MEETING REPORT

International Centennial Meeting on Pseudoxanthoma Elasticum: Progress in PXE Research

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A 2 day symposium entitled "International Centennial Meeting on Pseudoxanthoma Elasticum" was held in Bethesda, Maryland, on November 6 and 7, 1997. The meeting was planned by a committee consisting of the authors of this report and was chaired by Dr. Jouni Uitto, Department of Dermatology and Cutaneous Biology at Jefferson Medical College. Ms. Sharon Terry, President of PXE International, served as meeting coordinator. This meeting was attended by about 50 scientists and physicians from nine different countries, as well as several representatives of the patient advocacy organizations.

This meeting was considered extremely timely for several reasons. First, the initial definitive description of pseudoxanthoma elasticum (PXE) appeared in the medical literature just about 100 years ago, in 1896, and clearly delineated this disorder as an entity distinct from xanthomas (Darier, 1896). Second, 5 years had passed since the previous PXE symposium that was held at Jefferson Medical College in 1992 (Christiano *et al*, 1992). Finally, the progress in understanding various facets of PXE has advanced tremendously during the past 5 years, and in fact, the candidate gene underlying the majority of cases with PXE has been recently mapped to a distinct chromosomal region in the human genome at 16p13.1 (Struk *et al*, 1997; van Soest *et al*, 1997).

The keynote speaker of the meeting was Dr. Francis Collins, Director of the National Human Genome Research Institute, National Institutes of Health. Dr. Collins highlighted the advances of the human genome project that, he reported, is "on time and on budget." The goal of this project is to complete the sequencing of the entire human genome by year 2005 (Collins, 1997). It is clear that these efforts are making a major impact on elucidation of genetic defects in heritable diseases, such as PXE. Dr. Collins also emphasized the importance of collaborations with regard to studies on such relatively rare diseases. Dr. Collins further emphasized the fact that genetics has become the central science of medicine, with major implications for molecular diagnostics, prognostication, and genetic counselling. Identification of candidate genes and elucidation of mutations in heritable diseases form the basis for prenatal testing in families at risk for recurrence, and ultimately cure of these diseases in the form of gene therapy. At the same time, the progress in understanding the genetic basis of both monogenic as well as multifactorial diseases has raised several issues in the public policy arena, with the recognition of potential for genetic discrimination. Clearly, solutions for such issues have to be developed in order to provide maximum benefit from the latest genetic discoveries to the affected individuals and their families.

The ensuing symposium featured 21 internationally recognized scientists whose presentations described the pathophysiology and genetics of PXE and reviewed, in considerable detail, the clinical manifestations of the disease. The following is a summary of these presentations in the light of the identification of at least one major genetic locus for this heritable elastic tissue disorder.

GENETICS OF PXE

PXE is a systemic heritable connective tissue disorder primarily affecting the elastic tissue network in the body (Neldner, 1988). The precise incidence of PXE is unclear, its cause is unknown, and the underlying molecular gene defect remains to be delineated.

PXE demonstrates marked clinical and genetic heterogeneity (Pope, 1975; Neldner, 1988). The clinical variability is demonstrated by the fact that the involvement of the three major organ systems being affected, i.e., skin, eyes, and the cardiovascular system, is evident in some patients, whereas in others only a limited involvement of one of these organ systems can be found. For example, the presence of the disease may be suggested only by ophthalmologic examination, which may reveal the angioid streaks, without any further evidence of the disease. In the absence of information on a specific gene defect, this clinical variability raises critical questions concerning the mode of inheritance and accurate diagnosis (Lebwohl *et al*, 1994). Although genetic heterogeneity has been suggested in previous publications, and the existence of autosomal dominant and autosomal recessive patterns has been documented (Pope, 1974a, b, 1975), it appears that the majority of cases are sporadic. Among the families demonstrating a discernible mode of inheritance, autosomal recessive inheritance appears to be more common than autosomal dominant. This has clear implications for genetic counselling of families at risk for PXE regarding the possibility of an affected child in subsequent pregnancies and in future generations; however, assignment of an unequivocal inheritance pattern in most families, in the absence of genetic markers for carrier detection, is currently difficult. Furthermore, the elucidation of inheritance is complicated by clinical variability and late onset of the disease, the mean age of onset being ≈13 years. It was suggested that the disease may be primarily autosomal recessive, with heterozygous carriers demonstrating minimal, yet suggestive, signs of PXE. Alternatively, the disease could be autosomal dominant with incomplete penetrance. Consequently, the affected families should be counselled conservatively with the worst case scenario estimates of the risk.

The progress made towards improved understanding of the genetic basis of PXE was highlighted by the fact that a major PXE locus has been recently mapped to the short arm of human chromosome 16, spanning region 16p13.1 (Struk *et al*, 1997; van Soest *et al*, 1997). Although the gene for PXE has not been identified as yet, haplotype analyses spanning the region on chromosome 16p13 have suggested that the major mode of inheritance is autosomal recessive. On the other hand, it was suggested that ocular and cutaneous lesions may be inherited primarily in an autosomal recessive fashion, whereas vascular problems may be inherited in an autosomal dominant fashion, resulting from differential penetrance of the gene defect in different tissues.

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Human chromosome 16 consists of 98 Mb, demonstrates 24 cytogenetic bands, and has at least five fragile sites. Approximately 300 genes are known to be encoded by chromosome 16 and some 35 diseases are associated with abnormalities in these genes (Doggett *et al*, 1995). Although the region harboring the PXE gene is not particularly rich in expressed genes, several genes within the interval have been identified. These include members of the multiple drug resistance gene, myosin heavy chain polypeptide 11, and a calmodulin dependent phosphodiesterase. In addition, a polymorphic CCG repeat fragile site (FRA16 A) is within the interval. Mutations in any of these candidate genes would be unlikely to explain the pleiotropic manifestations noted in patients with PXE. Clearly, examination of additional families by utilizing informative markers flanking the PXE locus is required for identification of additional informative meioses in order to narrow the candidate gene interval. These efforts are currently being combined with continued mapping and sequencing of the corresponding region of chromosome 16, as well as the use of complementary approaches, including differential display. Using other heritable diseases as examples, one could predict that the actual gene responsible for PXE mutations residing on chromosomal region 16p13.1 will be identified in the near future. At the same time, it was noted that identification of the gene may well be only the beginning of a long journey towards understanding the mechanisms leading to PXE. Again, using several other heritable diseases as examples, such as cystic fibrosis and neurofibromatosis, it is conceivable that enormous efforts towards biochemical and cell biologic characterization of the gene products and their functions will be required to disclose how these gene defects result in the clinical signs of PXE.

ELASTIC FIBERS AND PXE

The pathology of PXE affects elastic fibers in several tissues, including skin, Bruch's membrane of the eye, and the arterial blood vessels. The histopathologic hallmark of PXE in the skin is accumulation of pleiomorphic, "elastotic" material in the mid-dermis. The morphology of these structures is clearly perturbed, suggesting functional alterations in the elastic fibers. Furthermore, a diagnostic feature of PXE is calcification of these elastic structures, which can be demonstrated by special histopathologic stains or by transmission electron microscopy of the skin. In addition, elastic structures in the arterial blood vessels as well as within the Bruch's membrane can become calcified.

It is now clear that elastic fibers consist of several multifunctional proteins (Mecham and Heuser, 1991; Rosenbloom, 1996). The major component is elastin, a well characterized connective tissue protein. Specific mutations in the elastin gene have been shown to underlie heritable diseases, including Williams syndrome, supravalvular aortic stenosis, and some forms of cutis laxa. In PXE, the primary genetic lesion does not reside in the elastin gene (ELN) that has been mapped to the long arm of chromosome 7 (Fazio *et al*, 1991).

Elastin is associated with multiple microfibrillar proteins that are now known to represent distinct groups, including fibrillins (FBN1 and FBN2), latent transforming growth factor-β binding proteins, microfibril associated glycoproteins, and others (Rosenbloom, 1996). Many of these proteins contain epidermal growth factor like domains and multiple calcium binding sites. Thus, any of the corresponding genes could potentially serve as a candidate gene for mutations in PXE; however, chromosomal mapping of many of these genes has thus far indicated loci different from 16p, thus excluding them as the primary site for mutations in PXE (Uitto and Rosenbloom, 1998). It should be noted, however, that some of the microfibrillar proteins have not been cloned as yet, and consequently the chromosomal locations of the corresponding genes are currently unknown.

As indicated above, a diagnostic feature of PXE is calcification of the elastic structures (Baccarani Contri *et al*, 1996). Some mechanistic insight into these abnormal calcifications has been provided by recent studies on extracellular mineral binding Gla proteins, a small family consisting at this point of osteocalcin and matrix Gla protein. Although the precise function of these proteins is still somewhat obscure, emerging evidence suggests that they play a role in endochondral ossification (Luo *et al*, 1995); however, it appears that their presence may be necessary to prevent abnormal calcification. The latter conclusion was drawn from studies examining knockout mice with ablated matrix Gla protein gene. A dramatic feature of these animals was extensive calcification of elastic structures in a variety of tissues, including arterial blood vessels (Luo *et al*, 1997). Again, human matrix Gla protein has been mapped to a chromosomal locus 12p, excluding it as a major candidate gene for PXE.

As noted from clinical, morphologic, and ultrastructural studies, changes in the extracellular matrix of connective tissue, primarily elastic tissues, is the pathologic hallmark of PXE; however, mapping of the known extracellular matrix genes has revealed loci distinct from 16p. Thus, the involvement of elastic tissues in PXE could be explained by two different predictions. First, PXE could be due to genetic alterations in a gene encoding a yet to be discovered extracellular matrix component of the elastic tissues. In this context, it should be noted that genes encoding certain extracellular matrix proteins (e.g., emilin; Bressan *et al*, 1993) have not been cloned as yet and their chromosomal location is unknown. The second possibility is that the changes seen in elastic structures, including morphologic alterations and calcification, are secondary to a primary defect in a gene that does not encode an elastic fiber component directly. Nevertheless, understanding of the biochemistry and cell biology of elastic fibers is critical for further understanding of the pathomechanisms of PXE. Delineation of the structural features and metabolic pathways leading to elastic fiber assembly may also provide a pharmacologic perspective with the potential to interfere with the deposition of abnormal material in the skin and other affected tissues in PXE.

CLINICAL FEATURES OF PXE

PXE primarily affects skin, eyes, and the blood vessels, with considerable morbidity. One of the intriguing features, however, is considerable phenotypic variability that can be both intra- and interfamilial. The intrafamilial variability could be explained, to a certain extent, by the mode of inheritance. For example, in the autosomal recessive families, heterozygous carriers could possibly show partial manifestations of the disease. This situation could be somewhat analogous to Alport's syndrome, an X-linked renal disorder in which the heterozygous carrier mother often demonstrates subtle clinical manifestations. Alternatively, the autosomal dominant inheritance would result in a relatively mild disease in individuals with a mutation in one allele only, whereas individuals homozygous or compound heterozygous for mutations in both alleles would manifest with a more severe phenotype. This intrafamilial heterogeneity also raises the question of diagnostic criteria for PXE (Lebwohl *et al*, 1994). It was agreed that angioid streaks in an individual without family history are not sufficient for diagnosis of PXE. At the same time, the presence of angioid streaks in an individual with a family history of PXE, particularly coupled with demonstration of calcified elastic fibers in the skin, is sufficient for diagnosis even in the absence of overt cutaneous manifestations.

The interfamilial phenotypic variability perhaps is best explained by genotype/phenotype correlations due to allelic heterogeneity. The latter possibility can obviously be addressed only after identification of specific mutations and analysis of an extended mutation database, coupled with careful clinical examination of the affected individuals. An intriguing possibility is that the genetic background of the affected individuals may alter the expression of the mutated gene. This would also raise the possibility of modifier genes that might alter the expression of the gene primarily affected.

Another set of factors modifying the disease severity may relate to nutritional, environmental, and life-style variables that should be considered during the evaluation of patients with PXE. These include, for example, intake of drugs, vitamins, hormones, and nutritional supplements. It is clear that avoidance of aspirin and nonsteroidal anti-inflammatory drugs, which result in prolonged bleeding times, can be beneficial to the patients with PXE during episodes of hemorrhaging. Similarly, cigarette smoking, which can worsen intermittent claudication, is a clear-cut risk factor in PXE; however, the influence of other drugs or dietary variables is less certain. For example, does postmenopausal hormone replacement therapy alter the clinical severity

of PXE? Can pregnancy affect the severity of PXE? Are vitamin D supplementation and excessive calcium intake risk factors for PXE? Does hyperlipidemia accelerate the clinical involvement of coronary arteries? Although individual physicians may have recommendations for patients with PXE in these areas, clinical data in support of such recommendations are largely lacking. In the area of prevention, avoiding head trauma to avoid retinal hemorrhaging certainly makes sense in children with PXE; however, the value of laser treatment for neovascularization of retina is still unclear. These kinds of issues should be addressed by controlled clinical trials that should incorporate novel technologies to evaluate consequences of PXE and treatment attempts. Finally, the efficacy of various treatment modalities as well as environmental influences could be potentially tested in animals if the ongoing search for animal models for PXE is to be successful.

RECOMMENDATIONS ON FUTURE RESEARCH

At the end of the meeting, a panel was convened to identify areas of importance in basic research and clinical emphasis in relation to PXE. Panel members concurred with the following recommendations. With regard to research, the following areas were considered of the highest importance.

1 Intensification of efforts to identify the gene or genes that harbor mutations causing PXE, through the use of state-of-the-art technologies. These efforts should take advantage of the human genome project, as well as other efforts to characterize gene loci within the candidate region on chromosome 16p.

2 Addressing phenotype/genotype correlations by examination of a mutation database, in the future, in the context of clinical variability.

3 Delineation of disease mechanisms by continued investigation of normal composition, biosynthesis, and assembly of elastic structures with an emphasis on structure–function relationships.

4 Development of clinical studies to identify confounding variables that may modify the severity and presentation of the disease, including hormonal aspects, dietary regiments, and blood lipid profiles. The importance of this approach is emphasized by the fact that identification of risk factors may provide opportunities to interfere with development of complications and progression of the disease through pharmacologic intervention and life-style changes.

5 Development of outcome studies regarding the efficacy of specific treatment modalities. An example of such a study is the use of lasers to treat retinal neovascularization.

6 Continued development of animal models of PXE, both through generation of transgenic animals and through screening of naturally occurring mutant mouse strains.

7 Intensified efforts to secure continued research funding from national sources to study the basic biology and pathophysiology of the skin, eyes, and cardiovascular system in the context of PXE.

8 Vigorous efforts to gain further public support for research on PXE and other heritable connective tissues disorders, through interactions with the Coalition of Heritable Connective Tissue Disorders (CHCTD), the Coalition of Patient Advocates for Skin Disease Research (CPASDR), the National Organization for Rare Diseases (NORD), and others.

In the clinical area of PXE, the following issues should be emphasized.

1 Identification of additional patients and families for increased sample collection of clinical material, through the PXE International Blood and Tissue Bank, for distribution to all qualified researchers.

2 Refinement of diagnostic criteria to identify individuals affected by PXE.

3 Careful examination of the pattern of inheritance, particularly after identification of the gene mutations, in families with clinical manifestations of PXE.

4 Continued monitoring of the evolution of the disease in individual patients to provide a database for prognostic indicators.

5 Continued fostering of international exchange of information and research material to enhance studies on PXE. This includes organizing workshops, similar to this one, at regular intervals.

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