Geusens P. P., Kitslaar P. J., et al, 2001 Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. *Arterioscler Thromb Vasc Biol* **21**:1998-2003.
- Farzaneh-Far A., Proudfoot D., Weissberg P. L., Shanahan C. M., 2000 Matrix gla protein is regulated by a mechanism functionally related to the calcium-sensing receptor. *Biochem Biophys Res Commun* **277**:736-40.
- Farzaneh-Far A., Weissberg P. L., Proudfoot D., Shanahan C. M., 2001 Transcriptional regulation of matrix gla protein. *Z Kardiol* **90**:38-42.
- Fraser J. D., Price P. A., 1988 Lung, heart, and kidney express high levels of mRNA for the vitamin K-dependent matrix Gla protein. Implications for the possible functions of matrix Gla protein and for the tissue distribution of the gamma-carboxylase. *J Biol Chem* **263**:11033-6.
- Hackeng T. M., Rosing J., Spronk H. M., Vermeer C., 2001 Total chemical synthesis of human matrix Gla protein. *Protein Sci* **10**:864-70.
- Hale J. E., Fraser J. D., Price P. A., 1988 The identification of matrix Gla protein in cartilage. *J Biol Chem* **263**:5820-4.
- Hale J. E., Williamson M. K., Price P. A., 1991 Carboxyl-terminal proteolytic processing of matrix Gla protein. *J Biol Chem* **266**:21145-9.
- Hermans M. M., Vermeer C., Kooman J. P., Brandenburg V., Ketteler M., Gladziwa U., et al, 2007 Undercarboxylated matrix GLA protein levels are decreased in dialysis patients and related to parameters of calcium-phosphate metabolism and aortic augmentation index. *Blood Purif* **25**:395-401.
- 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**:1302-6.
- Jono S., Ikari Y., Vermeer C., Dissel P., Hasegawa K., Shioi A., et al, 2004 Matrix Gla protein is associated with coronary artery calcification as assessed by electron-beam computed tomography. *Thromb Haemost* **91**:790-4.
- Luo G., D'Souza R., Hogue D., Karsenty G., 1995 The matrix Gla protein gene is a marker of the chondrogenesis cell lineage during mouse development. *J Bone Miner Res* **10**:325-34.
- Luo G., Ducy P., McKee M. D., Pinero G. J., Loyer E., Behringer R. R., Karsenty G., 1997 Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* **386**:78-81.
- Menger H., Lin A. E., Toriello H. V., Bernert G., Spranger J. W., 1997 Vitamin K deficiency embryopathy: a phenocopy of the warfarin embryopathy due to a disorder of embryonic vitamin K metabolism. *Am J Med Genet* **72**:129-34.
- Moe S. M., Reslerova M., Ketteler M., O'Neill K., Duan D., Koczman J., et al, 2005 Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). *Kidney Int* **67**:2295-304.
- Murshed M., Schinke T., McKee M. D., Karsenty G., 2004 Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. *J Cell Biol* **165**:625-30.
- Newman B., Gigout L. I., Sudre L., Grant M. E., Wallis G.A., 2001 Coordinated expression of matrix Gla protein is required during endochondral ossification for chondrocyte survival. *J Cell Biol* **154**:659-66.
- Nishimoto S. K., Nishimoto M., 2005 Matrix Gla protein C-terminal region binds to vitronectin. Co-localization suggests binding occurs during tissue development. *Matrix Biol* **24**:353-61.
- O'Donnell C. J., Shea M. K., Price P. A., Gagnon D. R., Wilson P. W. F., Larson M. G., et al, 2006 Matrix Gla Protein is associated with risk factors for atherosclerosis but not with coronary artery calcification. *Arterioscler Thromb Vasc Biol* **26**:2769-2774.
- Owen T. A., Aronow M. S., Barone L. M., Bettencourt B., Stein G. S., Lian J. B., 1991 Pleiotropic effects of vitamin D on osteoblast gene expression are related to the proliferative and differentiated state of the bone cell phenotype: Dependency upon basal levels of gene expression, duration of exposure, and bone matrix competency in normal rat osteoblast cultures. *Endocrinology* **128**:1496-504.

- Parker B. D., Ix J. H., Cranenburg E. C., Vermeer C., Whooley M. A., Schurgers L. J., 2009 Association of kidney function and uncarboxylated matrix Gla protein: data from the Heart and Soul Study. *Nephrol Dial Transplant* **24**:2095-101.
- Price P. A., Lim J.E., 2003 The inhibition of calcium phosphate precipitation by fetuin is accompanied by the formation of a fetuin–mineral complex. *J Biol Chem* **278**:22144-52.
- Price P. A., Williamson M. K., 1985 Primary structure of bovine matrix Gla protein, a new vitamin K-dependent bone protein. *J Biol Chem* **260**:14971-5.
- Price P. A., Urist M. R., Otawara Y., 1983 Matrix Gla protein, a new gamma-carboxyglutamic acid-containing protein which is associated with the organic matrix of bone. *Biochem Biophys Res Commun* **117**:765-71.
- Price P. A., Rice J. S., Williamson M. K., 1994 Conserved phosphorylation of serines in the Ser-X-Glu/Ser(P) sequences of the vitamin K-dependent matrix Gla protein from shark, lamb, rat, cow, and human. *Protein Sci* **3**:822-30.
- Price P. A., Thomas G. R., Pardini A. W., Figueira W. F., Caputo J. M., Williamson M. K., 2002 Discovery of a high molecular weight complex of calcium, phosphate, fetuin, and matrix gamma-carboxyglutamic acid protein in the serum of etidronate-treated rats. *J. Biol. Chem* **277**:3926-34.
- Price P. A., Chan W. S., Jolson D. M., Williamson M. K., 2006 The elastic lamellae of devitalized arteries calcify when incubated in serum: evidence for a serum calcification factor. *Arterioscler Thromb Vasc Biol* **26**:1079-85.
- Proudfoot D., Skepper J. N., Hegyi L., Bennett M. R., Shanahan C. M., Weissberg P. L., 2000 Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. *Circ Res* **87**:1055-62.
- Rennenberg R. J., Kessels A. G., Schurgers L. J., van Engelshoven J. M., de Leeuw P. W., Kroon A. A., 2009 Vascular calcifications as a marker of increased cardiovascular risk: a meta-analysis. *Vasc Health Risk Manag* **5**:185-97.
- Sato Y., Nakamura R., Satoh M., Fujishita K., Mori S., Ishida S., et al, 2005 Thyroid hormone targets matrix Gla protein gene associated with vascular smooth muscle calcification. *Circ Res* **97**:550-57.
- Schlieper G., Brandenburg V., Djuric Z., Damjanovic T., Markovic N., Schurgers L., et al, 2009 Risk factors for cardiovascular calcifications in non-diabetic Caucasian haemodialysis patients. *Kidney Blood Press Res* **32**:161-8.
- Schlieper G., Westenfeld R., Krüger T., Cranenburg E. C., Magdeleyns E. J., Brandenburg V. M., et al, 2011 Circulating nonphosphorylated carboxylated matrix gla protein predicts survival in ESRD. *J Am Soc Nephrol* **22**:387-95.
- Schurgers L. J., Cranenburg E. C., Vermeer C., 2008 Matrix Gla-protein: the calcification inhibitor in need of vitamin K. *Thromb Haemost* **100**:593-603.
- Schurgers L. J., Barreto D. V., Barreto F. C., Liabeuf S., Renard C., Magdeleyns E. J., Vermeer C., Choukroun G., Massy Z. A., 2010 The circulating inactive form of matrix gla protein is a surrogate marker for vascular calcification in chronic kidney disease: a preliminary report. *Clin J Am Soc Nephrol* **5**:568-75.
- Schurgers¹ L. J., Spronk H. M., Skepper J. N., Hackeng T. M., Shanahan C. M., Vermeer C., et al, 2007 Post-translational modifications regulate matrix Gla protein function: importance for inhibition of vascular smooth muscle cell calcification. *J Thromb Haemost* **5**:2503-11.
- Schurgers² L. J., Spronk H. M., Soute B. A., Schiffrin P. M., DeMey J. G., Vermeer C., 2007 Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. *Blood* **109**:2823-31.
- Schurgers¹ L. J., Teunissen K. J., Knapen M. H., Geusens P., van der Heijde D., Kwaijtaal M., et al, 2005 Characteristics and performance of an immunosorbent assay for human matrix Gla-protein. *Clin Chim Acta* **351**:131-8.
- Schurgers² L. J., Teunissen K. J., Knapen M. H., Kwaijtaal M., van Diest R., Appels A., et al, 2005 Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. *Arterioscler Thromb Vasc Biol* **25**:1629-33.

- Shanahan C. M., Cary N. R., Salisbury J. R., Proudfoot D., Weissberg P. L., Edmonds M. E., 1999 Medial localization of mineralization-regulating proteins in association with Monckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. *Circulation* **100**:2168-76.
- Shea M. K., O'Donnell C. J., Hoffmann U., Dallal G. E., Dawson-Hughes B., Ordovas J. M., et al, 2009 Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr* **89**:1799-807.
- Spronk H. M., Soute B. A., Schurgers L. J., Cleutjens J. P., Thijssen H. H., De Mey J. G., Vermeer C., 2001 Matrix Gla protein accumulates at the border of regions of calcification and normal tissue in the media of the arterial vessel wall. *Biochem Biophys Res Commun* **289**:485-90.
- Stafford D. W., 2005 The vitamin K cycle. *J Thromb Haemost* **3**:1873-8.
- Stheneur C., Dumontier M. F., Guedes C., Fulchignoni-Lataud M. C., Tahiri K., Karsenty G., Corvol M. T., 2003 Basic fibroblast growth factor as a selective inducer of matrix Gla protein gene expression in proliferative chondrocytes. *Biochem J* **369**:63-70.
- Sweatt A., Sane D. C., Hutson S. M., Wallin R., 2003 Matrix Gla protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. *J Thromb Haemost* **1**:178-85.
- Ueland T., Gullestad L., Dahl C. P., Aukrust P., Aakhus S., Solberg O. G., et al, 2010 Undercarboxylated matrix Gla protein is associated with indices of heart failure and mortality in symptomatic aortic stenosis. *J Intern Med* **268**:483-92.
- Wajih N., Borrás T., Xue W., Hutson S. M., Wallin R., 2004 Processing and transport of matrix gamma-carboxyglutamic acid protein and bone morphogenetic protein-2 in cultured human vascular smooth muscle cells: evidence for an uptake mechanism for serum fetuin. *J Biol Chem* **279**:43052-60.
- Wajih N., Sane D. C., Hutson S. M., Wallin R., 2004 The inhibitory effect of calumenin on the vitamin K-dependent gamma-carboxylation system. Characterization of the system in normal and warfarin-resistant rats. *J Biol Chem* **279**:25276-83.
- Wallin R., Cain D., Sane D. C., 1999 Matrix Gla protein synthesis and gamma-carboxylation in the aortic vessel wall and proliferating vascular smooth muscle cells—a cell system which resembles the system in bone cells. *Thromb Haemost* **82**:1764-7.
- Wallin R., Cain D., Hutson S. M., Sane D. C., Loeser R., 2000 Modulation of the binding of matrix Gla protein (MGP) to bone morphogenetic protein-2 (BMP-2). *Thromb Haemost* **84**:1039-44.
- Wang A. Y., 2009 Vascular and other tissue calcification in peritoneal dialysis patients. *Perit Dial Int* **29**:S9-S14.
- Zhao J., Warburton D., 1997 Matrix Gla protein gene expression is induced by transforming growth factor-beta in embryonic lung culture. *Am. J. Physiol* **273**:L282-7.
- Zhou Y. B., Jin S. J., Cai Y., Teng X., Chen L., Tang C. S., Qi Y. F., 2009 Lanthanum acetate inhibits vascular calcification induced by vitamin D3 plus nicotine in rats. *Exp Biol Med (Maywood)* **234**:908-17.

Received: 01 September 2011. Accepted: 06 October 2011. Published online: 07 October 2011.

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How to cite this article:

Silaghi C. N., Crăciun A. M., and Cristea V., 2011 Matrix Gla protein: the inhibitor of vascular and osteoarticular calcifications. *HVM Bioflux* **3**(3):178-190.