The genetics and immunology of Peyronie’s disease

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Peyronie’s disease (PD) is a condition characterized by localized and often progressive fibrosis and scarring of the penis. This condition has an unknown etiology although several hypotheses have been proposed. These include traumatic, immunologic and genetic causes. We studied the genetics and immunology of PD using both molecular biologic and molecular genetic techniques.

Men (n = 283) with PD were identified by retrospective chart review of one physician’s office practice. These men were contacted by telephone and asked to submit to an interview and blood test for genetic studies. Simultaneously, tissue and cells collected in the laboratory were examined by Western and Northern blot analysis for examination of protein and RNA for expression of HLA.

Of the first 107 men contacted, 24 were available and consented to interview and blood testing. The mean age was 60.3 y with an average duration of PD of 4.9 y. One patient had a family history of PD while no patients had Dupuytren’s contracture. Twenty patients were considered to have primary disease while four were secondary. Eleven patients had tissue prepared for Northern blot analysis and nine patients were the subject of Western blot analysis. All tissue, both Peyronie’s and control expressed class I MHC while no tissue expressed class II MHC. The expression of mRNA of class I MHC was equal for Peyronie’s and control patients while the expression at the protein level was less in the PD patients.

We conclude that PD may have multiple etiologic agents. One cannot exclude a class II MHC association but in our population, HLA DQ is not expressed. Class I MHC may be involved as the expression of class I MHC protein is different in Peyronie’s patients than in controls. Genetic studies are ongoing. International Journal of Impotence Research (2000) 12, Suppl 4, S127–S132.

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Introduction

Peyronie’s disease (PD) is a condition characterized by localized and often progressive fibrosis and scarring of the tunica albuginea of the penis. It is often associated with curvature of the penis, pain, and erectile dysfunction (ED). This condition was first described in 1743 by Francois Gigot de la Peyronie1 and its incidence has been reported to be as high as 1%.2 Although many theories exist as to the cause, the etiology of PD remains obscure. One theory suggests that trauma or mechanical stress may be responsible for the formation of a Peyronie’s plaque,3–5 ie, trauma to the penis results in delamination of the tunica albuginea with leakage of blood, fibrin deposition, and a subsequent inflammatory response.

Several other investigators believe that PD has a genetic or immunogenetic cause. Evidence for a genetic predisposition to PD was based on families in which a history of PD and Dupuytren’s contracture was found.6 In three families, an autosomal dominant pattern of inheritance was suggested based on the examination of pedigrees. A HLA analysis was performed to rule out an association with HLA-B7 complex. Chromosomal abnormalities have also been identified in patients with PD.7,8 Most commonly, an abnormality of the Y chromosome9 or an additional chromosome10 have been detected. Additional immunogenetic evidence stems from the association of PD with Dupuytren’s contracture and plantar fasciitis, conditions known to have an HLA association. Nyberg et al11 found an association between PD and the HLA B7 family of cross-reacting antigens. Although these results were subsequently refuted by Leffell and associates,12 other groups13–15 have also suggested associations with class II HLA antigens, specifically HLA DR and DQ. The purpose of this study was to determine whether a genetic or immunogenetic etiology can be attributed to PD.

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Methods and materials

All studies were performed according to protocols approved by the Internal Review Board of the Albert Einstein College of Medicine/Montefiore Medical Center.

Explant cell cultures

Smooth muscle cell cultures were derived from human corpus cavernosum tissue as previously described. Briefly, human corporal tissue obtained from patients undergoing placement of a penile prosthesis or gender reassignment for gender dysphoria was cut and placed in tissue culture dishes with sufficient nutrient medium. After the explants attached to the substrate, more medium was added. When the cells had migrated from the explant and undergone division, they were detached using 0.05% trypsin and 0.02% EDTA. The cells were subsequently grown in Dulbecco’s Modified Eagle Media (D-MEM) (Gibco BRL, Grand Island, NY) with 10% fetal bovine serum and antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml). Once the plates reach confluence, cells were either frozen, or passaged and processed for RNA or protein extraction. Only passages one to five were used for experiments. The cellular homogeneity was verified by immunofluorescence staining using anti-α-actin antibody, a human smooth muscle specific marker.

RNA preparation and Northern blot analysis

Total RNA was extracted from tissue and cell culture material using the TRIzol reagent (GibCo), adapted from the method of Chomczynski and Sacchi. Northern blots were prepared as described in Christ et al. Briefly, 20 μg of total RNA from each sample was electrophoresed in 1% agarose–formaldehyde gels. The RNA was capillary blotted in 10 x standard saline citrate (SSC) onto nylon membranes and fixed by heating. Hybridization was carried out in Rapid-hyb buffer (Amersham, Arlington Heights, IL) at 55°C with a biotin labeled human HLA A1-specific probe, corresponding to bases 879–1280 (GenBank accession d32131) for 2h. After washing with SSC, the membranes underwent detection steps using streptavidin and biotin alkaline phosphatase with CDP-Star substrate (New England Biolabs), and then exposed to Hyperfilm (Amersham).

Western blot analysis

Human corporal smooth muscle tissue were homogenized in 25 mM Tris-HCl buffer, pH7.4 containing 1 mM EDTA, 2 mM DTT, and 10 μg/ml each of leupeptin, aprotinin, PMSF, and centrifuged at 800 g for 10 min. The protein concentration of the supernatant was determined using the Micro BCA protein assay method (Pierce Chemical, Rockford, IL). Western blots were prepared. Briefly, 20–30 μg of protein from each sample were subjected to electrophoresis on 5% or 10% acrylamide gels. Proteins were then electrically transferred to nylon membranes. Blots were incubated overnight with 5% dried milk (BioRad) in phosphate buffered saline (PBS). Blots were then incubated with 1:100 dilution of human HLA (both class I and II) (Sigma, St. Louis, MO) for 2h. After incubation, blots were washed three times in PBS and incubated with 1:1000 dilution of horse-radish peroxidase (HRP) conjugated anti-mouse antibody. After three more washings, the HLA immunoreactive bands were detected with ECL reagents (Amersham Life Sciences, England).

Patient population

Patients for genetic studies were obtained from review of all charts from one urology practice. All patients with either a diagnosis of PD or those noted to have scarring of the penis were included. All patients were contacted by telephone and asked to submit to an interview and blood test after appropriate consent was obtained. The interview consisted of questions about nationality, duration of disease, family history of PD, co-morbidities, previous medical and surgical treatments and the presence of Dupuytren’s contracture or plantar fasciitis. Pedigrees were constructed of all patients with any family history of the condition and the blood of affected family members plus all available unaffected family members was tested.

Genomic DNA was prepared from 3 ml of human peripheral blood by using the Puregene Isolation kit (Genta, Minneapolis, MN). The DNA was diluted 1:50 and 1 μl was used for a PCR reaction. Lymphoblastic cell lines were established by using conventional procedures. This DNA was used for genetic dissection and identification of any genes that may be responsible for the condition. This was accomplished using the Family Core of the Program in Human Genetics at the Albert Einstein College of Medicine.

After the DNA from 200 patients has been collected, the genome will be mapped using polymorphic markers of tandem repeats of variable length. Based on patterns of inheritance, linkage
maps will be constructed by observing the frequency that two markers are inherited together. The closer the markers are located, the tighter the linkage between them.

Results

Two hundred and eighty three patients were identified as having PD. They were contacted by telephone and asked to submit to an interview and blood test. Of the first 107 patients contacted, 24 patients were available and agreed to participate. Nine patients refused to participate. The remainder were either unavailable at the time of this writing or the data were not evaluable.

The mean age of the patients participating was 60.3 y (range 47–88 y). One patient had a known family history with an affected son. The average duration of disease was 4.9 y (range 1 month–20 y). None of the first 24 patients had Dupuytren’s contractures or plantar fasciitis. Four patients had secondary disease, being attributed to intracavernous injection (three) and trauma (one). Fifteen patients were potent with three of these achieving partial erections sufficient for intercourse. Sixteen patients had been treated with medical therapy as first line therapy. The most common medical treatment was vitamin E, up to 2000 U/d, in 15 patients. Three patients were treated with colchicine, 1.2 mg/d, while one patient each received Potaba or verapamil intralesional injections. Ten patients were treated surgically with 8 modified Nesbit plications, one inflatable penile prosthesis and one semi-rigid penile prosthesis.

Cells from nine patients were processed for RNA purification. Five patients had PD while four patients served as controls. For protein purification, tissue or cells from four patients with PD were processed while four patients without PD served as control. Western blots were performed using purified protein from both patients and controls (Figure 1). Samples from both the Peyronie’s and control population expressed class I MHC, but there was less expression of class I protein in Peyronie’s patients than in the controls. There was no expression of class II HLA DQ in any of the samples, whether the patient had Peyronie’s or not (Figure 2). Northern blot analysis was performed using class I mRNA probes only and this revealed equal expression of mRNA in both Peyronie’s patients and controls (Figure 3).

Discussion

Peyronie’s Disease is a chronic condition characterized by localized and progressive scarring of the tunica albuginea of the penis. Patients with this disease often present to the urologist with curvature of the penis and pain, particularly with intercourse. The natural history of PD is that approximately 20% of patients will get better (defined as improvement in the curvature), 40% will get worse while 40% will remain stable. Examination of the Peyronie’s plaque has revealed alterations in the biochemical and structural make-up of the tunica albuginea. Luangkhot et al found that there was an increase in collagen fibers as well as distortion of the collagen.
distribution within the tunica albuginea of patients with PD. Similarly, Akkus et al.\textsuperscript{23} found disruption of the distribution of collagen and a decrease in elastin fibers within the tunica albuginea. The association of collagen alterations with genetic and immunologic diseases is well documented.\textsuperscript{24}

Several investigators have proposed an immunologic or immunogenetic cause for PD\textsuperscript{10–13,25–31} This evidence stems from an apparent association with HLA antigens. HLA, or human leukocyte antigen, is part of the major histocompatibility antigens (MHC).\textsuperscript{32} The gene for the MHC have been mapped to the p21.3 region of chromosome 6. The class I region encodes for the classical transplantation antigens HLA A, B and C. The class II region encodes the HLA D molecules, HLA DR, DQ and DP. There is a class III region which encodes several of the complement proteins and the enzyme 21-hydroxylase, as well as TNF\textsubscript{x} and \(\beta\) and HSP70. The MHC genes span approximately 3800 kb of DNA on chromosome 6.\textsuperscript{32}

HLA class I is expressed on most somatic cells, although levels vary from tissue to tissue. They are not found, however, on villous trophoblasts, neurons of the central nervous system, corneal epithelium or the exocrine portion of the pancreas. Lower levels of expression are found in endocrine tissue, myocardium, and skeletal muscle. Class II molecules are found on a more limited range of tissues. They are found on cell surfaces of B lymphocytes, monocytes, macrophages and dendritic cells, which are all antigen presenting cells of the immune system. They are also found in lower levels on vascular endothelium, breast and GI ductal epithelium and glomeruli.\textsuperscript{32}

Nyberg et al.\textsuperscript{6,9} demonstrated that PD was transmitted in an autosomal dominant fashion in three families who were HLA-B7 positive and that this antigen was present in 90% of the affected members. In their study, complete pedigrees were constructed from all three families, which revealed an extensive history of PD as well as Dupuytren’s contracture. All of the available family members, especially those affected, had HLA haplotyping by standard microtoxicity methods or mixed lymphocyte culture.

Zeigelbaum et al.\textsuperscript{31} reported on identical twins with PD who were typed to be HLA-B40 positive, a member of the B7 cross reacting antigen group which includes B7, B27, B40, BW22 and B13. It is interesting to note that both sons of one twin were B40 positive and were found to have induration of the penis at physical examination. Another brother, who was B7 group negative, was unaffected.

Ralph et al.\textsuperscript{27} recently performed tissue typing on 51 patients with PD. Fifteen of these patients also had Dupuytren’s contractures. They also cultured patients for infections known to cross react with HLA B27, ie, Campylobacter, Shigella, Salmonella, Yersinia and Chlamydia. They found a positive correlation only with HLA B27 and concluded that, because none of the bacterial cultures were positive, molecular mimicry or infection was not responsible for PD.\textsuperscript{27}

Rompel et al.\textsuperscript{12,13} performed HLA typing on 52 unrelated individuals with PD using a modified lymphotoxicity test for both class I and II MHC. They found a statistically significant association with HLA A1 and HLA DQw2, and in their 1994 study, they reported an association with HLA B8, Cw7 and DR3. The B7 family of antigen frequency, however, was similar to findings in the general population.

In a recent review, Leffell re-examined the evidence for an HLA association in PD\textsuperscript{23} and noted contradictory results from four studies investigating the B7 family. The first two reports that demonstrated a positive association evaluated only 17 patients combined,\textsuperscript{30,33} but two other studies, evaluating 80 patients,\textsuperscript{10,13} failed to demonstrate a positive association. With respect to class II MHC, only Rompel\textsuperscript{13} reported an association.\textsuperscript{25} In a study of 31 males, Nachtsheim and Rearden\textsuperscript{11} reported an association with HLA DQ5.

In the present study, we did not find expression of HLA DQ in any of our patients, even though HLA DQ is found in approximately 17% of the general population. Factors which may account for these findings are the following: (i) peripheral blood was not used for tissue typing and the HLA type of these patients was unavailable to us; (ii) corporal tissue may have been sampled from sites remote from the actual Peyronie’s plaque; and (iii) tissue storage (liquid nitrogen for up to 3 y) may also have affected changes in the tissue over time. We observed good expression of both RNA and protein to class I MHC, but have not to classify and type which class I MHC we are expressing.

It is interesting to note that, in spite of having equal expression of mRNA in both controls and Peyronie’s patients, the translation of message to protein was different.

A genetic etiology for PD has been examined by two other laboratories. Somers et al.\textsuperscript{18} performed chromosomal analysis and karyotyping in tissue derived from the plaques of 12 patients with PD, using lymphocytes or cells derived from elsewhere on the patient (dermis, normal tunica) as controls. Chromosome abnormalities were only detected in affected tunica albuginea. The most common abnormality noted was a Y deletion (45X,-Y) and two patients had a trisomy (of chromosome 7 and 8). Guerneri et al.\textsuperscript{7} performed similar studies on plaque tissue from 14 patients. This group also found an increased frequency of Y chromosome abnormalities, in eight of nine patients with an abnormal karyotype, although they discovered a high frequency of a supranumary Y chromosome. These authors did not comment, however, on their findings in the normal tissue studied.
We have proposed that PD may be a genetic condition and the identification of a gene may help further our understanding about it and eventually lead to approaches for gene therapy. In order to identify a gene responsible for a condition, gene mapping and genetic dissection must be performed. Next, linkage of genes must be established to map the genome. Three different methods of linkage analysis are possible. The best method is to evaluate multi-generational pedigrees and involves constructing a complete pedigree and DNA from all family members examined. The more family members with any given condition, and the more DNA available, the stronger an association can be made. Using this method in our study is difficult, if not impossible, as many of the men with PD are older and may not have surviving parents. Also, for those afflicted with PD, it is a very personal matter and finding family histories of this condition is difficult.

A second method is called the allele sharing method or affected sib pair analysis. In this method, siblings with and without the disease (preferably with the condition) are examined. Since siblings share 50% of their genetic material, two brothers with PD will share 100% of the DNA responsible for the condition. For an ideal analysis, approximately 500 sibling pairs would be required (R Kucherlapati, personal communication) and for an ideal analysis, approximately 500 sibling pairs would be required (R Kucherlapati, personal communication) and a multi-centered study would need to be undertaken. Currently, we have begun to contact investigators at major centers where PD is being undertaken. Currently, we have begun to contact investigators at major centers where PD is being studied to stimulate interest for such an under

The third method employs an ‘association’ or ‘case control’ approach, which do not concern familial inheritance patterns at all. These studies are based on homogeneous populations of unrelated individuals who are affected and unaffected. Examples of association studies are the association of HLA B27 with ankylosing spondylitis, where the allele is found in 90% of those affected and only 9% of the general population.

Once the population under study has been identified, genotyping can be performed. This is accomplished by examination of the chromosome and DNA by using markers of tandem repeats flanked by unique sequences (R Kucherlapati, personal communication). Any given individual will inherit 50% of his chromosomes from one parent and that region of tandem repeating sequences is conserved. Up to 300 markers are used to probe the genome in order to locate areas that are consistent. Once an area on a chromosome is identified, candidate genes in that area will be examined. For example, if the markers segregate to chromosome 1, where some collagen genes are located, then these areas will be sequenced in order to find a mutation in the collagen gene. This type of analysis has been performed on non-urologic condition with a known genetic association.

Conclusions

We have attempted to examine whether a genetic or immunologic association exists for PD. Our studies have not yet shown that such an association exists. Further work is needed to determine whether there is in fact an immunologic etiology to this condition. For example, subtyping our corporal tissue samples for class I MHC expression and studying these men for tissue typing would be of utmost importance. In spite of this, PD may not be solely an immunologic condition. Genetic studies are ongoing and may be paramount in identifying the cause of this disease.

Acknowledgement

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References

Appendix

Open discussion following Dr Melman’s presentation

Dr Broderick: What’s your opinion on the use of a lithotriptor to break up the plaques?

Dr Melman: I was aghast to see that people were using trauma to treat a disease that is induced by trauma. It doesn’t make any sense. I’d like to see people who are not invested in the device reporting on that. But there are no new medical therapies for these patients; no double blind studies that really show that anything is more efficacious, including vitamin E, caltracine, collagenous injections. The efficacy of verapamil injections, in my experience, has been pretty low.

Dr Carson: There’s one double blind study with Protava from Widener in Germany. He showed that there was a statistically significant improvement in curvature with Protava over placebo at twelve months.

Dr Melman: I just reviewed a paper on the use of an iontophoretic technique for getting Verapamil into the tissue. The results were statistically significant but it wasn’t a controlled study and it was rejected by the journal.

Dr Porst: This placebo controlled study with Protava didn’t show a significant improvement of the curvature, nor did it show a significant improvement of the plaques, whatever that means. In my opinion, it was not a successful trial.