The vascular phenotype in pseudoxanthoma elasticum and related disorders: contribution of a genetic disease to the understanding of vascular calcification

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Vascular calcification is a complex and dynamic process occurring in various physiological conditions such as aging and exercise or in acquired metabolic disorders like diabetes or chronic renal insufficiency. Arterial calcifications are also observed in several genetic diseases revealing the important role of unbalanced or defective anti- or pro-calcifying factors. Pseudoxanthoma elasticum (PXE) is an inherited disease (OMIM 264800) characterized by elastic fiber fragmentation and calcification in various soft conjunctive tissues including the skin, eyes, and arterial media. The PXE disease results from mutations in the ABCC6 gene, encoding an ATP-binding cassette transporter primarily expressed in the liver, kidneys suggesting that it is a prototypic metabolic soft-tissue calcifying disease of genetic origin. The clinical expression of the PXE arterial disease is characterized by an increased risk for coronary (myocardial infarction), cerebral (aneurysm and stroke), and lower limb peripheral artery disease. However, the structural and functional changes in the arterial wall induced by PXE are still unexplained. The use of a recombinant mouse model inactivated for the Abcc6 gene is an important tool for the understanding of the PXE pathophysiology although the vascular impact in this model remains limited to date. Overlapping of the PXE phenotype with other inherited calcifying diseases could bring important informations to our comprehension of the PXE disease.

Keywords: pseudoxanthoma elasticum, calcium, vessels, cardiovascular diseases, elasticity, ankle-brachial index

ARTERIAL CALCIFICATION IS AN INDEPENDENT RISK FACTOR OF CARDIOVASCULAR DISEASES

Arterial calcification is gaining an increasing interest as an independent marker for cardiovascular (CV) diseases in acquired metabolic diseases, such as type II diabetes (Becker et al., 2008) and chronic renal insufficiency (Goodman et al., 2000). Vascular calcification increases physiologically with age and studies from Egyptian mummies have revealed that arterial calcification is not a feature of modern life style due to the absence of risk factors such as smoking, high fat cholesterol diets encountered in these ancient civilizations (Allam et al., 2011).

Calcification of the intimal layers can complicate atherosclerotic plaques favoring the risk of rupture whereas deposit within the medial layer contributes to stiffen arterial wall leading to hypertension, cardiac hypertrophy, and heart failure (Demer and Tintut, 2008). Similarly to the bone, ectopic calcium sediment within the arterial wall is a dynamic and tightly regulated biological process involving a large number of cytokines and cellular pathways (Giachelli, 2004; Persy and D'Haese, 2009). Contrary to the bones, the artery is a tubular organ that should remain soft and flexible but resilient to the high distending blood pressure. The elastic properties of the arterial wall plays a key role in damping the cyclic pressure changes produced by the beating heart (O'Rourke et al., 2002). This dampening effect predominates within the large elastic vessels such as aorta and progressively decreases downstream as the vessel wall becomes more muscular in the medium and small-sized arteries allowing a continuous flow and to protect the thin walled capillaries against high pressures. Therefore, any changes in arterial wall elasticity, i.e., the recoiling force, and distensibility, (the capacity to be distended), either physiologically with aging (so called arteriosclerosis) or in response to abnormal metabolic conditions such as type II diabetes or chronic renal insufficiency, contribute to stiffen the arterial wall. Stiffening of the arterial wall will reduce the dampening effect leading to the increase in systemic arterial pressure, mainly pulse pressure, and ultimately damage the small capillaries in end-organs such as brain, kidneys, or heart (Laurent et al., 2009).

Our understanding of the direct or indirect contribution of calcification in the vascular system remains limited due to the multifactorial mechanisms. Although both elastic lamina and medial calcification could share similar genetic determinants, whether or not calcification precedes or follows the disruption and/or the degeneration of the elastic fibers is often difficult to establish (Wang et al., 2009). The resulting elasto-calcinosis refers to a timely

and well-balanced interaction between several local and remote factors (see review Atkinson, 2008). The mechanisms underlying calcification of the elastic fibers are multifactorial including physico-chemical conditions, inflammation and oxydative stress, metabolic dysfunction, and unbalanced promoters/inhibitors of calcification. This process can occur focally in the intimal layer and may complicate atheromatous plaques whereas it occurs more diffusely in the media.

The role of genetics in the calcification process is likely to take an important place since than >40% of the variance of aortic and coronary calcification phenotype could be under the control of genes. The roles of genes have been deciphered in various monogenic diseases but also in the general population (O'Donnell et al., 2002; Assimes et al., 2008; Rampersaud et al., 2008) and have been recently reviewed by Rutsch et al. (2011).

The pathophysiology of calcification in metabolic diseases is of a particular concern and the present review will focus mainly on pseudoxanthoma elasticum (PXE), an inherited disease displaying specific and unusual characteristics that belongs to a larger group of genetically and metabolically determined calcifying vascular diseases.

PSEUDOXANTHOMA ELASTICUM: AN ENIGMATIC CALCIFYING GENETIC DISEASE

Pseudoxanthoma elasticum is an inherited autosomal recessive multisystem disorder affecting connective tissues. Its phenotypic expression is characterized by the fragmentation and mineralization of elastic fibers in the skin termed elastorrhexis, the Bruch's membrane of the retina and the vasculature (Neldner, 1988; Hu et al., 2003; Uitto et al., 2011). Its prevalence is estimated from 1/25,000 to 1/50,000 and the causative mutations have been identified in the *ABCC6* gene encoding a trans-membrane ATP-binding cassette transporter, subfamily C, member 6 (ABCC6/MRP6; Le Saux et al., 2000) that is primarily expressed in the liver and the kidney, but with much lower expression in other affected tissues such as skin, eyes, or arteries (Kool et al., 1999). The biological function of the ABCC6 transporter and its substrates remains totally unknown to date. The phenotype seems to result from an unknown defect originating from the liver and the kidney leading to the extracellular calcium sediment but probably intracellular (Martin et al., 2012). Several studies have demonstrated that normal tissues exposed to the serum from PXE patients or mice knockout for Abcc6 are able to calcify (Le Saux et al., 2006; Jiang et al., 2007, 2010). Therefore, PXE is considered as a prototypical metabolic calcifying disease of genetic origin (Jiang and Uitto, 2006). PXE is also characterized by its delayed onset and a variability in its phenotypic expression suggesting that a large number of co-factors contribute to its phenotype. The classical risk factors involved in arteriosclerosis, such as tobacco, hypertension, dyslipidemia, could be greatly suspected to interfere with the severity of the vascular expression of the disease, although most of the vascular complications in PXE occur later during the life (>40 years) than the skin and eyes lesions, with an unexplained female preponderance (Uitto et al., 2010). The 2/3 female–1/3 male ratio in PXE leads to an unusual and unexplained prevalence of arterial disease in female compared to the general population. A PXE-like phenotype has also been reported in other genetic diseases such as beta-thalassemia and sickle cell anemia (Fabbri et al., 2009), cutis laxa (Vanakker et al., 2007), generalized arterial calcification in infancy (Kalal et al., 2012), a defect in gamma-glutamyl carboxylase (Vanakker et al., 2007), familial idiopathic basal ganglia (Wang et al., 2012) and more rarely induced by pharmacological substances such as seen with D penicillamine (Ratnavel and Norris, 1994). The fact that PXE phenotype could overlap with other genetic diseases suggests that these diseases share a common pathophysiology (Nitschke et al., 2012). The main phenotypical differences reported in the literature between the genetic calcifying diseases are summarized in **Table 1**.

A number of candidate substrates for ABCC6 have been hypothesized. The observation that PXE patients exhibit a low plasma vitamin K level and that anti-vitamin K drugs accelerate calcification in normal and in Abcc6$^{-/-}$ mice (Li et al., 2012) raised the hypothesis for a role of vitamin K in the calcification process. Vitamin K is a key factor for the activation of tissue calcification inhibitor factors such as matrix Gla proteins (MGPs). This hypothesis has not been confirmed due to no changes in the calcification process occurring in PXE animal models supplemented with vitamin K (Brampton et al., 2011; Fülöp et al., 2011; Gorgels et al., 2011). The implication of adenosine as a calcifying factor in PXE has also been hypothesized (Markello et al., 2011), as well as oxidative stress (Pasquali-Ronchetti et al., 2006; Zarbock et al., 2007), although the role of oxidative stress has not been evidenced by endothelial dysfunction at present in PXE.

THE VASCULAR LESIONS AND HISTOLOGICAL FINDINGS IN PXE

The elementary arterial lesions observed in PXE are characterized by mineralization of the elastic fibers of the medial layer, predominantly within the medium and small-sized musculo-elastic arteries. Abnormal elastic fibers are thought to be produced by the PXE skin fibroblasts (Quaglino et al., 2000), but could also occur with normal fibroblasts in the presence of PXE serum (Le Saux et al., 2006) or in presence of elastin degradation products (Simionescu et al., 2005). These findings suggest that PXE is a disorder of the mechanisms controlling the production of matrix constituents and that elastic fiber mineralization is caused by factors abnormally produced and entrapped within the fiber during elastin fibrogenesis (Baccarani-Contri et al., 1994). These finding called for the "elastosis hypothesis" as the primary mechanism in PXE and that calcification is secondary. A primary role for smooth muscle cells and fibroblasts in PXE is suspected as they are a source of many regulatory proteins involved in the calcification process such as alkaline phosphatase and MGP (Shanahan et al., 1999; Simionescu et al., 2005). Finally, an abnormal balance between elevated proteolysis activity with increased P-selectin (Gotting et al., 2008), matrix metallo-proteinase (MMP) 2 and 9 (Diekmann et al., 2009), suggests an abnormal remodeling of the extra cellular matrix (ECM) in PXE. Despite unknown mechanism sequence for elastosis and calcification, the nature and affected sites of calcification lead to different functional expression.

The macroscopic distribution of the calcification along the arterial tree can be mapped using standard X-ray, although 3D

Table 1 | Comparative characteristics of the arterial phenotype in PXE and other related disorders.

	Beta-thalassemia/ Sickle cell disease	PXE-like + cutis laxa	PXE	GACI	ACDC (or CALJA)
Elementary arterial phenotype					
Intima-media thickness	Increased	Unknown	Increased		
Endothelial dysfunction	Defective	Unknown	Unknown	Unknown	Unknown
Stenosis	Yes (26.7% mostly cerebral)	Yes (PAD 50%)	Yes (mostly coronary and cerebral)	Yes (mostly coronary, cerebral, and renal)	
Dilatation/aneurysm	Sporadic	Yes (mostly cerebral)	Infrequent		Popliteal
Calcification	Present	Present	Present	Present	Present
Arterial malformations			gastrointestinal, eyes (neovascularization) and carotids (?)		
Coagulation defects	Present (50%)	Present	Secondary?	Present	Present
Defective gene	HBB/HBF	GGCX	ABCC6	ENPP1	NT5E

For each disorder, bibliographical references for vascular phenotype are indicated. GACI, generalized arterial calcification of infancy (Rutsch et al., 2001; Kalal et al., 2012); ACDC, arterial calcification or CALJA, calcification of joints and arteries due to deficiency of CD73 (Markello et al., 2011); HBB/HBF, hemoglobinopathies Beta and S (Aessopos et al., 1998; Fabbri et al., 2009; Musallam et al., 2011); GGCX, gamma-glutamyl carboxylase (Vanakker et al., 2007, 2011; Li et al., 2009a); ABCC6, adenosine triphosphate-binding cassette (ABC) gene subfamily C (Boutouyrie et al., 2001; Germain et al., 2003; Kornet et al., 2004; Lefthériotis et al., 2011; Kupetsky-Rincon et al., 2012); ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase (Mackenzie et al., 2012; Nitschke et al., 2012); NT5E, Ecto-5′-nucleotidase (Markello et al., 2011; St. Hilaire et al., 2011).

reconstruction using helicoidal X-rays scans provides a more precise quantification and site identification (see **Figure 1**). In our experience, calcification accumulates mostly within the distal superficial femoral and below-knee arteries (tibialis and pedalis), a distribution mostly observed in the arteriosclerotic process associated with aging. Skin lesions are extensively documented in PXE, as skin biopsy are routinely done for diagnosis. However, histologic findings of the PXE-related vascular lesion are sparse and obtained from rare available autopsy samples. Ultrastructural analysis from the ascending aorta, iliac arteries, and vena cava from 2 males with PXE (36 and 80 years old), revealed that veins and arteries were similarly damaged (Gheduzzi et al., 2003). The alterations were not distributed homogeneously along the vessels with spotty alterations of elastic fibers, and aggregates of thin strands of amorphous elastin. The von Kossa staining revealed calcium sediment within the medial layers of the arterial wall of medium (e.g., carotids)- or small (radial)-sized arteries. In carotids, calcification was found extracellularly around elastin fibers although slight increase in intracellular calcium is also observed. Elastic fibers appears fragmented and proteoglycans accumulated preferentially within the media rather than intima compared to controls (Kornet et al., 2004). Qualitative and quantitative alteration in proteoglycans metabolism have been reported with increased heparin sulfate and decreased chondroitin sulfate in patient's urine (Maccari et al., 2003).

FUNCTIONAL CHANGES IN PXE ARTERY

The functional impacts of the arterial lesions, represented by elasto-calcinosis and proteoglycans accumulation, have been examined in a limited number of studies. Most of our knowledge is derived from non-invasive structural and dynamic observations using ultrasound techniques in living subjects and have provided important data for the understanding of the arterial lesions in PXE and their clinical expression. Three functional studies involving a small number (≈25) of PXE patients have focused on the carotid artery, an easily accessible large-sized musculo-elastic artery (Boutouyrie et al., 2001; Germain et al., 2003; Kornet et al., 2004). One of these studies has reported changes in the radial artery, a medium-sized muscular artery and the aorta, the main large-sized elastic artery of the body (Germain et al., 2003).

INCREASED INTIMA MEDIA THICKNESS

An increased carotid intima media thickness (IMT) has been reported in two human studies (Germain et al., 2003; Kornet et al., 2004) and another more recently involving mice (Kupetsky-Rincon et al., 2012). Compared to age and gender-matched patients, these changes were more marked in older patients than in younger ones under the age of 40. An increased carotid IMT is independently associated with a higher risk for CV events which could represent a relevant argument to explain an accelerated arteriosclerosis in PXE with higher than normal CV incidents (Eigenbrodt et al., 2008). This increase in IMT was not associated with an enlargement of the lumen size and was responsible for a 19% increase in the arterial wall mass. By contrast, the radial arteries exhibited a smaller lumen with a thicker IMT, suggesting an inward remodeling (Germain et al., 2003).

The larger IMT seems to result from the higher amount of proteoglycans without proliferative change in the vascular smooth muscle cells (VSMC). Proliferation of the VSMC toward the lumen leads to arterial stenosis and occlusion, and is observed in response to an abnormal mechano-transductive signaling in elastin deficiency diseases, such as Williams–Beuren syndrome (Li et al., 1998). But this may not be the sole mechanism since a higher IMT with a progressive loss of VSMC has also been reported in Progeria (Gerhard-Herman et al., 2011). More complex
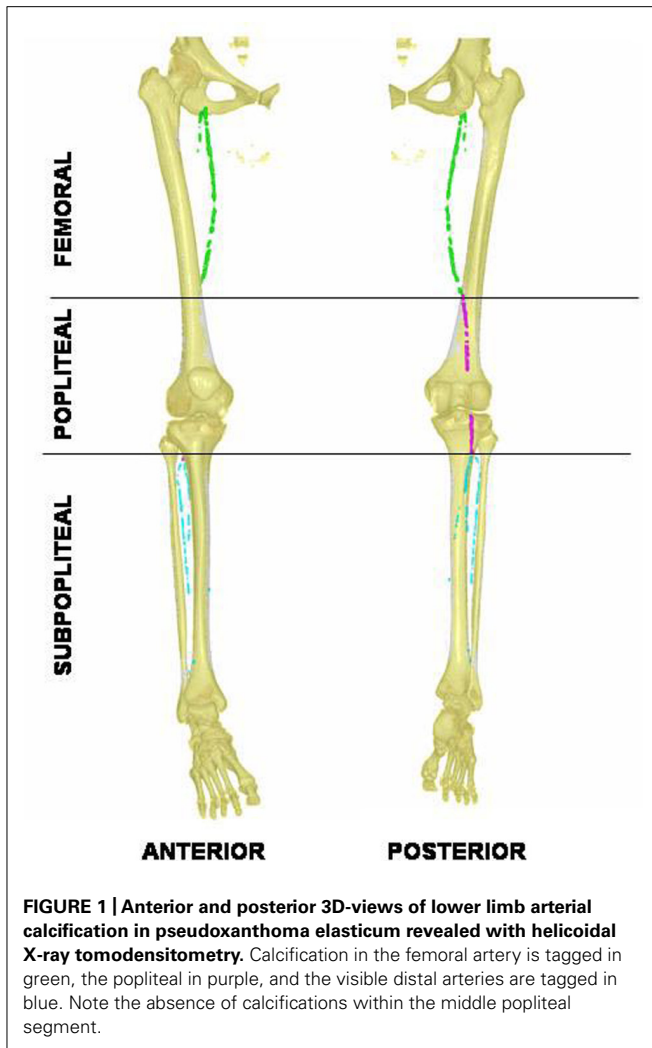
FIGURE 1 | Anterior and posterior 3D-views of lower limb arterial calcification in pseudoxanthoma elasticum revealed with helicoidal X-ray tomodensitometry. Calcification in the femoral artery is tagged in green, the popliteal in purple, and the visible distal arteries are tagged in blue. Note the absence of calcifications within the middle popliteal segment.

changes have also been reported with multilayered aspects in PXE coronary (Miwa et al., 2004) or mammary arteries (Sarraj et al., 1999).

Although these conclusions remain to be confirmed in larger cohorts, the arterial remodeling in PXE peripheral arteries exhibits differences from other arterial remodeling such as aging, hypertension, and atherosclerosis. This remodeling is characterized by intima-media thickening predominantly in the large and medium-sized musculo-elastic arteries (Boutouyrie et al., 1992). Aging is also defined by an outward remodeling whereas hypertension and atherosclerosis are rather characterized by an inward remodeling. This remodeling is variable along the arterial tree (Bortolotto et al., 1999; Bjarnegard and Länne, 2010) and with gender, women exhibiting a higher wall/lumen ratio than men (Green et al., 2010). The use of imaging techniques with higher spatial resolution will be very helpful to confirm and discriminate the relative changes of thickness between the intimal and medial layers, and between the different vascular beds.

Arterial wall stiffness and elasticity in PXE arteries: It is expected that both fragmentation of elastic fibers and calcification will affect the arterial elasticity of the PXE arteries.

Compared to age and sex-matched controls, the distensibility was found either unchanged (Germain et al., 2003) or higher (Kornet et al., 2004) in the large-sized artery, such as carotid, and in medium-sized arteries such as radial (Germain et al., 2003). Furthermore, in the older female patients, the elastic modulus was found unchanged in small-sized but was higher in the medium-sized artery. A lower or unchanged compressibility of the small-sized arterial wall was also reported in the ankle arteries of PXE patients (Leftheriotis et al., 2011).

Therefore, it is likely that the complex rearrangement of the extracellular matrix in PXE arterial wall resulting from the combination of elastin fragmentation associated to proteoglycans replacement, focal accumulation of calcium and activated MMP (Zarbock et al., 2010) in the media could mask a predicted arterial stiffness.

CLINICAL EXPRESSION OF THE VASCULAR PXE PHENOTYPE

The keystone clinical manifestations in PXE are represented by visual impairment due to the loss of central vision, large skin folds of esthetic concern, and CV complications (Neldner, 1988; Uitto et al., 2011). The vascular lesion in PXE takes a major place in the complications and the clinical outcome. Although, it seems that the lifespan of these patients is relatively preserved despite no clear reports on this point, the vascular impact of the disease is crucial and will be detailed in the following paragraphs.

ARTERIAL HYPERTENSION

Since the earliest reports, the presence of an arterial hypertension in PXE has been controversial with a highly variable prevalence reported in the literature ranging from 8% (Neldner, 1988) and up to 25% of the patients (Gotting et al., 2005). Even for the highest range, this reported prevalence remains within the overall limits of hypertension estimated to be 26.4% of the world's population (Kearney et al., 2005). In PXE, a higher than normal prevalence was likely explained on the basis of a renal artery stenosis or increased arterial stiffness resulting from the elasto-calcinosis (Goodman et al., 1963). A higher serum xylosyl-transferase (XT-1), a fibrosis marker of the extracellular matrix was found in hypertensive PXE patients (Gotting et al., 2005), though this is not supported by functional data (cf paragraph above). The association between ABCC6 and angiotensin polymorphisms (T174M and M235T) was not demonstrated in hypertensive PXE compared to normals (Gotting et al., 2005). Therefore, the higher prevalence of arterial hypertension in PXE remains to be demonstrated.

ANEURYSMS AND DISSECTIONS

An abnormally dilated and/or ruptured arterial wall is the most life-threatening complication of the PXE disease. Contrary to other genetic diseases where the connective tissues are affected, as in Marfan's or Elhers–Danlos diseases, reports of aneurysm in PXE patients are sparse and almost anecdotic.

In the cerebral vasculature, the prevalence of intracranial aneurysms is difficult to estimate. A Belgian PXE cohort ($n = 100$) based on self-reported data concluded that it was an unrelated association (van den Berg et al., 2000) while the association

between intracranial malformations, including aneurysms, and PXE may not be fortuitous (Vasseur et al., 2011).

Aneurysms in the other vascular beds such as aorta or lower limbs are very rarely reported. ABCC6 mutations were found in a minority of non-PXE patients (5/133) with abdominal aortic aneurysms (Schulz et al., 2005), but this was not statistically significantly different from healthy controls and could not be considered as a genetic risk factor for aortic aneurysms. Aorto-coronary aneurysm has also been reported (Heno et al., 1998) but seems not specific to PXE as it has also been reported in other PXE-like syndromes, such as beta-thalassemia (Farmakis et al., 2004). Common genetic factors underlie medial calcification, such as ABCC6 and aneurysm development, suggesting that although medial disruption and calcification may occur in parallel, medial disruption does not strictly occur as a result of vascular calcification (Wang et al., 2009). The possibility for a higher prevalence for arterial dissection, such as the spontaneous disruption of the internal layers of an artery, common in the carotids of PXE, is still under discussion (Brandt et al., 2005), but remains anecdotic at present. Although several missense mutations (H623Q, R3190W, and R1268Q) were found in the patients with carotid dissection, these mutations were not disease-causing as they were also detected in healthy subjects (Morcher et al., 2003).

ISCHEMIC STROKE

Beside the risk of stroke due to cerebral hemorrhage with ruptured intracranial aneurysms, the risk of ischemic stroke (IS) is another feared complication in PXE but remains difficult to establish. IS was reported in 15% of the PXE patients from a cohort of 38 patients compared to the general population (0.3–0.5%; Vanakker et al., 2008). In a cohort of 100 patients, IS was reported in seven patients with one patient having recurrent IS leading to a relative risk of 3.6 (95% confidence interval 3.3–4.0) of ISs in patients under 65 years (van den Berg et al., 2000). Focal cerebral ischemia in PXE was predominantly caused by small-vessel occlusive disease. Atherosclerotic plaques could co-exist with PXE lesions, but results from our cohort (unpublished data) showed that carotid plaques were absent in 55/93 (59.1%), unilateral in 17/93 (18.3%), and bilateral in 21/93 (22.6%) compared to age and gender-matched controls ($p = 0.987$) suggesting that it is not a primary mechanism for stroke in PXE. Transient cerebral ischemic attack could result from intermittent hemodynamic cerebral insufficiency due to intracranial arterial malformation (Vasseur et al., 2011).

CARDIAC DISEASES

Cardiac diseases in PXE are mainly represented by myocardial infarction, angina pectoris, and valvular malfunction (Neldner, 1988; Vanakker et al., 2008). Data from the largest cohorts (Neldner, 1988; Vanakker et al., 2008) have reported symptoms of myocardial origin ranging from 13 to 15% for angina pectoris but lower for infarction (1–5%) of the patients occurring at age <55 years and sometimes causing death. In the coronary arterial bed, the association with a heterozygous R1141X mutation in ABCC6 and ischemic vascular events including stroke, was not demonstrated in the general population ($n = 66831$

participants; Hornstrup et al., 2011), although a strong association was reported only with coronary artery disease (Koblos et al., 2010). Additionally, Abcc6 deficiency was found to increase infarct size and apoptosis in a mouse cardiac ischemia–reperfusion model, although there were no differences in cardiac calcification following ischemia/reperfusion (Mungrue et al., 2012). Abnormal coronary wall suggests that specific structural factors are likely present in these vascular beds (Miwa et al., 2004). Interestingly, the transferability of the PXE phenotype, i.e., calcification, to the arterial graft is still questioned (Sarraj et al., 1999; Iliopoulos et al., 2002; Song et al., 2004).

PERIPHERAL ARTERIAL DISEASE

Contrary to the other arterial beds, an early and severe peripheral arterial disease (PAD) described as a slowly worsening lower limb claudication is consistently and extensively reported in PXE patients without obvious CV risk factors. PAD is detected clinically by absence of ankle pulse, and the presence/absence of symptoms of a lower limb claudication such as a calf pain that limits or interrupts a walk. Additionally, the severity of PAD can be objectively determined by measuring the ankle-brachial systolic pressure index (ABI) which corresponds to the ratio of the systolic ankle and brachial pressures that normally ranges from 0.9 to 1.39, and the treadmill walking distance with the help of transcutaneous PO2 (Abraham et al., 2003). The prevalence of lower limb claudication, the symptomatic expression of PAD, is very high in PXE (53% in the Belgian cohort Vanakker et al., 2008 and 42% in ours) (Leftheriotis et al., 2011), a proportion clearly higher than the 9% men and 5% women with PAD reported in the general population. By contrast, the treadmill test, an objective evaluation of the arterial claudication, showed that only 56% of PXE with an ABI <0.90 were symptomatic during the test. The discrepancy between a high proportion of PAD detected by ABI with relatively less symptoms of intermittent claudication suggests that the PAD is well compensated by an efficient collateral circulation in PXE. This tolerance to ischemia was further demonstrated in our cohort by relatively well-preserved tissue oxygenation in PXE patients during walking. Interestingly, a relatively high incidence of subclinical peripheral artery disease (41%) was also reported in the carrier population ($n = 21$) suggesting that PAD could represent a frequent clinical manifestation, even in heterozygous patients. In addition to the presence/absence of ankle artery pulse, the ABI is a validated diagnostic tool for the detection of PAD and estimation of its severity, mainly in the asymptomatic patient (Diehm et al., 2009). PAD is also an independent marker of atherosclerosis associated with higher rates of CV diseases in the general population (Golomb et al., 2006). Calcifications in the tunica media of PXE patients are expected to increase arterial stiffness and thus decrease arterial wall compressibility (Kim et al., 2012). Although calcification predominates in the small-sized ankle arteries, the preserved arterial compressibility in these arteries remains unexplained.

HEMORRHAGE

Hemorrhages are a frequent ophthalmologic complication in PXE due to the rupture of proliferative choroidal neovascularization

secondary to angioid streak. To a lesser extent, gastrointestinal hemorrhage have also been reported and represent a life-threatening condition in PXE. The mechanism of hemorrhage remains unknown, but suggests sub-mucosal arterial malformations fragilized by the medial calcification with risk of rupture likely to the Bruch's membrane in the eyes. Neovessels and/or malformation could also result from defective regulatory pathways and vascular endothelial growth factor (VEGF) gene polymorphisms such as the c.-460T and the c.674C alleles that are independent risk factors for development of severe retinopathy (Zarbock et al., 2009). The genetic deficiency gamma-glutamyl carboxylase (GGCX), which is a PXE-like syndrome associated with an abnormal production of coagulation factors, are more prone to severe uncontrol bleeding (Li et al., 2009a).

WHAT DOES PXE MODELS TEACH US ABOUT CARDIOVASCULAR DISEASES?

Similar to other genetic diseases, the use of genetically engineered organisms such as mouse or zebrafish (*Danio rerio*) is helpful in our understanding of the PXE pathophysiology. Abcc6$^{-/-}$ mice exhibit a low-to-mild level of medial arterial calcifications (Gorgels et al., 2005; Klement et al., 2005), although most calcification processes develop markedly in specific organs such as the vibrissae, and represents an interesting marker of peripheral calcification (Le Corre et al., 2012). Other mouse strains such as C3H/HeOuJ are spontaneously prone to soft connective tissues calcification, due to the defective function of Abcc6, that can be easily followed and quantified using X-rays (Le Corre et al., 2012). Finally, the zebrafish model exhibits severe abnormal development that were fully rescued by co-injection of mouse Abcc6 mRNA (Li et al., 2010). Although the mechanism of the arterial lesions in PXE remains unexplained, the fact that exteriorized tissue lesions develop remotely from the predominant sites of ABCC6 demonstrates that modifying factors are at play and limits our conclusions from these mouse models. There is now a constellation of data arguing for the concomitant role of modifying genes (Li et al., 2007; Hendig et al., 2008), polymorphisms (Schon et al., 2006) and mutations, regulatory pathways (Martin et al., 2011) as well as nutritional and environmental factors (Zarbock et al., 2007, 2009, 2010; Pisciotta et al., 2009) considerably widening the phenotypic expression and severity of the disease. Furthermore, the selective involvement of anatomical sites such as skin, eyes, arteries is usually explained by the presence of elastic fibers (with the exception of the lungs), but recent data suggests that collagen fibers could also be involved (Gorgels et al., 2012).

THERAPEUTIC ISSUES IN THE PXE ARTERIAL DISEASE

In absence of validated and specific therapeutic targets in PXE, the treatment of arteriopathy remains limited. Vitamin K was not proven as an efficient treatment, although no data are presently available in human but the lack of a consistent result in proof-of-concept studies conducted in mice are not encouraging (Gorgels et al., 2011; Jiang et al., 2012). Despite disappointing initial results from a pilot study conducted in humans (Yoo et al., 2012), attempt to reduce calcification using phosphate binder (LaRusso et al., 2009) or magnesium supplementation

has recently gained renewed interest with conclusive results in Abcc6$^{-/-}$ mice (LaRusso et al., 2009; Li et al., 2009b; Kupetsky-Rincon et al., 2012). Treatment of acute ischemic complications is sporadically reported in the literature without obvious difference compared to the general population, although reports of limb amputation in the literature have never been made to our knowledge.

Finally, the careful management of CV risk factors remains an important point since atheroma could mask pre-existing lesions. Factors that could aggravate arterial calcification should be avoided in these patients such as anti-vitamin K which is an oral anticoagulant drug known to favor calcification (Price et al., 1998) that can now be advantageously replaced by anti-Xa oral therapy after the hemorrhage risk/benefit balance has been properly evaluated. The question of preventive anti-platelet therapy for IS is unsolved at present. In the general population, anti-platelet treatment such as aspirin is advised for secondary prevention of IS, but the higher prevalence of upper gastrointestinal hemorrhages in PXE remains a firm contraindication for the use of aspirin as well as for anticoagulants. A major challenge for all clinical trials attempted in PXE is the need for an objective and reproducible quantification of the soft-tissue calcification. In this way, quantification of the arterial calcification could be an interesting tool complementary to skin biopsy or ultrasound imaging and ophthalmologic follow-up since the severity of cutaneous manifestations and angioid streak of PXE are likely predictive of CV involvement (Utani et al., 2010).

In conclusion, although the arterial remodeling observed in PXE shares some of the features of arteriosclerosis and other calcifying vascular diseases of metabolic origin, it is not readily comparable to atherosclerosis. This raises challenging questions on the central role of the hepato-renal axis in the soft-tissue calcifying processes and PXE represents a prototypical systemic metabolic disease of genetic origin. The severity of the disease is progressive and highly variable in which CV symptoms and PAD seems a constant finding in these patients. Furthermore, the higher than expected compressibility of the arterial wall may represent a useful marker as well as quantification of the arterial calcium load. Data from larger cohorts are awaited with more detailed phenotypic descriptions. From a clinical point of view, the absence of efficient therapy, the follow-up of these patients first requires a tight control and management of the usual CV risk factors in addition to the limitation of pro-calcifying factors. The place of other anti-calcifying drugs, such as denosumab remains unknown at present. Finally a comparative analysis of the overlapping phenotypes such as PXE, generalized arterial calcification of infancy (GACI), and other PXE-like diseases is likely to add valuable information on the elusive mechanism of calcifying genetic diseases.

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