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Primary pulmonary hypertension: immunogenetic response to high-mobility group (HMG) proteins and histone

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SUMMARY

HLA class II alleles (DNA typing) and antibodies to HMG-1,2,14,17 proteins and H1 histone were determined in three predominantly Caucasian groups of patients with pulmonary hypertension (PHT). Forty-four adults had primary pulmonary hypertension (PPH), 42 children had PPH, and 41 children had PHT associated with anatomically large congenital pulmonary to systemic shunts (PHT+shunt). The HLA class II alleles in the Caucasian patients were compared with those of 51 healthy Caucasian controls. Eight (18%) of 44 sera from adults with PPH bound HMG-14 and 23 (52%) bound H1. None of 42 sera from children with PPH bound either HMG-14/17 or HMG-1/2, whereas four (10%) bound H1. In the PHT+shunt group of 41 children, two (5%) bound HMG-14, one (3%) bound HMG-17, four (10%) bound HMG-1 and/or HMG-2, and six (15%) bound H1. Among the 12 HMG antibody-positive patients, HLA-DQ6 was present in nine of 10 HLA typed patients (six PPH adults and three PHT+shunt children), seven of whom had antibodies to HMG-14 and one to HMG-17. The 100% frequency of HLA-DQ6 in seven Caucasian patients with antibodies to HMG-14/17 was statistically significant when compared with the 41% frequency of -DQ6 present in 51 healthy Caucasian controls ($pc = 0{\cdot}027$, pc = Bonferroni correction, $OR = 21{\cdot}3$). In contrast, when compared with controls, 25 patients with PPH and anti-H1 antibodies (21 adults and four children) had increased frequencies of HLA-DQ7 and -DR5 (60% *versus* 29%, $P = 0{\cdot}010$, $OR = 3{\cdot}6$ and 48% *versus* 22%, $P = 0{\cdot}018$, $OR = 3{\cdot}4$), which were not significant after correction. In essence, antibodies to HMG-14 and to H1 proteins were present predominantly in adults with PPH, suggesting that the pattern of response to HMG-14/17 was similar to that previously reported in systemic lupus erythematosus (SLE) and drug-induced autoimmunity. This is the first report of an association between autoantibodies directed against HMG and H1 with immunogenetic markers. These data suggest that a subset of patients with PPH may have an autoimmune disease.

Keywords anti-HMG antibodies HLA typing anti-nucleosome antibodies autoimmunity major histocompatibility locus

INTRODUCTION

Primary pulmonary hypertension (PPH) is a well described clinical entity of unknown etiology [1]. The increased frequencies of Raynaud's phenonon [1], antinuclear antibodies [2], and the anti-Ku autoantibody [3] found in patients with PPH have prompted the hypothesis that PPH could be an autoimmune disease [4–7]. Pulmonary hypertension (PHT), often resembling PPH, can also be a clinical component of connective tissue diseases (CTD) including systemic lupus erythematosus (SLE) [8,9], scleroderma [10], rheumatoid arthritis (RA) [11], dermatomyositis [12] and mixed connective tissue disease [13]. HLA-class II alleles encoded within the MHC appear to play important roles in autoimmune diseases [14]. The associations of HLA with PPH and with CTDs and PHT have been varied and complex. Increased frequencies of HLA-DR52 have been found in patients with scleroderma and PHT [15] and of HLA-DR3 and -DR52 in children with PPH [16]. More recently an increased frequency of HLA-DQ7 was reported in both children and adults with PPH [17]. There have been no reports of genetic/autoantibody associations specific for PPH, although HLA-DQ1 has been correlated with the anti-Ku autoantibody which has been identified in some patients with PHT [18].

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Table 1. Frequencies of antibodies to HMG proteins and H1 histone in pulmonary hypertension (PHT) patients

	Patients (predominantly Caucasian)		
Antibodies	PPH adults, $n = 44$ *n* (%)	PPH children, $n = 42$ *n* (%)	PHT+shunt children, $n = 41$ *n* (%)
Anti-histone H1	23 (52)	4 (10)*	6 (15)†
Anti-HMG-14	8 (18)	0 (0)	2 (5)
Anti-HMG-17	0 (0)	0 (0)	1 (3)
Anti-HMG-1/2	0 (0)	0 (0)	4 (10)

*Anti-histone, primary pulmonary hypertension (PPH) children *versus* PPH adults; pc = 0·008; OR = −10·4.

†Anti-histone, PHT+shunt children *versus* PPH adults; pc = 0·008; OR = −6·4.

The purpose of this study was to determine whether a genetic/autoantibody subset of PPH could be delineated by the presence of antibodies to HMG proteins. The five main types of mammalian HMG proteins are HMG-1, -2, -14, -17, and HMG-I/Y. HMG-14 and -17 bind to core histones and are preferentially associated with transcriptionally active chromatin, whereas HMG-1 and HMG-2 are associated with internucleosomal DNA [19,20]. Anti-HMG antibodies have been previously reported in SLE [21], drug-induced autoimmunity [22], scleroderma [23], juvenile rheumatoid arthritis (JRA) [24–26], and canine SLE [27]. Since there have been no reports of anti-HMG antibodies in PPH or of HLA typing in any patient group with these antibodies, we determined the presence of these antibodies and the HLA-DRB1,3,4,5 and -DQB1 alleles in three patient groups with PHT. The patient groups were adults with PPH, children with PPH, and children with PHT associated with congenital heart disease consisting of anatomically large pulmonary to systemic communications (PHT+shunt).

PATIENTS AND METHODS

Patients, controls and sera

Sera were obtained from 44 adults with PPH, 42 children with PPH and 41 children with PHT+shunt for this study after approval by the institutional review board of Columbia University College of Physicians and Surgeons. Children were 18 years or younger. All patients, an extension of three previously reported groups with PHT, underwent cardiac catheterization and the diagnosis of PPH was established using the algorithm developed by the PPH NIH registry [28]. The PHT+shunt children, many described previously [16], had anatomically large congenital pulmonary to systemic communications but with unexplained pulmonary vascular disease. The unexplained pulmonary vascular disease is defined as pulmonary vascular disease occurring earlier than normally seen in children with anatomically large shunt lesions, or developing in children followed closely from early infancy without previously demonstrating hyperkinetic high flow PHT.

Racial composition of patients was: 44 adults with PPH (38 Caucasians, three orientals, and three Indians), 42 children with PPH (40 Caucasians, one Indian and one Black), and 41 children with PHT+shunt (39 Caucasians and two orientals). Serum samples were obtained from most patients before the institution of chronic vasodilator (nifedipine and/or prostacyclin) therapy. Except for two adults on hydralazine, none of the other patients received drugs associated with drug-induced autoimmunity, such as procainamide, isoniazid, quinidine, D-pencillamine, acebutolol, alpha-methyl-dopa, sulfasalazine, or chlorpromazine [29].

None of the patients fulfilled the American College of Rheumatology diagnostic criteria for SLE [30], scleroderma [31] or rheumatoid arthritis [32] or the suggested criteria for JRA [33]. As part of an ongoing study of immunogenetic profiles in PHT patients, patient determinations were antinuclear antibodies, autoantibodies to DNA, Ro, La, Sm, RNP and centromere, serum complement, immunoglobulin IgG, A, and M quantification, and rheumatoid factor.

Normal controls included 51 healthy adult Caucasian volunteers from Columbia Presbyterian Medical Center (CPMC) personnel.

Immunoblotting

HMG proteins and histone H1 were extracted from calf thymus nuclei with 5% perchloric acid (PCA) and precipitated in acetone/hydrochloric acid, as described by Nicholas & Goodwin [34]. HMG fractions were purified by cation exchange high pressure liquid chromatography (HPLC). The HMG proteins in the HPLC fractions were identified by their elution positions and mobilities in acid-urea polyacrylamide gels [22]. To characterize the HMG fractions in SDS-polyacrylamide (15%) gels, the HPLC-purified fractions were used for initial immunoblotting experiments. Thereafter, the reconstituted acid-acetone-precipitated PCA extract was used as the antigen source for immunoblotting. Electrophoresis and immunoblotting adapted the protocols previously described [22,23]. Sera were diluted to 1:100 in blocking solution (3% milk and 0·05% Tween 20 in PBS). Detection of bound antibody was by autoradiography with ^{125}I-protein A (specific activity > 30 mCi/mg; ICN Biomedicals, Costa Mesa, CA), diluted in blocking solution to $125–250 \times 10^3$ ct/min per ml, or with biotin-avidin peroxidase anti-human IgG (Vectastain ABC kit; Dimension Labs, Missisauaga, ON).

Histocompatibility studies

HLA class II typings (DRB1,3,4,5 and -DQB1) were performed using the UCLA PCR-SSP (sequence-specific primers) or SSOP (sequence-specific oligonucleotide probes) [35]. Antigen frequency was obtained by direct count for the patients and for 51 normal Caucasian controls at CPMC.

Statistical analysis

χ^2 and Fisher's exact tests were used to compare the HLA-DR,DQ allele frequencies of the patient groups with the control group. The odds ratios (OR) were calculated using Mantel–Haenzel methods [36]. Bonferroni correction (pc) was used to correct for the number of alleles tested ($\times 7$ for HLA-DQ and $\times 10$ for -DR alleles).

RESULTS

Antibodies to HMG proteins and H1 histone in PHT patients

Table 1 illustrates the frequencies of antibodies to HMG-1/2, HMG-14/17 and H1 proteins found in the sera of 44 adults and

Table 2. HLA-DRB1,3 and DQB1 alleles and clinical parameters in pulmonary hypertension (PHT) patients with antibodies to HMG proteins

PHT patients	Anti-HMG antibodies	Clinical parameters	HLA class II alleles		
			HLA-DRB1	HLA-DRB3	HLA-DQB1
1 PPH	14		1301,1501		**0604,0602**
2 PPH	14	C2 deficiency, anti-hep C Increased IgG,A,M	1301,1302	0101,0301	**0603,0604**
3 PPH	14	Increased IgA	1501,0401		**0601,**0301
4 PPH	14	Increased IgG,A	1501,1101/4	0202	**0602,**0301
5 PPH	14		1502,0701		**0601,**0201
6 PPH	14	Increased IgG Decreased protein S	1302,0701	0301	**0604,**0201
7 PPH	14		NT		
8 PHT+shunt	14	Anti-hep C	NT		
9 PHT+shunt	1,2,14	Down's syndrome	1502,0102		**0601,**0501
10 PHT+shunt	1,2,17		1501		**0602,**0501
11 PHT+shunt	1 or 2		1301,0401	0101	**0602,**0302
12 PHT+shunt	1,2	Charge syndrome, thyroiditis, hypothyroid	0701,1101/4	0202	0201,0301

Nos 1–7, adults with primary pulmonary hypertension (PPH); nos 8–12, children with PHT+shunt. Patients were Caucasian except for no. 3 (Korean) and no. 8 (Vietnamese). NT, Not tested.

42 children with PPH and of 41 children with PHT+shunt. Eight of 44 (18%) adults with PPH had antibodies to HMG-14 and 23 (52%) had antibodies to H1. None of the 42 children with PPH had antibodies to HMG-1/2 or HMG-14/17, whereas four (10%) had anti-H1 antibodies. In the PHT+shunt group of 41 children, six (15%) had anti-H1 antibodies. Four of these children with anti-H1 antibodies had antibodies to HMG-1 and/or HMG-2, two of whom had either an anti-HMG-14 or anti-HMG-17 antibodies. The other anti-H1-positive child had an anti-HMG-14 antibody. Of interest, antibodies to HMG-1/2 were not found in adults with PPH. Normal sera have previously been shown not to have antibodies to HMG proteins or to H1 [22,23].

The most important statistically significant finding was the decreased frequency of anti-histone H1 antibodies in both PPH and PHT+shunt children compared with their frequency in adults with PPH (Table 1). Both comparisons had $pc = 0{\cdot}008$ when children were compared with adults, but the $OR = -10{\cdot}4$ for PPH children was greater than the $OR = -6{\cdot}4$ for PHT+shunt children.

There did not appear to be any uniform features which clinically distinguished PHT patients with (Table 2) and without antibodies to histone and/or HMG proteins. As noted previously, positive antinuclear antibodies, an occasional autoantibody, and autoimmune parameters within families were found in all three categories of these patients [16], although none of the patients fulfilled the American College of Rheumatology diagnostic criteria for SLE [30], scleroderma [31], or RA [32]. Raynaud's phenomenon was present infrequently. One child (no. 9) with PHT+shunt and antibodies to H1, HMG-1/2 and HMG-14 had Down's syndrome, and another (no. 12) with antibodies to HMG-1/2 had CHARGE syndrome. Sera from the vast majority of patients, regardless of their anti-HMG antibody status, were obtained before initiation of vasodilator therapy. Two adults received hydralazine: one had anti-HMG-14 and anti-H1 antibodies and the other did not have antibodies.

HLA class II alleles in PHT patients with antibodies to HMG proteins

HLA class II typing was available for 36 of 44 adults with PPH, 37 of 42 children with PPH, and 38 of 41 children with PHT+shunt. Table 2 illustrates the HLA-DRB1,3,4,5 and -DQB1 alleles found in 10/12 patients with antibodies to HMG-14/17 and HMG-1/2. HLA-DQ6 was present in nine of the 10 HLA class II typed patients (eight Caucasians and one Korean) having antibodies to either or both families of HMG proteins. Seven patients with antibodies to HMG-14 (six adults and one PHT+shunt child) and an additional PHT+shunt child with an antibody to HMG-17 typed for HLA-DQ6. HLA-DQ6 was present in three of the four children with PHT+shunt and antibodies to HMG-1 and/or HMG-2, including the two children with antibodies to HMG-14/17. Most HLA-DQ6-positive patients from the three clinical groups with PHT did not have antibodies to HMG proteins. For example, of the 38 HLA class II typed adults with PPH, 16 had HLA-DQ6 but only six of these 16 (38%) had antibodies to HMG-14.

HLA-DQ6 is an allele in linkage disequilibrium with both the -DR15 and -DR16 subtypes of -DR2 and the -DR13 and -DR14 subtypes of -DR6. However, in these patients positive for HLA-DQ6 and antibodies to HMG-1/2 and HMG14/17, only the more prevalent -DR15 and -DR13 subtypes were present. In fact six of eight of the HLA-typed patients with anti-HMG-14/17 antibodies had the HLA-DRB1*1501/1502, -DQB1*0601/0602 haplotypes. The 75% frequency of these haplotypes was significant when compared with the 18% frequency found in controls ($P = 0{\cdot}0023$, $OR = 11{\cdot}6$).

HLA class II alleles in Caucasian HMG antibody-positive patients and Caucasian controls

Table 3 compares the frequencies of selected HLA-DRB1 and -DQB1 alleles found in the Caucasian antibody-positive patients with those present in 51 Caucasian controls typed concomitantly.

Table 3. Comparisons of frequencies of selected HLA-DRB1 and -DQB1 alleles in pulmonary hypertension (PHT) patients, HMG antibody-positive patients and healthy controls (Caucasians only)

HLA alleles	Controls, $n = 51$ (%)	PPH, $n = 66$ (%)	PHT+shunt, $n = 36$ (%)	Anti-HMG-14/17, $n = 7$ (%)	Anti-HMG-1/2,14/17, $n = 9$ (%)
HLA-DR2	(22)	(21)	(39)	(71)	(56)
HLA-DR6	(26)	(23)	(31)	(43)	(44)
HLA-DQ5	(35)	(18)‡	(44)	(29)	(22)
HLA-DQ6	(41)	(36)	(53)	(100)*	(89)
HLA-DQ7	(29)	(54)†	(31)	(29)	(33)

*Anti-HMG-14/17-positive patients *versus* controls; HLA-DQ6, pc = 0·027, OR = 21·3.
†Primary pulmonary hypertension (PPH) patients (adults+children) *versus* controls; HLA-DQ7, pc = 0·049, OR = 2·9.
‡PPH patients *versus* PHT+shunt children; HLA-DQ5, pc = 0·035, OR = −3·6.

Table 3 also compares the frequencies of selected alleles in patients (children and adults) with PPH and PHT+shunt children with each other and with the same controls. The most statistically significant finding was the increased frequency of HLA-DQ6 in the seven patients with antibodies to HMG-14/17, 100% *versus* 41% (pc = 0·027, OR = 21·3). The associations between HLA-DQ6 and HMG-14 alone and between HLA-DQ6 and both HMG-1/2 and 14/17 families combined were not significant when confined to Caucasians. There were no significant increases in any of the HLA-DQB1*0601-0604 subtypes of -DQ6. The increased frequencies of HLA-DR2 and of HLA-DR6 in patients with anti-HMG14/17 were not significant after Bonferroni correction. Although three of the four children with PHT+shunt and antibodies to HMG-1 and/or HMG-2 (including two with antibodies to HMG-14 and HMG-17) also had HLA-DQ6, the small sample size precluded statistical comparisons. Although these data evaluated Caucasians, the frequencies of HLA-DQ6 in normal orientals, 42% [37], Blacks, 47% [38] and Caucasians, 41% [39], were similar.

The significantly increased frequency of HLA-DQ7 found in this enlarged group of patients with PPH compared with normal controls (pc = 0·04, OR = 2·8) was reported previously in a smaller group of children and adults with PPH [17]. The significantly decreased frequency of HLA-DQ5 in patients with PPH compared with children with PHT+shunt ($P = 0·035$, OR = −3·6) is a new finding which requires further study. However, neither the patients with PPH nor the children with PHT+shunt had significant changes in HLA-DQ5 compared with its frequency in normal controls.

HLA Class II alleles in PPH patients with anti-histone (H1) antibodies

Table 4 compares the frequencies of selected HLA-DRB1 and -DQB1 alleles found in 25 patients with PPH (21 adults and four children) and anti-H1 antibodies with those found in 51 healthy Caucasian controls. No significant differences were apparent when corrected for the number of alleles tested (Bonferroni correction). Before correction, there was an increased frequency of HLA-DQ7 and of -DR5 in the predominantly Caucasian patients with PPH and antibodies to H1 compared with controls, 60% *versus* 29% ($P = 0·010$, OR = 3·6) and 48% *versus* 22% ($P = 0·018$, OR = 3·4), respectively.

DISCUSSION

This is the first study of the prevalence of antibodies to the nucleosome-associated protein HMG-14 and H1 histone in the sera of adults with PPH and of the immunogenetic correlation between antibodies to HMG-14/17 and HLA-DQ6. Antibodies to HMG-14 were present in 18% of adults with PPH, in none of the children with PPH and in 5% of children with PHT+shunt. All patients with PHT and antibodies to HMG proteins had antibodies to H1 histone. Antibodies to H1 histone were also more prevalent: approximately 50% in adults with PPH and 10–15% in children with PPH or PHT+shunt.

There was nothing clinically apparent to distinguish patients with antibodies to HMG or histone H1 proteins from the antibody-negative patients. We had expected that antibodies to HMG-1/2 and H1 might define patients with active and/or more severe disease, since antibodies to HMG-1/2 are more common in systemic sclerosis than in other CTDs [23] and antibodies to histone in these patients identified a subset of scleroderma patients with more severe disease [40]. However, in the current study of PPH and PHT+shunt patients, this was not the case. The failure to find antibodies to any type of HMG protein in children with PPH is interesting, since adults with PPH had antibodies to HMG-14 and a few children with PHT+shunt had antibodies to both the HMG-1/2 and HMG-14/17 families. This may reflect the small sample size, the decreased frequencies of autoantibodies in children compared with adults, or immaturity and/or anergy of the immune system in these children. Our clinical experience has been that children with PPH, although initially found to be antinuclear antibody-negative, have developed low titre antinuclear antibodies over time [16].

The pattern of autoantibodies to HMG-14 found in the subset of adults with PPH was similar to that found in SLE [21] and drug-induced autoimmunity [22]. The pattern of anti-HMG-1/2 antibodies found in children with secondary PHT was also consistent with the autoantibody specificity reported for JRA [24,25]. However, none of these patients fulfilled the diagnostic criteria for SLE [30] or JRA [33], although PHT may present as a *forme fruste* of these or any CTDs. None of the PPH or PHT+shunt patients had elevated titres of antibodies to dsDNA, Sm or Ku.

The patients did not receive drugs commonly associated with drug-induced lupus with the exception of two adults on

Table 4. Frequencies of selected HLA-DRB1 and -DQ1 alleles in primary pulmonary hypertension (PPH) patients with antibodies to histone (H1) compared with healthy controls (Caucasians only)

HLA alleles		Controls, $n = 51$ *n* (%)	PPH patients, $n = 25$ *n* (%)	*P*	OR
HLA-DR1	DRB1*0101-3	13 (26)	2 (8)	0·123	– 3·9
HLA-DR5	DRB1*1101-4 DRB1*1201/2	11 (22)	12 (48)	0·018	3·4
HLA-DQ7	DQB1*0301	15 (29)	15 (60)	0·010	3·6

P = NS after Bonferroni corrections.

hydralazine, one of whom had antibodies to HMG-14 and histone H1. None of the patients received procainamide, isoniazid, quinidine, D-penicillamine, acebutolol, alpha-methyldopa or sulfasalazine. Neither of the two vasodilator drugs used, nifedipine and/or prostacylin, has been implicated in drug-induced autoimmunity.

The 100% frequency of HLA-DQ6 in Caucasian patients with antibodies to HMG-14/17 (OR = 21) was the most significant immunogenetic finding. The significantly increased frequency of HLA-DQ7 in patients with PPH and the failure to find an HLA association for children with PHT+shunt compared with normal controls have been reported previously for smaller patient numbers [17]. There are no previous reports of HLA testing in patients with antibodies to HMG proteins with which to compare our immunogenetic findings. In patients with PPH and anti-histone H1 antibodies, the increased frequencies of HLA-DR5 and of -DQ7 were only significant prior to correction. Anti-histone antibodies have been reported to be associated with an increased frequency of HLA-A2 (class I) and not with any -DR allele in children with chronic juvenile arthritis [41].

Whether the strong immunogenetic correlation between antibodies to HMG-14/17 and HLA-DQ6 will persist regardless of disease origin remains to be determined. An increased frequency of HLA-DQ6 was reported in patients with scleroderma and antibodies to fibrillarin, a U3-RNP antigen found in the fibrillar region of the nucleolus [42]. In scleroderma, this autoantibody, more frequent in Blacks, was also associated with a higher frequency of PHT, radiographic small bowel involvement, and diffuse skin disease (reviewed in [43]). The HLA-DQB1*0602 subtype has been associated with anti-Sm autoantibodies in SLE [44], but antibodies to Sm were not found in our PPH or PHT+shunt patients. Uninvestigated is whether anti-HMG antibodies were also present in the HLA-DQ6-positive scleroderma patients with anti-fibrillarin antibodies and in the -DQ6-positive SLE patients with anti-Sm antibodies.

The HLA-DQ6/anti-HMG-14/17 association found here did not have specificity for a particular HLA-DQB1*0601-4 subtype. It is difficult to define what is unique to these four subtypes compared with the other HLA-DQB1 alleles. In the first domain of the HLA-DQB1 chain, only the HLA-DQB1*0602-4 subtypes have phenylalanine at the aa position 86 and aspartate at aa position 57 which is shared by -DQ4,7 and 9. In contrast, -DQB1*0601 has tyrosine at position 86 (like -DQ5) and valine at position 57 (like -DQB1*0501). The aa position 57 has been implicated in autoimmunity [45] and position 86 guards an entrance to the antigen-binding cleft [46], each site contributing to binding with the T cell receptor.

The HLA-DQ6/HMG-14/17 association could also result from linkage to neighbouring molecules encoded within the MHC which were not determined here. These include class III complement components C2, C4 and Bf, tumour necrosis factor-alpha (TNF-α), TAP proteins, proteosomes, hsp70, and other undefined molecules. Particular haplotypes rather than individual MHC specificities may also provide more important susceptibility factors for disease and for autoantibody production. For example, the HLA-DRB1*1501, DQA1*0102, DQB1*0602 haplotype has been implicated in conferring susceptibility to SLE [47]. This is also suggested in our data by the presence of HLA-DRB1*1501/1502, -DQB1*0601/0602 haplotypes in six of the eight patients with anti-HMG-14/17 antibodies.

These data raise several interesting immunogenetic aspects which require further investigation and substantiation in larger numbers of patients. Since the genes for human HMG-14, -17, -1 and -2 have been cloned [20], B and T cell epitope mapping studies are possible. Such studies could define whether there are private or shared antigens within or between HMG families. The results of this type of investigation may help to define the distinctive relationship of HLA-DQ6, or of other linked MHC molecules, and the specificity of the antibody response to HMG proteins. The presence of antibodies to HMG-14/17 proteins, which bind nucleosomal core histones of transcriptionally active chromatin, has suggested to several investigators that the nucleosome or nucleosomal subunit, and not DNA or histone alone, is the immunogen in autoimmune disease (reviewed in [43,48]). This suggestion, if substantiated, supports our hypothesis that a subset of patients with PPH have an autoimmune disease and an increased frequency of a particular HLA class II allele.

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