

**The Prevalence of Pseudoxanthoma Elasticum-like Connective Tissue Changes in  
an Oral Biopsy Service**

Thesis

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in the Graduate School of The Ohio State University

By

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## Abstract

Pseudoxanthoma elasticum (PXE) is a connective tissue disorder affecting elastic fibers of the skin, eyes and heart primarily. This autosomal recessive disorder is caused by mutation of the ABCC6 gene. Initial investigations suggested a prevalence of 1 in a million; however, further research has suggested that PXE may occur more frequently. PXE has been found worldwide. Both Chassaing and Ringpfeil (2005) reported rates between 1:25,000 to 1:100,000 (0.004%-0.001%). Li (2008) suggested that 1:50,000 to 1:70,000 (0.002%-0.0014%) was representative. The true prevalence is uncertain for at least two reasons. First, the condition has a wide range of expression with some affected individuals having obvious signs of the disorder, while others show no outward changes. Secondly, many physicians are not familiar with signs and symptoms of PXE, therefore patients may not be diagnosed with this disorder. Further complicating the situation, it is known that individuals who are heterozygous for the trait can show signs of PXE, including the connective tissue changes seen by histopathologic examination. Very little has been documented about the carrier rate of PXE. Neldner (1988) reported it to be 1:160 (0.625%) in the general population. Chassaing (2005), using the Hardy-Weinberg law, calculated the frequency as 1:80 (1.25%) assuming a prevalence of PXE as 1:25,000; but postulated that it could be as high as 3%. Histopathologically the classic connective tissue change of PXE is the presence of fragmented calcified elastic fibers confirmed by

using the Verhoeff-von Gieson and von Kossa stains. Connective tissue changes of PXE have been documented in oral biopsy specimens. In addition, PXE-like connective tissue changes have been identified as an incidental finding in specimens evaluated by Oral Pathology Consultants at The Ohio State University College of Dentistry. Investigating the frequency of these changes could make a contribution to answering the question of actual prevalence of heterozygotes of PXE, or of PXE itself. These histopathologic changes alone are not diagnostic for PXE. Conditions exist that have similar histopathologic features to those of pseudoxanthoma elasticum, such as PXE-like fibers within scars, and some inflammatory skin diseases. For example, according to Nielsen (1978), calcific elastosis without perforations, calciphylaxis after chronic renal failure, saltpeter and penicillamine intoxications are histopathologically indistinguishable. To confirm PXE or heterozygosity of PXE, further studies would have to be undertaken including medical and genetic evaluation. There is no cure for PXE. As it progresses, skin changes can cause cosmetic and psychological problems, eye changes can cause central vision loss and cardiac changes can lead to coronary artery disease. Early diagnosis has a significant impact on management of this disorder by aiming to avoid or reduce the morbidity of its effects. The purpose of this pilot study was to determine the frequency of PXE-like changes in oral mucosal biopsy specimens.

## Dedication

Dedicated to my husband Matthew, the toughest guy I know.

## Acknowledgements

It is with sincere appreciation and thanks I would like to acknowledge the following people: my thesis committee Drs. John Kalmar, Carl Allen, and Mike Beck for their guidance, support and invaluable input; histotechnician Mary Lloyd for her professional services and personal support; Mary Marin for her constant encouragement; Dr. Susan Mallery for her unselfish willingness to help; Dr. Kristin McNamara, and my fellow residents Drs. Brent Martin and Gisele Mainville for all they have taught me and for the pleasure of their company.

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Aggressive osteoblastoma of the maxilla: a case report and review of the literature. Head  
Neck Pathol 2011;5:165-70.

#### Fields of Study

Major Field: Dentistry

Minor Field: Oral and Maxillofacial Pathology



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## Chapter 1: Introduction and Statement of the Problem

Pseudoxanthoma elasticum (PXE) is a heritable multi-system disorder of elastic tissue which can involve many organs, but primarily affects the skin, eyes, and blood vessels particularly those of the heart and gastrointestinal system [1-6]. Cutaneous findings characteristic of PXE were probably first documented in 1881 by Rigal, a French dermatologist. In 1884, Balzer described a patient who died of pulmonary tuberculosis, but who in addition, had yellow papular lesions of the skin and heart. At the time he felt this represented an unusual variant of xanthelasma. In 1889, Chauffard reported a patient with massive gastrointestinal bleeding who also had xanthoma-like skin lesions. The term “*pseudoxanthoma elasticum*” was first proposed by Darier in 1896 who studied skin biopsies from the patient reported by Chauffard and found that the skin lesions were not xanthomas, but were due to calcified elastic fibers [1,3]. The xanthoma-like clinical appearance was underscored by the name *pseudoxanthoma elasticum* [2,3]. Angioid streaks of the retina were also described in PXE patients by others [1,6] and were independently related to the skin changes by Grönblad and Strandberg in 1929 [6-8]. Because of this, PXE has also been known as Grönblad-Strandberg syndrome [8,9]. In 1944, Carlborg reported cardiovascular elastic fiber calcification in 29 patients with PXE [6].

While considered a genetic disorder, the pathway in PXE has been controversial [3,5,10-14], and only within the past decade has an exclusively autosomal recessive pattern of transmission been determined [3,6,10,15-18]. A variable phenotype has added to the difficulty in making the diagnosis of PXE [1,2,11]. Additionally, clinically normal skin of patients with PXE can show histopathologic changes [1,4,19]. Although heterozygotes often show no evidence of PXE, clinical signs may be observed [3,11,12] and microscopic changes of the elastic fibers have been documented as well [11]. The clinical differential diagnosis can be broad, but the histopathologic findings of abnormal, calcified elastic fibers in the connective tissue are specific for PXE [1-3,12,20,21].

In order to improve the accuracy and consistency of a diagnosis of PXE, previously established criteria have been recently updated [22,23]. The discovery of the ABCC6 gene associated with PXE [12,13,24,25] has led to the theory that PXE is a systemic metabolic disorder [6,15, 26-28]. Localized acquired PXE and some genetic disorders can have clinical and histopathologic similarities to PXE [20,21,29,30]. Consistent use of defined criteria and genetic testing has been recommended to establish a diagnosis of PXE. In addition to variations in diagnostic criteria, the relative rarity of PXE and its variable clinical expression has made it difficult for accurate estimates of prevalence and incidence to be determined. We have found PXE-like connective tissue changes incidentally in oral biopsy specimens that were excised for diagnosis other than PXE. The aim of our pilot study was to examine the prevalence of PXE-like fibers in oral biopsy specimens, and compare the prevalence of these changes to the current estimate of the prevalence of PXE as well as the estimated prevalence of the mutated ABCC6 gene.

## Chapter 2: Materials and Methods

This prevalence study was approved by the Biomedical Sciences Institutional Review Board of The Ohio State University, protocol number 2010H0006. Samples were obtained from the biopsy service of Oral Pathology Consultants at The Ohio State University College of Dentistry. Five hundred oral mucosal biopsy specimens were accessed from January 2009 to June 2009. Four groups were compiled based on age: 0-20 years, 21-40 years, 41-60 years and over 60 years old. These cases were accessioned consecutively until there were 125 cases for each group. Cases with no surface oral epithelium, cases from an age group which had met the maximum (125 each group), and specimens from skin were excluded. Inclusion criteria were presence of reticular fibrous connective tissue and surface oral epithelium.

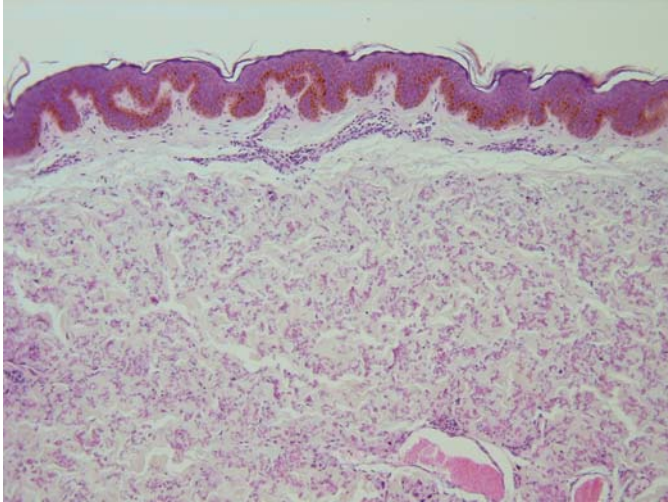
Three slides of 4-micron sections were prepared for each case. Each specimen was evaluated by light microscopy after staining with hematoxylin and eosin (H&E), Verhoeff-van Gieson stain (VH) for elastic fibers, and von Kossa (VK) stain for calcified fibers. Tissue from a known case of PXE was used as control for H&E, VH and VK (Figures 2.1-2.4). The age and sex of each patient was recorded. We considered a finding positive for fragmented calcified elastic fibers, as those seen in PXE, only if both stains were positive, regardless of the degree of positivity. The slides were reviewed by two examiners (CH and JK). Each stained section was evaluated, with quantitation for the



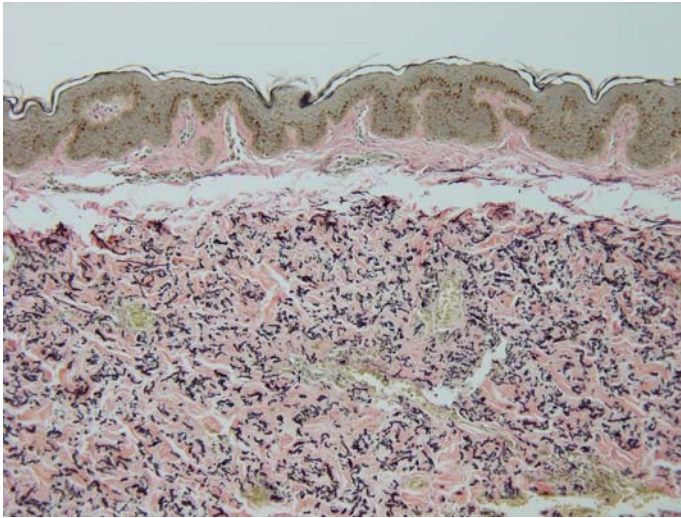
amount/intensity of abnormal fibers as negative (0), mild/focal (+1), moderate (+2), or widespread/heavy/generalized (+3). Slides were reviewed independently, with inter-examiner consultation on the discrepancies. Results were by consensus agreement. Prevalence values and corresponding 95% confidence intervals were calculated overall and for each decade and sex.



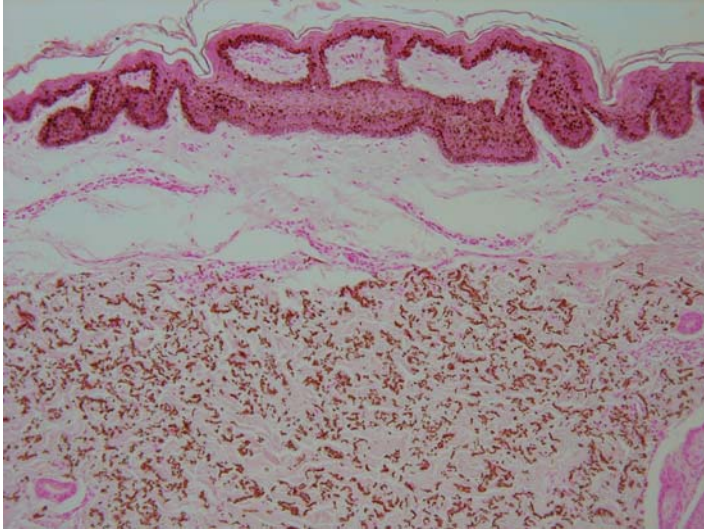
**Fig. 2.1.** Lax, folded neck skin of a PXE patient.  
*(photograph courtesy of Dr. Tim Bartholomew)*



**Fig. 2.2.** Low-magnification photomicrograph of a hematoxylin and eosin-stained section of lesional tissue from the patient in Fig. 1.



**Fig. 2.3.** Low-magnification photomicrograph of a Verhoeff-van Gieson-stained section of lesional tissue from the patient in Fig. 1.



**Fig. 2.4.** Low-magnification photomicrograph of a von Kossa-stained section of lesional tissue from the patient in Fig. 1.

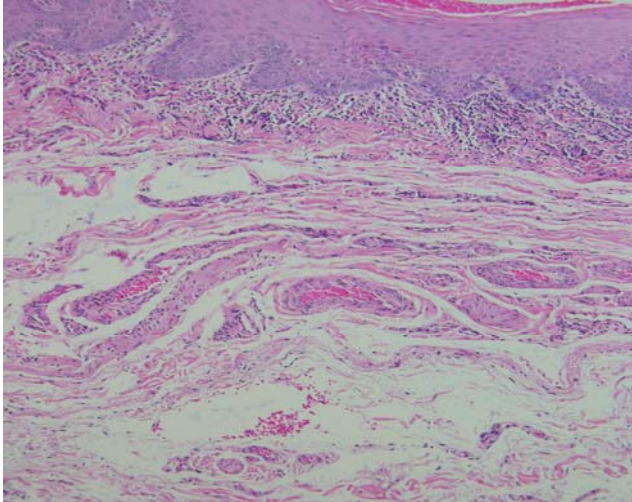
### Chapter 3: Results

A prevalence of 9.8% (n=49) was identified for PXE-like changes in the connective tissue of oral biopsy specimens submitted to this biopsy service. The lower confidence bound is 7.3% and upper confidence bound is 12.8%, with a confidence interval of 95% (Table 3.1). A result was considered positive for the microscopic findings of PXE-like fibers only if both VH and VK were positive, regardless of degree of positivity.

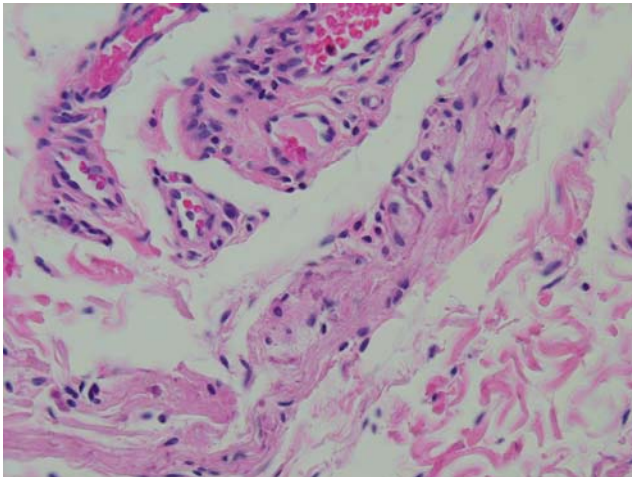
**Table 3.1. Prevalence of Positive Findings by Decade, Gender and Overall**

| <b>GENDER</b>        | <b>First</b> | <b>Second</b> | <b>Third</b> | <b>Fourth</b> | <b>Fifth</b> | <b>Sixth</b> | <b>Seventh</b> | <b>Eighth</b> | <b>Ninth</b> | <b>All</b>  |
|----------------------|--------------|---------------|--------------|---------------|--------------|--------------|----------------|---------------|--------------|-------------|
| <b><i>Female</i></b> |              |               |              |               |              |              |                |               |              |             |
| LCB <sub>.95</sub>   | 0.0          | 0.0           | 0.0          | 1.9           | 2.6          | 4.7          | 6.2            | 12.7          | 2.5          | 6.8         |
| Estimate             | <b>0.0</b>   | <b>0.0</b>    | <b>0.0</b>   | <b>8.8</b>    | <b>12.0</b>  | <b>12.5</b>  | <b>16.2</b>    | <b>27.6</b>   | <b>20.0</b>  | <b>10.1</b> |
| UCB <sub>.95</sub>   | 0.0          | 0.0           | 0.0          | 23.7          | 31.2         | 25.3         | 32.0           | 47.2          | 55.6         | 14.3        |
| <b><i>Male</i></b>   |              |               |              |               |              |              |                |               |              |             |
| LCB <sub>.95</sub>   | 0.0          | 0.0           | 0.1          | 3.3           | 0.1          | 0.7          | 13.8           | 0.2           | 9.4          | 5.9         |
| Estimate             | <b>0.0</b>   | <b>0.0</b>    | <b>3.5</b>   | <b>11.8</b>   | <b>8.0</b>   | <b>6.5</b>   | <b>28.1</b>    | <b>8.3</b>    | <b>66.7</b>  | <b>9.4</b>  |
| UCB <sub>.95</sub>   | 0.0          | 0.0           | 17.8         | 27.5          | 26.0         | 21.4         | 46.8           | 38.5          | 99.2         | 14.0        |
| <b><i>All</i></b>    |              |               |              |               |              |              |                |               |              |             |
| LCB <sub>.95</sub>   | 0.0          | 0.0           | 0.1          | 4.4           | 3.3          | 4.5          | 12.7           | 10.6          | 9.1          | <b>7.3</b>  |
| Estimate             | <b>0.0</b>   | <b>0.0</b>    | <b>1.9</b>   | <b>10.3</b>   | <b>10.0</b>  | <b>10.1</b>  | <b>21.7</b>    | <b>22.0</b>   | <b>30.8</b>  | <b>9.8</b>  |
| UCB <sub>.95</sub>   | 0.0          | 0.0           | 9.9          | 20.0          | 21.8         | 19.0         | 33.3           | 37.6          | 61.4         | <b>12.8</b> |

None of our study specimen cases stained as strongly positive as the control tissue, which was a cutaneous biopsy of a known PXE patient. The microscopic slides for a study specimen scored as moderate, (+2), are shown (Figures 3.1-3.4).

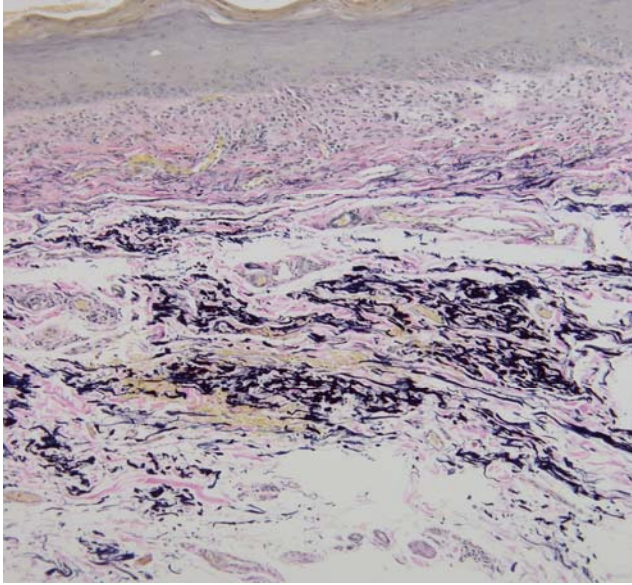


**Fig. 3.1.** Low-magnification photomicrograph of a hematoxylin and eosin-stained section of a study specimen quantified as +2 for the presence of PXE-like fibers.

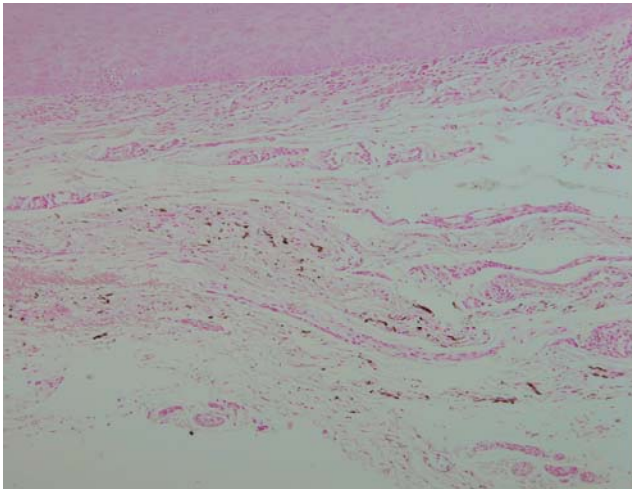


**Fig. 3.2.** High-magnification (40X) of a hematoxylin and eosin-stained section of an area from Figure 3.1 with PXE-like fibers.





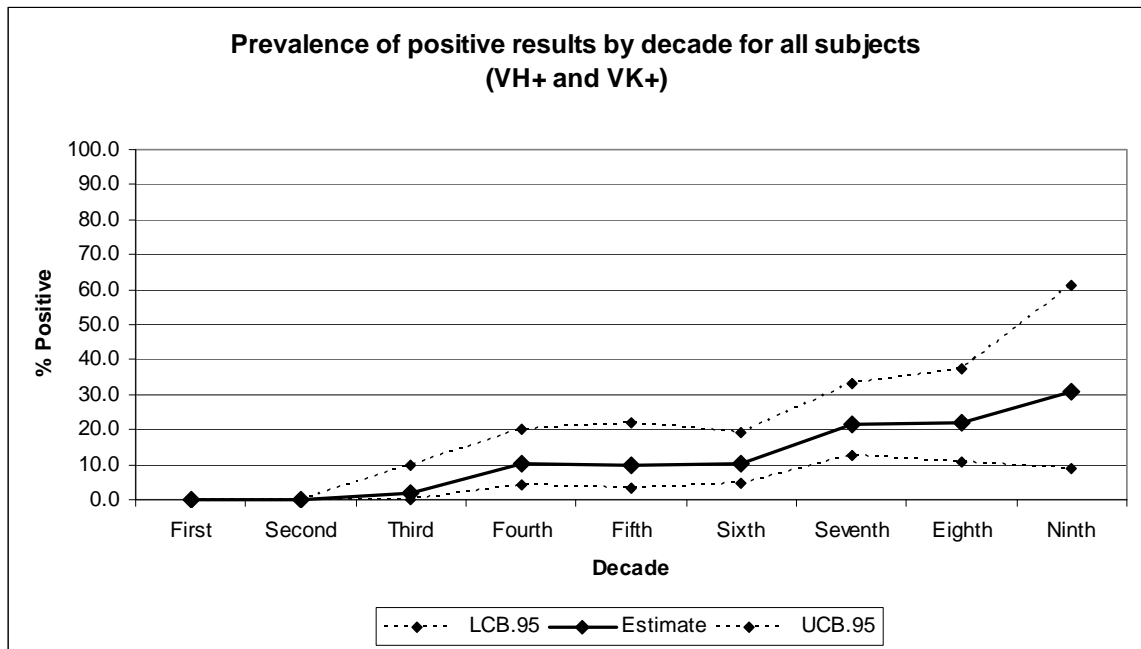
**Fig. 3.3.** Low-magnification photomicrograph of a Verhoeff-van Gieson-stained section of the specimen in Fig. 3.1 showing fragmented elastic fibers.



**Fig. 3.4.** Low-magnification photomicrograph of a von Kossa-stained section of the specimen in Fig.3.1 showing calcified elastic fibers.

Of the 500 cases in our study, no connective tissue changes were found in the first two decades. Dividing the groups into males and females, one positive finding was found in

the third decade in a 28-year-old male, with no positive findings in females in that decade. Overall female positivity was 5.6% (n=28), and male positivity was 4.2% (n=21). Positive findings correlated directly with increasing age (Figure 3.5, Table 3.2).



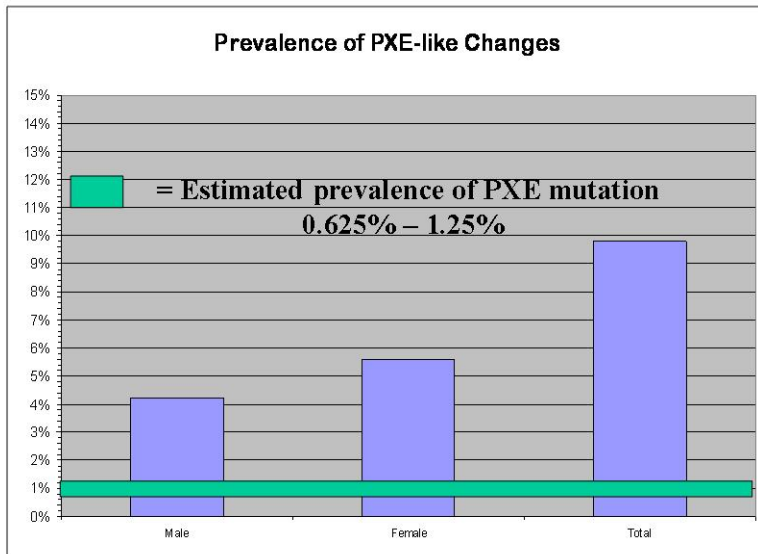
**Fig. 3.5. Prevalence of Positive Results by Decade for All Subjects**

**Table 3.2. Number of Positive Cases by Decade, Gender, and Overall**

| GENDER   | DECADE |        |       |        |       |       |         |        |       | All |
|----------|--------|--------|-------|--------|-------|-------|---------|--------|-------|-----|
|          | First  | Second | Third | Fourth | Fifth | Sixth | Seventh | Eighth | Ninth |     |
| Female   |        |        |       |        |       |       |         |        |       |     |
| Positive | 0      | 0      | 0     | 3      | 3     | 6     | 6       | 8      | 2     | 28  |
| Negative | 17     | 52     | 25    | 31     | 22    | 42    | 31      | 21     | 8     | 249 |
| Total    | 17     | 52     | 25    | 34     | 25    | 48    | 37      | 29     | 10    | 277 |
| Male     |        |        |       |        |       |       |         |        |       |     |
| Positive | 0      | 0      | 1     | 4      | 2     | 2     | 9       | 1      | 2     | 21  |
| Negative | 10     | 47     | 28    | 30     | 23    | 29    | 23      | 11     | 1     | 202 |
| Total    | 10     | 47     | 29    | 34     | 25    | 31    | 32      | 12     | 3     | 223 |
| All      |        |        |       |        |       |       |         |        |       |     |
| Positive | 0      | 0      | 1     | 7      | 5     | 8     | 15      | 9      | 4     | 49  |
| Negative | 27     | 99     | 53    | 61     | 45    | 71    | 54      | 32     | 9     | 451 |
| Total    | 27     | 99     | 54    | 68     | 50    | 79    | 69      | 41     | 13    | 500 |

Our results showed a higher overall prevalence of PXE-like connective tissue changes than the suspected prevalence of PXE reported by Neldner of 0.001% to 0.004%. The results were higher than the estimated frequency of the mutation of the ABCC6 gene, which is 0.625%-1.25%. Additionally, our results were higher than the 3% prevalence of the ABCC6 mutation Chassaing postulated in 2005 (Figure 3.6) [6].





**Fig. 3.6.** Prevalence of PXE-like changes seen in the study compared to the estimated prevalence of the PXE mutation.

## Chapter 4: Discussion and Review of the Literature

A literature search was done using Medline with the following key words:

pseudoxanthoma elasticum, Grönblad-Strandberg syndrome, PXE, gene pseudoxanthoma elasticum, and using references from identified articles. No date range was specified.

### Section 1: Clinical Presentation

The clinical presentation of PXE can be broad [2] and may vary within the same family [11], but the cutaneous lesions are considered the hallmark of this condition [3,20]. They are characterized by asymptomatic 1-3 mm yellowish-white papules especially found on flexor surfaces of the skin and particularly the axilla, neck and inguinal folds [1,2,20]. The clinical appearance has been described as resembling an orange peel [2] or “plucked chicken skin” [21]. With progression, the papules may coalesce to form plaques with a “cobblestone” appearance [6]. Eventually, the skin becomes thickened, soft and lax [1,20]. Mucous membranes of the lips, oral cavity, rectum and vagina can also be involved [1-3,6,21,30]. Lesions in the oral cavity may resemble Fordyce granules [2]. In some cases, cutaneous lesions can be minimal [1,2], but biopsy of even apparently normal tissue has confirmed the microscopic features of PXE [1]. Changes in normal skin of patients with PXE have also been seen by the use of indirect immunofluorescence using antibodies against elastin and fibronectin, although the changes are less dramatic than with lesional skin [4].

Ocular findings include a *peau d'orange* appearance of the retina [3] and angioid streaks. Although not specific to PXE, these features are considered suggestive of the disorder [1,2,20-23, 31]. Angioid streaks, however, are also found in at least 41 other conditions, including beta-thalassemia, sickle cell anemia and osteitis deformans [23,31]. They are caused by tears in Bruch's membrane (an elastic tissue) and can be seen by slit lamp examination. [22,23]. They usually appear in the second or third decade [31] and can lead to central vision loss by neovascularization, a growth process of new small blood vessels which subsequently rupture and bleed. Isolated angioid streaks in the absence of other clinical features or a positive family history of PXE are not considered diagnostic [22], although their presence raises the possibility of PXE [23]. Together with the typical skin changes of PXE, ocular findings can help strengthen or confirm the diagnosis. Interestingly, ocular features may not be present in early life, but are found in nearly every affected adult [3,17,22]. In the experience of Vanakker *et al.*, all PXE patients develop angioid streaks in their second or third decade, and the authors suggested that this sign should be mandatory for the diagnosis in patients over 30 years old [17]. Two additional ocular signs considered more specific to PXE are "comets" and "wings" [23,31]. A comet is a small, round, white punched-out lesion of the retinal pigment epithelium and underlying choroid with or without a slightly depigmented tail. Comets are asymptomatic, located in the mid-periphery of the retina, and are said to be pathognomonic for PXE. Wings are asymptomatic hyperpigmented paired smudges on each side of an angioid streak.

The cardiovascular system may be affected by premature vascular degeneration, causing intermittent claudication, hypertension, cerebral or coronary occlusion [1,2,20,21]. The elastin-rich arterial blood vessels become calcified, leading to myocardial infarctions at a relatively younger age than expected [32]. Internal hemorrhage of the gastrointestinal tract attributed to calcified gastric vessels is another problem caused by PXE [1,2,20,21,32].

Heterozygous carriers of the ABCC6 gene can have a varied phenotypic presentation as well [5,11,12]. Typically, the dermal manifestations are less severe compared to patients who are homozygous [11,12]. In one study, vascular symptoms were found in 40%-50% of heterozygotes [5]. Sherer, in a report of 4 cases, found ocular findings without skin lesions, skin lesions without ocular findings, and mild skin and ocular findings in parents of affected children [33]. It can be difficult to discern mild PXE from mild expression of clinical signs in a heterozygote patient. In the Martin *et al.* (2006) study, despite clinical evaluation, skin biopsy and ABCC6 genotyping, it was not possible to distinguish between PXE with a limited phenotype and heterozygosity with clinical signs suggestive of PXE in five of their cases. In these 5 cases, all the patients were older than 45 years. Although they did not fulfill all the criteria and could not definitively be placed into a specific category, the authors believed these 5 patients also had PXE. The observations supported two conclusions: 1) heterozygotes with severe ophthalmologic and cardiovascular manifestations should be diagnosed as having PXE, and 2) extending the definition of PXE to heterozygotes with significant manifestations of the disease should be considered. The authors also suggested physicians should be

more cautious regarding prognosis of heterozygous relatives of a patient with PXE, and have closer clinical follow-up for the detection of complications [34].

## Section 2: Epidemiology

A 2:1 female predominance has been reported for PXE however, few population-based studies have been performed [1,3,30]. Reasons for this gender predilection are unknown but it is possible that females may be more likely to seek medical advice for PXE due to cosmetic concern [3]. The cutaneous lesions of PXE typically present in the second decade [3,20] with an average age of 13 years [3]. Affected patients experience normal growth, development, intelligence and life span [3]. Early literature suggested a PXE prevalence of 1 in a million [3]. In 1993, Hacker reported the prevalence to be 1 in 100,000 to 160,000 [21]. Both Chassaing and Ringpfeil (2005) estimated rates between 1:25,000 to 1:100,000 without an apparent geographic or racial predilection [6,35]. Li (2008) proposed 1:50,000 to 1:70,000 as the prevalence of PXE [36]. The true prevalence of PXE remains unknown for many reasons. It is not uncommon for a diagnosis of PXE to be delayed for several years after the appearance of characteristic skin lesions (average age: 22 years). This delay may be related to minimal clinical signs or symptoms, patients postponing medical evaluation or care, or because many physicians are not aware of this disorder [3]. Due to such delays and subsequent difficulties in establishing the diagnosis, PXE may be more common than previously suggested. It has been suggested that the prevalence of PXE and of the mutation have been underestimated in the general population [6,16,34]. In 1988, Neldner reported the carrier rate for the PXE gene in the

general population as 1 in 160 (0.625%) [3]. Chassaing (2005) calculated it to be 1:80 (1.25%) but speculated that it could be as high as 3% [6].

### Section 3: Histopathologic Features

PXE is characterized microscopically by fragmented, calcified elastic fibers [2,3,20]. Advanced cases are easily detected by hematoxylin and eosin (H&E), but early changes can be very subtle [21]. Although fragmentation and calcification are essential for diagnosis of PXE, these microscopic features are not specific to PXE [22,37,38]. With H&E staining, the rigid calcified elastic fibers show basophilia at the periphery [3,20]. They are irregularly arranged, sometimes as fibrillar clumps, and located in the mid-reticular area of the connective tissue [2]. The epithelium and papillary area of the connective tissue are not involved [2]. A mild infiltration of chronic inflammatory cells may be noted [2]. The most sensitive stain for calcium in PXE is the von Kossa (VK) technique [3,21]. The calcium deposits (specifically the carbonate and phosphate components of these deposits) have an affinity for silver salts and are highlighted by VK staining [2,20]. Its specificity for calcified fibers is important, but VK positivity alone is not diagnostic for PXE [3]. The fully developed elastic fiber with abundant elastin is the primary target for PXE [3]. Elastic stains such as Verhoeff elastic stain (VH) highlight the fragmented and clumped fibers [2,3]. Changes in collagen have also been noted in PXE patients by some researchers [2,4]. Irregular bundles and a “thready” appearance to the collagen in the affected area have been reported [11]. Using indirect immunofluorescence, Lebwohl found the normal pattern of collagen bundles was disrupted and appeared as irregular clumps in the deep dermis in lesional skin of patients

with PXE, but the pattern was normal in non-lesional skin. This area corresponded with the area of fragmentation of elastic tissue [4]. Interestingly, the characteristic changes of PXE have also been found in non-lesional tissue of patients with the inherited form of PXE [4,19]. Lebowitz noted fragmentation and clumping of elastic tissue in the middle and deep dermis using immunofluorescence, although these changes were not as dramatic as those seen in lesional skin [4]. Pfenninger found typical histological characteristics of PXE in the absence of macroscopic skin lesions [19]. A study in 1999 by Bacchelli *et al.* found histopathologic alterations, although not as severe, in the dermis of asymptomatic, heterozygote carriers of PXE. Carrier status was determined by haplotype analysis using markers surrounding the locus of the PXE gene on chromosome 16p. Fragmented, calcified elastic fibers were found, as well as irregular-sized collagen bundles. None of these findings were noted in the dermis of an unaffected relative in one family who was identified as a non-carrier by haplotype analysis. The authors concluded that these alterations could be indicative of carrier status of PXE even in the absence of clinical signs [11]. Reinforcing Bacchelli's 1999 conclusions, histopathologic changes of elastic fibers of skin were a constant finding in heterozygote carriers of PXE in a 2007 study [39].

#### Section 4: Genetics

Our understanding of the genetic basis for PXE has undergone continual evolution. In his 1975 report, Pope proposed two autosomal dominant, and two autosomal recessive phenotypes of PXE [10]. Neldner, in 1988, favored an autosomal recessive pathway of inheritance for PXE, although he indicated that autosomal dominant cases might

represent a relatively small percentage of total affected patients [3]. A 1992 consensus conference in Philadelphia attempted to address the need to modify and expand the existing clinical and genetic classification of PXE. The report generated from that meeting defined major and minor criteria for the diagnosis of PXE, and reduced the subtypes to recessive, dominant and sporadic. The classification was further divided into categories based on combinations of the major and minor criteria. Difficulties were acknowledged in the classification of patients without characteristic skin signs. The authors postulated that these patients could be heterozygote carriers of the recessive gene, or have a mild form of the autosomal dominant type [22]. Two-generation involvement has since been explained by pseudodominance [12,15,18,40]. While the majority of PXE cases appear to be sporadic without a family history [13,35,41], PXE is now known to be inherited exclusively by the autosomal recessive pathway [6,10,15-18,42].

Consanguinity is strongly associated with PXE, but may be difficult to confirm, either due to reluctance of current family members to discuss or disclose this possibility or because a confident determination is simply not possible with past generations [3].

In 2000, the gene for PXE, ABCC6, was discovered [12,13,24], and over 300 mutations of the ABCC6 gene have been found to date [30]. ABCC6, also known as “multi-drug resistance-associated protein 6,” (MRP6), is a member of the C-family of ATP-binding cassette proteins located on chromosome 16 [7,13]. ABCC6 is primarily found in the liver and kidneys. These transmembrane transporters, fueled by adenosine triphosphate, are involved in the transport of various substrates, such as ions and lipids, across cell membranes [39], and may also play a role in cellular detoxification [12].



Although the exact function of ABCC6 has not been determined, it is believed it may serve as an export pump facilitating the removal of certain metabolites from hepatocytes. The gene is most active in the liver, corresponding to the predominant site of gene product synthesis. Little to no gene product is found in tissues typically affected by PXE [28,43]. Additionally, Martin *et al.* (2007) studied histopathologic skin changes in heterozygote carriers of mutations in ABCC6. Findings of their study supported the hypothesis that having a single ABCC6 mutation is enough to disrupt the elastic fibers, suggesting that a continuum exists with the PXE process [39]. With another study in 2008, Martin again described heterozygous individuals with signs suggestive of PXE [34].

Including ABCC6 in a classification system is appropriate. Vanakker developed a flowchart in 2008 categorizing patients as “definite,” “probable,” and “probably not” PXE based on skin biopsy, ophthalmological manifestations, and molecular diagnosis within the diagnostic work-up. The study re-affirmed that to make a clinical diagnosis of PXE, dermatologic inspection and fundoscopy are essential. According to Vanakker, if characteristic skin and ocular lesions are present, as well as confirmation with ABCC6 analysis, skin biopsy may not be needed. The analysis is also helpful if classic cutaneous or fundus lesions are not present, in which case a skin biopsy is a must [17]. Plomp proposed an update to the classification system in 2010 that also takes into account the systems and organs most frequently involved in PXE. Major and minor diagnostic criteria are defined, with delineation of requirements for the diagnosis of PXE as “definitive diagnosis,” “probable diagnosis,” and “possible diagnosis” (Table 4.1) [23].

**Table 4.1. Revised Diagnostic Criteria for Pseudoxanthoma Elasticum (PXE)**  
**[Plomp *et al.* 2010]**

**Major diagnostic criteria**

1. Skin
  - a. Yellowish papules and/or plaques on the lateral side of the neck and/or flexural areas of the body; *or*
  - b. Increase of morphologically altered elastin with fragmentation, clumping and calcification of elastic fibers in a skin biopsy taken from clinically affected skin
2. Eye
  - a. Peau d'orange of the retina; *or*
  - b. One or more angioid streaks, each at least as long as one disk diameter. When in doubt, fluorescein or indocyanine green angiography of the fundus is needed for confirmation.
3. Genetics
  - a. A pathogenic mutation of both alleles of the *ABCC6* gene; *or*
  - b. A first-degree relative [parent, sib, child] who meets independently the diagnostic criteria for definitive PXE

**Minor diagnostic criteria**

1. Eye
  - a. One AS shorter than one disk diameter; *or*
  - b. One or more 'comets' in the retina; *or*
  - c. One or more 'wing signs' in the retina
2. Genetics
  - a. A pathogenic mutation of one allele of the *ABCC6* gene

**Requirements for the diagnosis of PXE**

- a. Definitive diagnosis
 

The presence of two [or more] major criteria not belonging to the same [skin, eye, genetic] category
- b. Probable diagnosis
 

The presence of two major eye or two major skin criteria, *or*  
 The presence of one major criterion and one or more minor criteria not belonging to the same category as the major criterion
- c. Possible diagnosis
 

The presence of a single major criterion *or*  
 The presence of one or more criteria

Sickle cell anemia, beta-thalassemia, and PXE-like phenotype with cutis laxa and multiple coagulation factor deficiency should be excluded, if mutational analysis of *ABCC6* is negative or not available

Signs and symptoms in PXE may arise with increasing age. If a patient is <30 years a probable or possible diagnosis of PXE should be considered provisional and dermatologic and ophthalmologic examinations should be repeated at 5 years.

PXE was initially thought to be a connective tissue disorder [1,3,4,20,21] and it has been associated with several autoimmune conditions, including rheumatoid arthritis, sicca syndrome, and lupus erythematosus [9,44,45]. Discovery of the *ABCC6* gene in 2000 was an important milestone in understanding PXE [12,13,24,25], providing a basis to examine the cellular and molecular events that result in the clinical manifestations of PXE [12].

## Section 5: Pathogenesis

Several theories have been proposed to explain the pathogenesis of PXE, including a biochemical abnormality of elastic fibers, production of abnormal collagen, abnormalities of fibroblast structure and function, an increase in mucopolysaccharides in the dermis, abnormal proteases, oxidative stress, and increased dietary calcium [3,6,8,21,31]. With respect to diet, increasing magnesium intake has been shown to prevent ectopic mineralization, whereas decreased intake results in acceleration of the mineralization process, suggesting that diet may affect the severity of PXE [30]. A combination of abnormal elastic fiber composition and dysregulated inhibition of calcium deposition may ultimately be responsible for fiber degeneration [46]. Beside the effect of diet, it is known that tissue injury or inflammation can cause neutrophils to secrete proteolytic enzymes that can cleave elastin. As a result, the damaged fibers exhibit negatively charged residues that attract positively charged calcium ions. Cell membrane damage at these sites may allow calcium influx until the intracellular calcium concentration rises and crystallization occurs [46]. The “PXE cell hypothesis,” pertaining in particular to skin fibroblasts and arterial smooth muscle cells, postulates that changes in morphology, migration, and/or by biosynthetic profiles of the cells results in local mineralization due to the absence, or even normally low levels of ABCC6 activity [30,47]. Cultured skin fibroblasts from patients with PXE have been shown to display enhanced synthesis of elastin and glycosamino-glycan/proteoglycan complexes, along with degradation because of elevated MMP2 [30,47]. Zarbock *et al.* conducted an analysis of MMP2 in a cohort of

PXE patients. They found MMP2 to be a genetic cofactor for PXE that was present more often in PXE patients than in healthy controls [43].

The hypothesis that PXE is a metabolic disorder was put forth by Uitto *et al.* in 2001 [26], and further developed with Jiang *et al.* in 2009 [28]. This conclusion was supported by the finding that ABCC6 is not present in the tissues typically affected by PXE [43]. Mutation may result in accumulation of compounds that lead to progressive calcification of elastic tissues [12]. The “metabolic hypothesis” postulates that the absence of normal ABCC6 leads to changes in circulating factors which normally prevent aberrant mineralization within the connective tissue. The Jiang *et al.* study, using wild-type and knockout mice, confirmed that circulatory factors were a critical component of the mineralization process. Additionally, they supported the idea that mineralization could be prevented or even reversed [28]. The 2006 study by Le Saux *et al.* also concluded that PXE is a primary metabolic disorder with secondary connective tissue manifestations, a conclusion that is currently accepted by most authorities [27].

#### Section 6: Acquired Pseudoxanthoma Elasticum

In addition to the difficulties in diagnosis associated with a variable phenotype, and inter- and intra-familial heterogeneity, PXE-like clinical and histopathologic manifestations can be found in unrelated acquired or genetic conditions which lack the ABCC6 mutation [35]. “*Localized acquired PXE*” is the preferred term for a non-inheritable form of PXE, as has been described by Neldner [3]. It has an identical clinical and histopathologic appearance to the cutaneous lesions of inherited PXE [3,20,21], but is characterized by late onset, a negative family history and absence of cutaneous flexural

and retinal lesions or any other manifestations of the inherited disease [3,21]. Some of these forms, such as saltpeter-induced skin lesions, were noted in the 1992 consensus conference [22]. Other conditions that resemble PXE clinically and histopathologically are periumbilical perforating pseudoxanthoma elasticum, papular lesions on the forearms of miners after exposure to a mixture of calcium and ammonium nitrates, and calcific elastosis [20-22,38]. Many clinically visible PXE-like skin lesions are recognized, but not all have the characteristic microscopic findings. Likewise, conditions exist that exhibit the same histopathologic changes with the absence of typical skin findings. Some genetic conditions also have PXE-like changes. Patients with  $\beta$ -thalassemia [48] and sickle cell anemia have been found to have both the cutaneous lesions and the ocular findings seen with PXE, in the absence of ABCC6 mutation [29,30].

#### Section 7: Therapeutic Recommendations and Treatment

There is no cure for PXE, but factors that exacerbate its signs and symptoms should be avoided [3,21]. High childhood dietary calcium intake has a positive correlation with cardiovascular manifestations of PXE, so it has been advised that calcium intake be limited to the Recommended Dietary Allowance per the United States Department of Agriculture [3]. Universal agreement is lacking, however, on the benefits of dietary calcium restriction. Because the skeleton serves as a labile reservoir of calcium, reducing or maintaining low serum calcium is difficult and can increase the risk of osteoporosis [49]. The concept that dietary changes may be potentially helpful in the treatment of PXE and alter the age of onset or the extent of mineralization was discussed by Uitto *et al.* (2010) [30]. In patients with angioid streaks, retinal hemorrhage with the potential for

central blindness is a major concern. It would be prudent to counsel the patient to avoid head contact sports or activities that include intense straining (i.e. weight lifting), and to consult with an ophthalmologist [3]. As with non-PXE patients, and especially because PXE patients are predisposed to develop atherosclerosis, smoking should be avoided and regular exercise is helpful in preventing cardiovascular complications [3]. Controlled studies to prove the impact of smoking and exercise on the actual course of the disease have not been published. Plastic surgery is an effective option to improve the cosmetic appearance of affected skin [50].

From an experimental standpoint, Jiang *et al.* reported promising results related to a potential treatment for PXE. Their studies with mice have shown that by re-introducing the circulating anti-mineralization factors that are missing due to mutation of ABCC6, connective tissue mineralization can be halted or reversed [28,47].

It is advisable that all PXE patients and their families undergo genetic counseling [3]. PXE support groups are available and may help reduce a patient's sense of isolation and provide them with current information about the disease. Annual follow-up evaluations by a cardiologist and ophthalmologist are generally warranted, as well as with any other health care specialist specific to the individual patient's needs.

## Chapter 5: Conclusion

### Section 1: Summary of Results

The overall results of this study revealed a prevalence of 9.8% for PXE-like connective tissue changes in oral mucosal samples submitted to diagnose other oral conditions. This prevalence is markedly higher than the estimated prevalence of patients who have a confirmed diagnosis of PXE (0.001%-0.004%) or of the estimated prevalence of the PXE mutation (0.625%-1.25%). The PXE-like changes that were observed could represent tissue alterations indicative of patients who are either homozygous or heterozygous for PXE. The connective tissue abnormalities could also be related to mechanical stress, tissue injury [46,37], or the normal ageing process. It is also possible that some or all of the changes observed in the archived tissue specimens represented acquired forms of PXE.

The average age at clinical presentation for PXE is 13 years old, however; no positive histopathologic findings were in specimens from patients less than 20 years of age. An increase in the amount of elastic tissue changes was expected with older age groups but we did not anticipate an increase among middle-aged patients. Angioid streaks of PXE may not be seen until 30 years of age [17], so perhaps the dystrophic elastic fiber changes seen in oral biopsy specimens are not clinically apparent until an older age. Bowen described incidental PXE-like fibers in the inflamed skin of patients who presented with a

variety of conditions, but had no clinical lesions suggestive of PXE. Upon genetic analysis, two of the patients were found to have mutations in the ABCC6 gene [37].

As expected, the highest percentage of PXE-like tissue changes were found in the oldest age group of patients. Previous studies have shown that the light microscopic changes of PXE can be minimal or even undetectable in heterozygotes. Even in these cases, though, transmission electron microscopy has identified discrete electron dense precipitates identical to yet smaller than those seen in typical PXE elastic fibers [11]. In addition, mineral precipitation may progress with age [11]. Delayed onset of PXE is another consideration, as it may not manifest until later decades.

Another potential reason for our finding of a higher overall prevalence of PXE-like connective tissue changes could be that some or all positive patients were affected by some form of acquired PXE. Many of these conditions present with an advanced age of onset, are not systemic, and most often are limited to the skin [38]. Some however, like  $\beta$ -thalassemia, are not localized disorders [48].

The following table provides a visual overview of our results (Table 5.1). It is based on degree of tissue positivity using VK staining, as VK was considered the most sensitive microscopic indicator of PXE-like elastic fiber change, although the results with VK and VH were nearly identical. All of our positive cases were quantified as either +1 or +2. None of them had widespread/heavy/generalized staining (+3).



**Table 5.1. Summary of Quantitation of Results**

| <b>Quantitation (VK result)</b> | <b># of Cases</b> | <b>% of Total</b> |
|---------------------------------|-------------------|-------------------|
| <b>+1</b>                       | 38                | 7.6%              |
| <b>+2</b>                       | 11                | 2.2%              |
| <b>+3</b>                       | 0                 | 0%                |
| <b>Combined +1 and +2</b>       | 49                | 9.8%              |

## Section 2: Areas for Further Study

If this study were to be repeated, adding the location of the biopsy would provide useful information, particularly if a specimen was in an area of frequent trauma or flexure since local factors have been shown to affect the elastic fibers. A thorough medical history would be beneficial and could add important study information. No more than one specimen per patient should be included. The elastic fibers of mucocoeles are often clumped. Mucocoeles and cases of squamous cell carcinoma or other malignancies should be excluded.

One of the difficulties with the study was the relatively arbitrary method of quantitation of positive findings. It would be helpful to spend time calibrating the examiners, with mutual agreement of the amount/intensity of abnormal fibers that would qualify as +1, +2, or +3. The majority of positive cases in our study, (n=38), were quantified as +1. Subtle changes can be difficult to appreciate on H&E examination, so it is more likely cases in this group would go unnoticed. It may be more likely that cases

quantified as +2 and higher would be noticed. In our study 11 cases were quantified as +2 for a prevalence of 2.2%. Interestingly, this prevalence result closely approximates the range for the estimated prevalence of the PXE mutation (0.625%-1.25%), and is well within the range supposed by Chassaing *et al.* (2005) of as high as 3%.

Whether the changes observed in oral biopsy specimens represent acquired PXE, genetic PXE, or some other process is not known. Further studies, including genetic testing with DNA analysis, would be required to determine the significance of these findings. It would be important to include documentation of homogeneity or heterogeneity if the mutation is present. A study such as this, designed to assess the clinical relevance of fragmented calcified PXE-like fibers seen incidentally in oral biopsy specimens, seems warranted. Plomp's 2010 proposed update for the PXE classification system gave guidelines for diagnosis, but gave no mention of how to proceed when PXE-like findings are incidental. Ideally, the finding of fragmented calcified elastic fibers would serve to trigger the next step in a diagnostic algorithm. Should the patient have a dermatologic examination? Should the patient be referred for an ophthalmologic examination? Would it be best to recommend a genetic test? Or, are these changes merely due to the ageing process? Having sound clinical and genetic information to correlate with the histopathologic findings would be of immense value in determining the nature of these fibers and the implications, if any, of their presence. The pathologist could then convey microscopic findings to the clinician in a report that would be more helpful and relevant to patient management. Additionally, knowing the specific findings would be useful in further determining the prevalence of PXE or carriers of PXE. It should be

noted that although molecular testing is the most reliable method to diagnose PXE [41], in less than 10% of PXE patients, no mutation of the ABCC6 gene can be found [51].

PXE can cause life-shortening cardiovascular disease [5,52]. Coronary artery disease is prevalent in the general population, so limiting studies to patients who developed it at a younger than average age would be helpful. Utani *et al.* recently reported a relationship between PXE skin and mucous membrane lesions and cardiovascular involvement, with a direct correlation between the severity of the lesions and the degree of cardiovascular involvement [52]. Investigations including retrospective and prospective studies of patients with coronary artery disease could reveal more specifically the role of PXE. For example, if PXE is a metabolic disease, how does it relate overall among the many etiologic factors of cardiovascular disease [5,47]? If carrier status of PXE makes a person more susceptible to cardiovascular disease, as some studies have suggested [5,53,54], the impact may be more far reaching than previously thought. Trip *et al.* (2002) reported a case-control study of a cohort of patients under 50 years old with a history of premature coronary artery disease (CAD) to assess the relationship with ABCC6 mutation. Results showed 3.2% of patients with premature CAD and 0.8% of the control population carried the ABCC6 mutation, indicating this mutation is not rare in the general population, and appears to contribute to increased risk of CAD [53]. It would be interesting to repeat the study with another cohort, and additionally include oral mucosal biopsy. Such work may lead to a more detailed understanding of the prevalence and heterogeneity of PXE as well as other conditions with PXE-like tissue changes.

An intriguing follow-up study would involve patients with angioid streaks, including documentation of comets and wings. After excluding patients with the major known risk factors for angioid streaks, oral mucosal biopsies could be evaluated for PXE-like changes. The prevalence of positive findings simultaneously in these two sites could be compared to other PXE prevalence studies supplemented with DNA analysis.

Genetic testing for PXE can be done by collection of a blood sample or by using a buccal swab kit. A genetics laboratory will evaluate the ABCC6 gene to determine if there are any mutations [55]. Although DNA testing can be costly, it may be comparable to the sum of the costs of an eye exam and mucosal biopsy. Additionally, the patient could have a more definitive diagnosis without the need for a surgical procedure. The choice to have genetic testing is an important personal decision, as the results can have profound and sometimes unintended consequences. Before having a genetic test, consultation with family members and healthcare professionals is advised.

With genetic disorders it is important to not only identify affected patients, but also carriers of the mutation [11]. If a mutation is identified, genetic counseling is warranted [11]. We have commented on the fact that PXE has a variable phenotypic expression, even within the same family [12], and that when classic clinical signs are not present, PXE can remain undiagnosed. Heterozygotes can also have a variable phenotypic expression [3,11,30], and the histopathologic findings seem to vary as well. Heterozygote carriers of a mutation in the PXE gene have shown histopathologic features similar to PXE, although not as dramatic [11]. However, Plomp *et al.* compared skin biopsies from PXE patients who were homozygous and heterozygous for the same ABCC6 mutation, as

well as with healthy control subjects, and found no calcification of elastic fibers in heterozygotes [23].

### Section 3: Comments

Because of the wide range in clinical and histopathologic signs, knowing the significance of PXE-like changes in oral mucosal biopsies has merit. If the changes we see in our biopsy service are in fact consistent with carrier status of PXE, or of PXE itself, this finding would be notable. It would impact not only the actual prevalence of PXE, but more importantly, the long-term health and well-being of the patient and the patient's family by allowing earlier identification of the condition.

Because the amount of change seen in elastic fibers can be minimal, and because the Verhoeff and von Kossa stains are typically only ordered to confirm the presence of fragmented calcified elastic fibers, the pathologist should have an awareness of the microscopic appearance of these abnormal elastic fibers when examining H&E slides. Some pathologists are more experienced with PXE-like changes in the connective tissue, especially when they are subtle, and so are more likely to observe them when they are present. Another reason PXE-like changes could be missed is that the pathologist may be primarily concentrating on the clinical setting or the primary impetus for tissue biopsy. Being cognizant of the potential for elastic fiber alterations will increase the likelihood of recognizing them, which in turn could impact the health of the patient. In a reported case by Goette, the patient had angioid streaks, but otherwise had no relevant medical history, and the characteristic skin changes of PXE were not present. Only the oral mucosa was clinically involved, showing characteristic yellow papules of PXE. Biopsy permitted the

diagnosis of PXE, demonstrating the importance of recognizing the mucosal appearance of PXE [2]. A wider understanding of the clinical and histopathologic signs of the disorder could help solve this confounding diagnostic dilemma, and lead to earlier identification of this rare condition.

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