Pseudoxanthoma elasticum: diagnostic features, classification and treatment options

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Introduction: Pseudoxanthoma elasticum (PXE), a multisystem orphan disease, clinically affects the skin, the eyes and the cardiovascular system with considerable morbidity and mortality. The clinical manifestations reflect the underlying pathology consisting of ectopic mineralization of peripheral connective tissues.

Areas covered: The diagnostic criteria of PXE include characteristic clinical findings, together with histopathology of accumulation of pleiomorphic elastic structures in the dermis with progressive mineralization, and the presence of mutations in the ABCC6 gene. PXE-like cutaneous changes can also be encountered in other ectopic mineralization disorders, including generalized arterial calcification of infancy (GACI) caused by mutations in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene. In some cases, overlapping clinical features of PXE/GACI, associated with mutations either in ABCC6 or ENPP1, have been noted. PXE demonstrates considerable inter- and intrafamilial heterogeneity, and consequently, accurate diagnosis is required for appropriate classification with prognostic implications. There is no effective and specific treatment for the systemic manifestations of PXE, but effective therapies to counteract the ocular complications are in current clinical use.

Expert opinion: A number of observations in the animal model, the Abcc6−/− mouse, have indicated that the mineral composition of diet, particularly the magnesium content, can influence the severity of the mineralization phenotype. These observations suggest that appropriate dietary interventions, coupled with lifestyle modifications, including smoking cessation, might alleviate the symptoms and improve the quality of life of individuals affected with this, currently intractable, orphan disease.

Keywords: ectopic mineralization disorders, generalized arterial calcification of infancy, heritable orphan diseases, pseudoxanthoma elasticum

1. Introduction

Pseudoxanthoma elasticum (PXE) is a prototype of heritable ectopic mineralization disorders with clinical involvement of the skin, the eyes and the cardiovascular system (Table 1) [1,2]. French dermatologists provided the original description of skin findings, in association with elastic fiber degeneration in the skin and heart, in late 1800s with the diagnosis of ‘xanthelasmas’. Following extensive re-examination of previously published cases, Darier in 1896 called the skin lesions ‘pseudo-xantome elastique’ differentiating them from xanthomas, thus the name ‘pseudoxanthoma elasticum’ [3]. Subsequently, Grönblad and Strandberg, an ophthalmologist and a dermatologist from Sweden, respectively, established the...
association of cutaneous manifestations with eye findings, particularly angioid streaks, giving rise to the now obsolete eponym Grönblad–Strandberg syndrome [4,5]. The cardiovascular manifestations were recognized as part of the disease spectrum of PXE several decades later [6]. Finally, the recent progress in molecular genetics, with identification of the underlying mutant genes, primarily ABCC6, has helped to clarify the clinical constellations of this complex multisystem disorder [7].

The precise prevalence of PXE is currently unknown, but estimates suggesting 1 in 50,000 would imply that there are ~7000 – 8000 PXE patients in the USA. With the same global prevalence, there could be as many as 150,000 patients affected with PXE worldwide. Even this number may be an underestimate, since PXE, like many genetic conditions, has variable expressivity and its milder forms often remain undiagnosed for years or even for the lifetime [1]. Although PXE is a rare disease, it presents with significant clinical manifestations similar to common conditions, with considerable morbidity and occasional early mortality. Specifically, its ocular and cardiovascular manifestations mimic age-related macular degeneration and atherosclerotic vascular calcification, respectively, serving as a genetic model to unravel the pathomechanisms of these common, ‘acquired’ age-associated degenerative disorders.

2. Clinical features

PXE can present a diagnostic challenge to practicing physicians for several reasons. First, the clinical manifestations are late-onset, and the early cutaneous findings, frequently the first diagnostic sign of PXE, are subtle and usually not recognized until the second or third decade of life. Therefore, as is typical with many rare diseases, the ‘diagnostic odyssey’ lasts a decade on average or even longer. Often, individuals are not diagnosed until the development of ocular and vascular complications. Second, there is considerable interfamilial heterogeneity, and even within some families the involvement of different organ systems may predominate. The reasons for this phenotypic heterogeneity remain currently undiscovered, and specifically, attempts to establish genotype/phenotype correlations have to date failed to establish one [8,9]. It is conceivable that genetic modifier genes and epigenetic factors, in addition to dietary and lifestyle variables, can explain the phenotypic variability [2]. Finally, adding to the diagnostic challenge are the observations that skin findings similar to those in PXE have been encountered in a number of both acquired and heritable disorders.

2.1 Skin manifestations

The primary cutaneous lesion in PXE is a small, discrete papule with yellowish hue, and these develop initially on predilection sites, such as lateral neck and axillae, as well as antecubital and popliteal fossae [1]. These papules slowly coalesce into larger plaques that eventually render the skin leathery and inelastic (Figure 1A). Although the skin findings are primarily of cosmetic concern, they signify the potential for ocular and vascular complications with considerable morbidity. It should be noted, however, that the skin findings can be minimal even in the presence of significant ocular or vascular manifestations.

The characteristic histopathologic finding in the skin lesions is accumulation of pleiomorphic elastotic material in the mid and lower dermis that reveals progressive mineralization, as detected by routine hematoxylin–eosin stain or more readily by mineral-specific stains, including Alizarin red and von Kossa. Analysis of the ion composition of the mineral deposits by energy-dispersive X-ray has demonstrated the presence of calcium and phosphorus in an approximately 2:1 ratio, consistent with calcium hydroxyapatite [1,10,11].

2.2 Eye findings

The characteristic eye finding is the angioid streaks present in essentially all patients after 20 years of the disease [1,12]. However, the earliest ocular finding in PXE is the presence of peau d’orange, an appearance of the fundus reminiscent of the name it bears [12]. Following peau d’orange, angioid streaks develop in almost all cases as a result of mineralization...
of Bruch’s membrane, an elastin-rich sheath separating the retinal pigment epithelium from the choroid layer (Figure 1B). As this membrane becomes progressively calcified, it has a propensity to fracture, allowing choroidal neovascularization, that is, the growth of fragile blood vessels through the membrane. These vessels may leak fluid or bleed, causing distortion of vision. The natural history of subretinal hemorrhage in PXE is to heal with scarring, which in the macula can cause central visual loss and legal blindness.

2.3 Vascular involvement

The clinical manifestations of the vascular involvement include loss of peripheral pulses, intermittent claudication, renovascular hypertension, acute upper gastrointestinal hemorrhage, intestinal angina, coronary artery disease presenting with angina pectoris and less frequently myocardial infarction [13-15]. Cerebrovascular disease presents as transient ischemic attacks, ‘ministrokes’ and multi-infarct dementia [16]. These findings result from progressive mineralization of the elastic media and intima primarily in mid-sized arteries, associated with intimal hyperplasia and arterial narrowing, as well as mineralization of other cardiovascular tissues (Figure 1C).

Overall, increased risk of cardiovascular complications has been observed not only in patients with PXE but also in heterozygous ABCC6 mutation carriers. In particular, heterozygous carrier status of the loss-of-function mutation p. R1141X, the most common mutation in the Caucasian populations, is associated with a strong increase in the prevalence of coronary artery disease [17-19].

Table 1. Clinical manifestations and tissue involvement in different genetic variants of PXE.

| Variant of PXE | Predominant organ involvement | Characteristic clinicopathological features |
|---------------|-------------------------------|-------------------------------------------|
| ABCC6 associated | Skin, eyes, arteries | Late-onset, slowly progressive mineralization; loss of visual acuity; cardiovascular manifestations |
| ENPP1 associated | Arterial blood vessels | Prenatal or early postnatal mineralization; demise at <1 year of age |
| GGCX associated | Skin | Mineral deposits in mid-dermis; loose and sagging skin; vitamin K-dependent coagulation defect |

ENPP1: Ectonucleotide pyrophosphatase/phosphodiesterase 1.

Figure 1. Clinical findings in patients with PXE. (A) Characteristic skin lesions on the predilection site on the neck, with yellowish papules coalescing into a plaque of inelastic and leathery skin. (B) Presence of angioid streaks. (C) Mineralization of the aortic valve revealed by echocardiography in a 6-year-old patient with PXE-like skin findings and cardiovascular involvement due to mutations in the ABCC6 gene.

Adapted from [85].
PXE: Pseudoxanthoma elasticum.

3. Diagnosis and classification

In developing a classification for a disease, such as PXE, there are several considerations. For example, the diagnostic criteria should be specific based on diagnostic findings, they should be sensitive and not limited to a minority of patients, and they should be easily detectable and quantitatively measurable. Such criteria should also show high inter-observer reliability, should have been validated and should be applicable to different age groups. Such criteria are necessary for the development and standardization of clinical trials, understanding the histopathology and correlate the phenotypic findings with pathogenetic observations, thus helping to translate the meaning of the molecular data. The presence of diagnostic criteria is also important for disease registries to produce meaningful and comparable data.

The early attempts to devise a classification system for PXE date back to 1970s, when Pope described four subtypes based on clinical findings and the proposed mode of inheritance, autosomal dominant versus autosomal recessive, in a cohort of 121 patients [20]. One of the difficulties with this classification was that the clinical signs were not particularly well delineated. Furthermore, with the advent of molecular genetics and identification of the mutant genes, it has been demonstrated that the inheritance of PXE is exclusively autosomal recessive, and there is no autosomal dominant form of this disease [21,22]. In 1988, Neldner proposed that histopathological diagnosis of a lesion on the neck or a flexural area should be an inclusion criterion, but this approach eliminates potential cases without characteristic skin lesions or with atypical histopathology [1].
Table 2. Diagnostic criteria for PXE – 2014.

Definitive PXE*

- Two pathogenic mutations in the ABCC6 gene

OR:

- Ocular findings – angioid streaks > 1 DD or peau d’orange in an individual < 20 years of age

Together with

- Skin findings
  - Characteristic pseudoxanthomatous papules and plaques on the neck or flexural creases
  - Diagnostic histopathological changes in lesional skin: Calcified elastic fibers in the mid and lower dermis, confirmed by positive calcium stain

Note that if definitive findings are present only in the skin or eyes, the presence of two pathogenic ABCC6 mutations revealed by subsequent genetic testing would confirm the diagnosis of PXE even in the absence of a complete phenotype

Possible PXE

- Without having met the above criteria, a patient could be considered to have ‘possible PXE,’ the degree of probability depending on the presence of other factors, including family history and particularly affected siblings; microcalcifications in arterial blood vessels and other organs; histopathological changes in apparently unaffected skin; presence of a single PXE-associated mutation in either ABCC6 or ENPP1, especially if the same mutation has been found in an affected sibling

*Note that the diagnosis of definitive PXE requires the exclusion of β-thalassemia, sickle cell disorder and the atypical PXE-cutis laxa phenotype due to γ-glutamyl carboxylase deficiency as a result of mutations in the GGCX gene.

ENPP1: Ectonucleotide pyrophosphatase/phosphodiesterase 1;

PXE: Pseudoxanthoma elasticum.

In 1992, a consensus conference proposed classification of patients into two major categories, based on histopathology and clinical findings in the skin and the eyes. A combination of these criteria allowed classification of patients either into ‘certain PXE’ (Category I) or ‘uncertain PXE’ (Category II) [23]. Vanacker et al. in 2008 classified PXE patients into ‘definite’, ‘probable’ and ‘probably not’ categories, based on skin evaluation and ophthalmologic examination [24].

In 2010, Plomp et al. presented a classification that incorporated the identification of mutations in the ABCC6 gene to complement clinical signs and symptoms [25]. These authors proposed the following guidelines for diagnostic criteria: i) examination of the skin by a dermatologist or physician familiar with PXE; ii) a skin biopsy from an affected lesion, or if not applicable, from the lateral side of the neck or from a scar, stained with hematoxylin-eosin, Verhoeff-von Gieson (for elastin) and von Kossa (for calcium deposits) stains; iii) fundoscopy by an experienced ophthalmologist for angioid streaks, peau d’orange, macular degeneration, ‘comets’ and ‘wing’ signs; and iv) mutation analysis of the ABCC6 gene. In this approach, a definitive diagnosis of PXE cannot be made on the basis of clinical criteria alone while histopathology and/or mutation analysis is required to complement the phenotypic findings. This classification also excludes conditions with PXE-like cutaneous findings due to mutations in genes other than ABCC6. The criteria proposed by Plomp et al. also take into account the late onset of the disease, noting that if the diagnosis of PXE is not definitive in suspected individuals under 30 years of age, the disease should be considered provisional, and clinical examinations should be repeated subsequently at 5-year intervals [25].

Several recent developments relating to PXE, including expansion of the clinical spectrum of PXE and identification of mutations in patients with PXE-like cutaneous features in another gene, ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), in addition to ABCC6, has prompted us to refine the classification of PXE (Table 2) [26,27]. In general, the 1992 consensus conference criteria have served clinicians and researchers rather well, despite the fact that the use of subtypes has not been widely accepted, and the diagnosis still remains ambiguous in some cases of PXE. A significant improvement in the diagnostic criteria is based on identification of the ABCC6 as the major gene harboring mutations in PXE in 2000 [28-31]. More recently, a second genetic locus, the ENPP1 gene, has been identified [26,32]. Furthermore, with expansion of the PXE phenotype to include conditions with an overlap with generalized arterial calcification of infancy (GACI), it is felt that refinement of the classification criteria is timely.

The gold standard for diagnosis of PXE is finding either homozygosity or compound heterozygosity for known disease-causing mutations in the ABCC6 gene. There may sometimes be difficulty in distinguishing some missense mutations from inconsequential polymorphisms, but a recently developed mRNA phenotypic rescue assay in zebrafish can assist in distinguishing between these two possibilities [33,34]. There are, to our knowledge, no cases in which the presence of two mutations in the ABCC6 gene has not been associated with clinical findings of PXE in individuals older than 30 years of age. It is felt, therefore, that the presence of two mutations in ABCC6 alone would establish the diagnosis of PXE. In this context, it should be noted that mutations in the ENPP1 gene have recently been identified in patients with PXE-like clinical presentation. Some of these patients have predominantly vascular presentation, together with characteristic skin findings of PXE, suggesting a phenotypic overlap with GACI [27,35]. It is debatable, if these patients presenting with vascular and cutaneous manifestations at an early age should be diagnosed as having GACI with PXE-like cutaneous findings or as pediatric PXE. Perhaps these patients should be appropriately characterized as a PXE/GACI overlap condition.

The presence of one or two major eye findings (i.e., peau d’orange and/or angioid streaks > 1 DD) in conjunction with one or two skin findings, that is, i) classical pseudoxanthomatous skin lesions, papules or plaques with or without cutaneous...
laxity; and/or ii) a positive skin biopsy taken from lesional skin, or taken from the neck or flexural area in the absence of physical lesions, should be diagnostic of PXE. It should be noted that this classification proposes exclusion of β-thalassemia, sickle cell disease and multiple vitamin-K-dependent coagulation factor deficiency due to GGCX mutations as being diagnosed as PXE. As noted in recent studies of genetically distinct patient populations, phenotypic expression of PXE can be highly variable, but essentially all classic PXE patients with two ABCC6 mutations had ocular findings and skin lesions [24,36]. Thus, PXE may not be definitively diagnosed if either skin or eye findings are absent, unless two ABCC6 mutations are found in molecular genetic testing. It should also be noted that the proposed classification does not take into account the presence of vascular manifestations due to the fact that most of them are relatively common in populations at large, and consequently, these signs lack specificity.

Typical skin lesions, even when confirmed by histopathology, cannot confirm the diagnosis of PXE when occurring without ocular findings. In this case, the diagnosis could be ‘possible PXE’, unless there are two pathogenic ABCC6 mutations. Ocular findings in PXE are usually unequivocal, but in adults in whom no definitive angioid streaks are found or in whom the streaks are not typical in appearance, fluorescein angiography or indocyanine green angiography should be performed. The presence of unequivocal angioid streaks on fundus angiography would fulfill one of the major diagnostic criteria for PXE.

One of the main limitations of molecular genetic testing is that ~10% of individuals who fulfill the current diagnostic criteria for definitive PXE have either no or only one detectable mutation in ABCC6. Several possible reasons have been postulated, including the fact that the entire introns and 5’-regulatory regions of the gene are not sequenced when PCR amplification of the exons and flanking intronic sequences is carried out [8]. Furthermore, in a limited number of cases with PXE-like cutaneous changes, often associated with extensive vascular involvement, mutations in the ENPP1 gene have been disclosed [26,32]. Therefore, although finding two pathogenic ABCC6 mutations is the gold standard for the diagnosis of classic PXE, the lack of sensitivity of current genetic testing and potential locus heterogeneity make the accompanying clinical criteria especially important for accurate diagnosis.

4. Differential diagnosis

The difficulty in diagnosing PXE, particularly by non-dermatologists, often relates to the observation that PXE-like cutaneous lesions, with and without histopathological findings of ectopic mineralization, can be encountered in unrelated acquired and heritable conditions. Skin findings similar to those in PXE, but without evidence of mineralization of the elastic structures of the skin, can be encountered in a number of situations. For example, age-associated cutaneous findings on the sun-exposed areas, particularly on the neck, including actinic elastosis and cutis rhomboidalis nuchae, can be reminiscent of those in PXE [37]. Based on clinical observations, papillary dermal and mid-dermal elastolysis, white fibrous papulosis and sequelae of long-term treatment with high doses of penicillamine, as well as late-onset focal dermal elastosis can present with findings suggestive of PXE [37,38]. However, histopathology of these lesions does not reveal tissue mineralization. PXE-like cutaneous changes, with ectopic mineralization, can be encountered in patients with β-thalassemia and sickle cell anemia, two genetic conditions in which, however, there are no mutations in the ABCC6 gene [39]. A rare acquired form of PXE, with characteristic ultrastructural and histopathologic findings, has also been described in the antecubital fossae of farmers as a result of topical exposure to calcium nitrate-containing fertilizers [40]. Skin findings with mineral deposits have also been reported in patients with idiopathic hypercalcemia, hyperphosphatemia, familial tumoral calcinosis and calciphylaxis, but these conditions frequently demonstrate skin ulcerations and necrosis, which are not features of PXE [41].

Recent studies have demonstrated considerable genotypic and phenotypic overlap between GACI and PXE [32,35]. In their classic forms, these two conditions are distinct (Table 1). Typical GACI presents with extensive vascular mineralization characteristically noted at birth. The diagnosis is often made by prenatal ultrasound, and the majority of these patients die within the first 6 months of life from complications of vascular mineralization [42,43]. This contrasts with the typical presentation of PXE, with late onset of manifestations and relatively normal lifespan in most affected individuals. Initially, GACI was shown to result from mutations in the ENPP1 gene, which encodes an enzyme, ENPP1 [43]. This enzyme converts ATP to AMP and PPi, the latter being a powerful physiologic inhibitor of tissue mineralization. In the absence of ENPP1 activity, the PPi levels are significantly reduced allowing local precipitation of calcium and inorganic phosphate in the form of hydroxyapatite. While PXE was originally shown to result from mutations in the ABCC6 gene, which encodes a transmembrane transporter protein primarily expressed in the liver (Figure 2), recent studies have demonstrated that some patients with PXE-like findings can also harbor mutations in ENPP1. In some of these cases, the clinical findings are indistinguishable from those in patients with mutations in the ABCC6 gene. On the other hand, recent studies have demonstrated that patients with GACI with extensive arterial mineralization and early demise as a result of cardiovascular complications can also harbor mutations in the ABCC6 gene [26,27]. In some cases, these mutations are the same as those found in patients with relatively mild, slowly progressing PXE. These observations suggest that the biological processes controlled by ABCC6 and ENPP1 gene products may, as a result of loss-of-function mutations, converge to similar pathways leading to ectopic mineralization [27,32,44]. At the same time, the considerable intrafamilial heterogeneity, even as a result
of the same mutations in the ABCC6 gene [45], suggests the influence of modifier genes on the genetic background of affected individuals, epigenetic modulation and/or influence of environmental and lifestyle factors, including diet. Collectively, the observations on GACI and PXE, together with recognition of other ectopic mineralization disorders affecting the skin and the arterial blood vessels, including normophosphatemic familial tumoral calcinosis resulting from SAMD9 mutations [46], and CD73 deficiency due to mutations in the NT5E gene [47], suggest the presence of an intricate mineralization/anti-mineralization network in tissues.

Recent observations of the presence of PXE-like cutaneous findings in patients with vitamin K-dependent multiple coagulation factor deficiency due to mutations in the GGCX gene have also been thought to have pathomechanistic implications for PXE [48,49]. Specifically, the patients with mutations in the GG CX gene, which encodes γ-glutamyl carboxylase, show PXE-like cutaneous findings together with excessive folding and sagging of the skin similar to cutis laxa. However, the histopathologic findings are characteristic of PXE and show mineralization of elastic structures, a finding not present in patients with cutis laxa. These patients also demonstrate an excessive bleeding phenotype due to the fact that the γ-glutamyl carboxylase is required for activation of vitamin K-dependent coagulation factors, that is, prothrombin and factors VII, IX and X [50].

The observations of PXE-like cutaneous changes in patients with GG CX mutations initially suggested that defective
of its derivatives, including vitamin K3 demonstrated that ABCC6 does not transport vitamin K or some gate [57]. These experiments indicated that the PXE phenotype is not a direct consequence of vitamin K deficiency.

The considerable overlap noted in some patients with PXE and GACI, with overlapping gene defects in ABCC6 and ENPP1, has suggested another potential treatment for a subset of patients with PXE. Specifically, ENPP1 mutations in patients with GACI result in deficiency of PPi, and a few clinical studies have suggested that feeding of these patients with bisphosphonates, pyrophosphate analogs, such as etidronate, may be helpful in counteracting the mineralization [70,71]. Recent observations on Abcc6−/− mice have suggested that they also may have reduced content of PPi in their plasma [72], and indirect evidence on PXE patients’ fibroblasts suggests altered PPi metabolism [73], potentially implying the presence of its derivatives from liver into the circulation and the deficiency of such factor(s) in peripheral tissues could result in predominance of the uncarboxylated, inactive form of MGP, allowing the mineralization processes to ensue [53]. This hypothesis was largely discounted, however, by subsequent demonstrations that feeding of Abcc6−/− mice, an animal model of PXE, with excessive amounts of vitamin K did not counteract the ectopic mineralization [54-56]. Furthermore, in vitro studies demonstrated that ABCC6 does not transport vitamin K or some of its derivatives, including vitamin K3-gluthione conjugate [57]. These experiments indicated that the PXE phenotype is not a direct consequence of vitamin K deficiency.

5. Treatment options

In spite of considerable progress made in understanding the genetic basis of PXE, the pathomechanistic details leading from mutations in the ABCC6 gene to the ectopic mineralization of the peripheral connective tissues remain unclear. In particular, the specific function of ABCC6 and the identity of physiologically transported substrates are unknown [2]. Consequently, there is no specific treatment for the systemic manifestations of PXE. However, effective treatment for ocular involvement has been recently developed, and a number of promising avenues for counteracting the systemic mineralization are being followed at the preclinical level (Table 3).

Significant progress has been made in local treatment of eye manifestations of PXE by intravitreal injection of VEGF antagonists, and a number of reports have demonstrated that such agents are effective in controlling choroidal neovascularization [58-61]. The VEGF antagonists have proven so effective in treatment of the eye manifestations, the major complication of PXE, that this approach has largely replaced previously used treatment modalities for retinal manifestations, including laser coagulation and photodynamic therapy.

A number of recent studies have focused on the role of mineral composition of the diet as a potential modifier of the severity of PXE. Initially, a retrospective study suggested that high intake of dairy products, rich in calcium and phosphate, during childhood and in adolescence might develop a more severe form of PXE later in life [62]. A number of studies utilizing Abcc6−/− mice as a model system of PXE [63,64] revealed that in these mice, dietary magnesium, but not calcium, can profoundly influence the extent of ectopic mineralization [65,66]. Specifically, an increase of the magnesium content of the mouse diet by fivefold completely abolished the ectopic mineralization, while reduction of the magnesium to 20% of the normal value resulted in significant acceleration of calcium deposition [65,67]. Based on these and related preclinical experiments, a clinical trial exploring the effects of supplementary dietary magnesium on the progression of cutaneous findings and vascular mineralization is currently underway (NCT01525875). In addition, small clinical trials on a limited number of patients with PXE have suggested that feeding of phosphatidylcholine may be helpful in diminishing the progress of PXE [68,69]. However, no controlled clinical trial testing phosphatidylcholine has been published as yet.

Table 3. Potential treatment of PXE.

| Treatment possibility                              | Mechanism of action                                      | Ref.                  |
|---------------------------------------------------|----------------------------------------------------------|-----------------------|
| **Clinical**                                      |                                                          |                       |
| Anti-VEGF injection                               | Prevent choroidal neovascularization                     | [58-61]               |
| Oral magnesium supplementation                    | Ongoing clinical trial to prevent systemic manifestations | NCT01525875          |
| **Ongoing studies on molecular therapies**        |                                                          |                       |
| Transfusion of mineralization inhibitor(s)        | To prevent systemic mineralization                       | [75]                  |
| Oral statins                                      | Control of lipid metabolism to prevent cardiovascular mineralization | [84]                  |
| PTC read-through drug(s)                          | Potential read-through of nonsense mutations in the gene | [76]                  |
| Cell transplantation                              | Liver regeneration with hepatic lineage cells            | [78]                  |
| Administration of chaperone molecules             | Membrane targeting of mislocalized ABCC6 protein         | [34,77]               |
| Pyrophosphate analog(s)                           | Prevent systemic calcification in ENPP1-associated PXE/GACI | [70,71]               |

PXE: Pseudoxanthoma elasticum.

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of pathomechanistically similar pathways of mineralization in GACI and PXE. Consequently, these observations would suggest that bisphosphonates might also be useful for treatment of PXE, a possibility that can be readily tested in Abcc6 
 mice before extending them to clinical practice.

While the pathomechanistic details of pathways leading to ectopic mineralization in PXE as a result of mutations in the ABCC6 gene are currently largely unknown, there are a number of different general approaches that could be exploited toward treatment of PXE. One such approach would involve introduction of proteins into circulation, which would serve as a powerful anti-mineralization factor. One such protein is \( \alpha \)-fetuin, which under physiologic homeostatic conditions is synthesized in the liver and secreted into plasma, and is able to prevent aberrant mineralization, as attested by knockout mice developed through targeted ablation of the \( \alpha \)-fetuin gene; these mice develop extensive mineralization of arterial blood vessels [74]. In fact, it has already been demonstrated in Abcc6 
 mice that transgenic overexpression of \( \alpha \)-fetuin counteracts the development of mineralization [78].

A number of additional strategies could also be considered for treatment of PXE in a manner that has been applied to other heritable diseases, including other ABC transporter gene defects, such as cystic fibrosis. For example, recent observations suggest that small-molecular-weight compounds that facilitate the translational read-through of premature termination codon mutations (PTCs) can result in synthesis of full-length protein from the mutant allele [76]. Approximately 40% of all ABCC6 gene mutations in patients with PXE consist of PTCs, including p.R1141X, which accounts for ~25% of all mutations in PXE [8]. Another allele-specific approach would involve facilitated trafficking of proteins that are initially misfolded and mistargeted due to pathogenic missense mutations by using chemical chaperone molecules that directly or indirectly modify the protein conformation. Such molecules have been suggested to be effective in some cases of cystic fibrosis with CFTR gene mutations, and the applicability of this approach for PXE has been demonstrated in mice transiently expressing human ABCC6 with missense mutations that result in intracellular retention of the protein [84,77]. Cell-based strategies have also been explored by targeting liver through transplantation of cells with hepatoblastic lineage, either established cell lines or differentiated from induced pluripotent stem cells [78]. While the development of these novel strategies is currently at the preclinical level, meanwhile, a combination of therapies, such as an appropriate diet, supplemented with magnesium or possibly phosphate binders and pyrophosphate analogs, can be complemented with lifestyle modifications, such as immediate cessation of smoking and continuous moderate physical exercise. It is anticipated that such approaches, while not a cure, might slow the progression of the clinical phenotypes, with improved quality of life for patients with PXE, a currently intractable orphan disease.

6. Expert opinion

Orphan diseases, defined by the United States FDA Orphan Drug Act as any diagnosis with <200,000 affected individuals, demonstrate as a group tremendous need and potential opportunity to discover therapeutics. Since there are as many as 7000 orphan diseases, the total number of individuals affected by an orphan disease in the United States is estimated to be ~26 million [79]. Among those 7000 rare diseases, only ~250 have specific and effective treatments available, which has prompted the International Rare Disease Research Consortium to set a goal to develop therapies for 200 additional rare diseases between 2010 and 2020. A special effort of such discovery is directed at development of allele-specific treatments of heritable diseases as part of the personalized medicine [80].

For development of allele-specific therapeutics, understanding the disease mechanism is critical with identification of pathomechanistic pathways as targets. In case of PXE, the molecular defects in the ABCC6 gene are in most cases well delineated, but the precise pathways leading from such mutations to the mineralization phenotypes in peripheral tissues are less well understood. In addition, an unexplored area of PXE relates to the causes of the tremendous intra- and interfamilial heterogeneity, which has been suggested, largely on the basis of animal studies, to reflect influence of modifier genes and epigenetic factors [81,82]. As the cost of sequencing of an individual’s entire genome, or a subset of it in the form of whole exome sequencing, continues to dramatically decrease, it is conceivable that a number of modifier genes influencing the mineralization phenotypes will be identified in the near future. It is also possible that individuals with relatively mild disease that has not previously been brought to the attention of healthcare providers will be diagnosed, and in some cases such diagnosis might even be predicted even before the manifesting signs and symptoms develop. It is expected that the spectrum of phenotypic presentations, with potential targets for pharmacologic intervention, will be identified within the next 5 years. It should be noted that serendipity often plays a role in identifying treatments for orphan diseases, and drug repurposing, that is, finding new indications for an existing drug with established safety profiles, may result in discovery of novel therapeutics for PXE and other orphan diseases [83].

Declaration of interest

The authors were supported by the National Institutes of Health (NIH), USA and by PXE International, lay organization advocating on behalf of patients and families with PXE. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
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