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A one-day workshop focusing on the clinical, genetic, and molecular aspects of PXE was held at Jefferson Medical College, Philadelphia, Pennsylvania, on June 10, 1992. The Organizing Committee consisted of the authors of this report, and the conference was chaired by Dr. Jouni Uitto, Professor and Chairman of the Department of Dermatology at Jefferson Medical College. Approximately 40 physicians and scientists representing six countries participated in the meeting (Fig 1). The patient advocate organization, the National Association of Pseudoxanthoma Elasticum (NAPE), was represented and an update on NAPE programs was provided.

The featured speaker of the workshop was Dr. Victor McKusick (Johns Hopkins University School of Medicine), who provided a historical perspective and suggested future directions for PXE research with lessons from other heritable connective tissue disorders, such as the Marfan syndrome [1]. Dr. McKusick pointed out that the previous PXE symposium of the same size was held at Johns Hopkins University over a decade ago, in 1980, and was organized by Dr. McKusick. His presentation illustrated the clinical variability and diagnostic difficulties in PXE, as well as the problems in establishing the pattern of inheritance. Dr. McKusick indicated that understanding of the underlying molecular mechanisms of PXE should be amenable to approaches of molecular genetics and cellular biology.

The other speakers and the topics discussed in the workshop were as follows.

Dr. F. Michael Pope (Dermatology Research Group, Harrow, Middlesex, UK): Classification of PXE into Clinical Subtypes.

Dr. Mark G. Lebwohl (Mt. Sinai School of Medicine, New York, NY): Dominant PXE is an Underrepresented Disorder.

Dr. Lis Danielsen (Bisbjerg Hospital, Copenhagen, Denmark): Experimental Models of Calcification of Elastic Fibers in PXE.

Dr. Kenneth Neldner (Texas Tech University Health Sciences Center, Lubbock, TX): Evolution of the PXE Phenotype.

Dr. Anne de Paepe (University Hospital, Gent, Belgium): Specific Recessive Forms of PXE.

Dr. Charles D. Boyd (UMDNJ-RWJ Medical School, New Brunswick, NJ): The Candidate Gene Approach to PXE.

Dr. Angela M. Christiano (Jefferson Medical College, Philadelphia, PA): Linkage Studies in PXE Families.

Dr. Maurice Godfrey (University of Nebraska, Omaha, NE): Fibrillin Expression in PXE Fibroblast Cultures.

Dr. Harry C. Dietz (Johns Hopkins University School of Medicine, Baltimore, MD): Fibrillin Mutations in the Marfan Syndrome — Lessons for PXE.

Ms. Carol Daugherty (Texas Tech University Health Sciences Center, Lubbock, TX): Discussion on the Establishment of a Centralized PXE Database and Update on NAPE.

The following is a summary of the presentations and discussions from the Workshop on PXE.

CLINICAL ASPECTS OF PXE

Pseudoxanthoma elasticum (PXE) is a relatively rare clinical condition affecting tissues rich in elastic fibers. The diagnosis of PXE can be made without difficulty if the cardinal signs of the disease, i.e. characteristic skin involvement, ocular findings, and cardiovascular manifestations, are present [2]. However, in clinical practice, the diagnosis can be difficult to make in patients with limited clinical findings. Furthermore, although most cases of PXE appear to be inherited, the precise mode of inheritance in many families is difficult to establish due to delayed onset and variable expression of the phenotype within families. Finally, although the elastic fibers are clearly abnormal in the affected tissues, the underlying mutations and the etiologic mechanisms are largely unknown.

The precise diagnostic criteria for PXE are not well defined. Diagnosis of PXE in an individual without a family history may be difficult due to delayed onset of clinical manifestations and limited expression of the disease. For example, the question was raised whether a positive skin biopsy with characteristic dermal calcification of the elastic tissue is a prerequisite for diagnosis in cases without family history. It was also debated whether the diagnosis can be made solely on the basis of ocular findings (such as angiod streaks) in the absence of cutaneous manifestations if there is a positive family history for PXE. These and similar questions suggested that there is a need to establish more precise guidelines for the diagnosis.
of PXE, in order to assign the phenotype in families used in linkage studies. Consequently, a committee was formed, consisting of Drs. Lebwohl, Neldner, Pope, and de Paepe, with the charge to review and establish criteria for the diagnostic of PXE. Meanwhile, however, the following tentative classification was proposed. Category I contains individuals with PXE with classic manifestations of the disease, including angiod streaks and skin involvement with characteristic histopathologic features of deranged elastic fibers with calcium deposition. Category II contains sub-groups of individuals with limited evidence of PXE, such as the presence of angiod streaks in the absence of skin findings and a positive family history of the first degree. The groups within category II will warrant closer investigation of the immediate family as well as follow-up for possible development of other signs of PXE and eventual re-classification to category I.

GENETIC HETEROGENEITY OF PXE

It is clear that in some families PXE is inherited in an autosomal dominant fashion [3], but in most cases recessive inheritance or sporadic new mutations can be assumed. In the absence of affected family members or a genetic marker, it is impossible to decide whether these cases represent new dominant mutations or a recessively inherited homozygous mutant allele.

Determination of the precise mode of inheritance in many families is also uncertain because of variable expression. Attempts to establish the precise mode of inheritance within a given family are of critical importance for genetic counseling, as is establishment of genetic linkage, using either the candidate gene approach or positional cloning (see below).

Patients with PXE-like cutaneous changes can also be recognized in some acquired conditions [2]. The acquired forms include D-penicillamine-induced cutaneous changes, which differ from PXE in the lack of calcification of the elastic fibers, and peribulbar cutaneous PXE. PXE-like cutaneous changes ultrastructurally indistinguishable from genuine PXE have also been described in Scandinavian patients exposed to Norwegian saltpeter (a calcium-containing fertilizer).

MOLECULAR PATHOLOGY OF ELASTIC FIBERS IN PXE

The cardinal manifestations of PXE result from abnormalities in the elastic fibers of affected tissues, i.e., the skin, Bruch's membrane of the eye, and arterial blood vessels. The underlying pathology involves fragmentation and calcification of the elastic structures, as well as apparent accumulation of disorganized elastic material [2]. The mutated gene(s) and the etiologic mechanisms leading to the clinical phenotype are currently unknown.

Recent advances in understanding the basic biochemistry and molecular biology of the elastic fibers have provided insights into the potential mechanisms leading to abnormal elastin fibrillogenesis [4,5]. It is now known that the major component of the elastic fibers consists of elastin, which is initially synthesized as a ~70 kDa polypeptide encoded by a 3.5-kb mRNA. The elastin gene, which has been unequivocally mapped to human chromosome 7 at the locus 7q11.2, consists of 34 separate exons spanning 45 kb of genomic DNA [6,7]. Elastin is synthesized at a relatively high level by cultured smooth muscle cells, which may be the primary cell type responsible for elastin synthesis in blood vessels. In addition, cultured human skin fibroblasts clearly express the elastin gene at lower levels and may be the primary source of elastin in the human dermis.

In the extracellular space, the elastin polypeptides align into a fibrillar structure that is stabilized by formation of covalent interchain cross-links known as desmosines. The formation of desmosines is catalyzed by a copper-dependent enzyme, lysyl oxidase, that
Table I. Candidate Genes in Pseudoxanthoma Elasticum

<table>
<thead>
<tr>
<th>Gene</th>
<th>Symbol</th>
<th>Map Locus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastin</td>
<td>ELN</td>
<td>7q11.2</td>
<td>[6]</td>
</tr>
<tr>
<td>Fibrinil 15</td>
<td>FIB-1</td>
<td>15q15–21</td>
<td>[10]</td>
</tr>
<tr>
<td>Fibrinil 5</td>
<td>FIB-2</td>
<td>5q23.3–31.2</td>
<td>[10]</td>
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<tr>
<td>Fibrinil 17</td>
<td>FIB-3</td>
<td>17q</td>
<td>[10]</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>LOX</td>
<td>5q23–31</td>
<td>[8]</td>
</tr>
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has recently been cloned and mapped to human chromosome 5q23 [8,9]. Lysyl oxidase is also required for the cross-linking of collagen fibers.

Electron microscopic examination of normal elastic fibers has revealed that the elastin core is surrounded by a microfibrillar component. The fibrillins, a family of newly characterized proteins, are the major component of these microfibrils. Fibrillins are large molecular weight (~350 kDa) polypeptides rich in cysteine and contain epidermal growth factor-like repeat subdomains that participate in calcium binding. Thus far, there is evidence for at least three distinct fibrillin genes [10]. The best characterized fibrillin (FIB-1) has been mapped to human chromosome 15, and mutations in this gene underlie the classic Marfan syndrome, as elegantly summarized by Dr. Dietz [11,12]. Mutations in this fibrillin gene would explain the abnormalities in the elastic structures in the cardiovascular system, such as aneurysms and dilatation of the aorta, encountered in the Marfan patients. The distribution of fibrillin 15 is not limited, however, to elastic structures, but is also present in other tissues, including the periosteum and zonular ligaments, thus explaining the pleiotropy of the Marfan syndrome. The second fibrillin gene has been mapped to chromosome 5 (FIB-2) and has been linked to a Marfan-like syndrome, congenital contractual arachnodactyly [13]. Finally, there is evidence for a distinct fibrillin gene on chromosome 17 (FIB-3); however, this gene has not been extensively characterized.

An interesting and pathognomonic feature of PXE is calcification of the elastic structures in the skin and blood vessels. Dr. Dietz indicated that fibrillin contains several calcium binding domains within the epidermal growth factor-like repeats of the protein. Site-directed mutagenesis studies have suggested that a change in the amino acid sequence within these domains can increase the calcium binding activity by as much as a hundredfold. Thus, mutations affecting this segment of a fibrillin molecule could explain the increased calcification of the elastic fibers observed in PXE.

RECOMMENDATIONS FOR FUTURE AREAS OF RESEARCH AND CLINICAL EMPHASIS

At the conclusion of the workshop, Dr. Uitto summarized the critical areas for future research and clinical emphasis, from the discussions. In the clinical area, the following issues were suggested to deserve the highest priority.

1. Establishment of diagnostic criteria that allow more precise identification of patients with PXE.
2. Improved delineation of the mode of inheritance in families affected with PXE in order to establish genetic linkage in families utilizing informative markers.
3. Continued monitoring of the evolution of the disease in individual patients, to provide a database for prognostic indicators.
4. Development of carefully controlled outcome studies regarding the use of specific treatment modalities, including lasers for the treatment of retinal hemorrhage.
5. Development of clinical studies to examine the role of confounding variables that may modify the severity of the disease. These include the role of calcium intake and the level of plasma lipids in affected individuals.
6. Continued support of the patient advocate organization, the National Association of Pseudoxanthoma Elasticum, in its efforts to disseminate information to the professional and lay audience alike.
7. Identification of additional patients through establishment of a National PXE registry which could serve as a clearing house for clinical data and as a repository of cells and other tissue material from patients.

In the area of research directions towards identification of the underlying cause of PXE, the following recommendations were developed.

1. Continued application of genetic linkage analyses with the candidate gene approach utilizing informative RFLP in these genes or with markers flanking the mapped loci of the candidate genes.
2. In the event that the candidate gene approach leads to exclusion of the genes tested, approaches of positional cloning should be considered, provided that well-characterized families with clearly recognizable patterns of inheritance are available.

3. Delineation of the mechanisms of the disease through further assay of enzymatic activities of lysyl oxidase and elastolytic proteases in fibroblast cultures.

4. Continued fostering of the international exchange of information and tissue material to promote studies on PXE. This includes organization of workshops similar to this one at regular intervals.

5. Vigorous efforts to secure stable research funding to study the basic biology and pathophysiology of the elastic fibers, especially in the context of PXE.

6. Intensification of efforts to increase the public support for research on PXE, in the context of the Coalition of Heritable Disorders of Connective Tissue.

REFERENCES


PATIENT RECRUITMENT ANNOUNCEMENT

Researchers in the Department of Dermatology at Jefferson Medical College, Philadelphia, PA, have an ongoing interest in the molecular biology of pseudoxanthoma elasticum (PXE). We recently held a one-day workshop on progress in research on PXE, and determined that an expanded network of patient material is critical for the continuation of this work. In a large collaborative effort with Mt. Sinai School of Medicine and UMDNJ-Robert Wood Johnson Medical School, we are seeking families that would be interested in participating in genetic linkage studies. As summarized in the report of the Workshop on PXE (see above) we are testing for genetic linkage to PXE using RFLP in candidate genes for this disorder. We ask readers of this announcement aware of PXE patients interested in participating in our ongoing research efforts, to please contact Dr. Angela Christiano, Department of Dermatology, Jefferson Medical College, 233 South 10th Street, Room 431, Philadelphia, PA 19107; Tel. (215) 955-2176; Fax. (215) 955-5788.