ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE PROMOTER OF APOLIPOPROTEIN H WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease that targets the vascular system, and can result in premature atherosclerotic vascular disease. The causes of SLE and many of the SLE associated problems, such as thrombosis, anitphospholipid syndrome, and atherosclerosis, which are significant public health concerns, are thought to be multifactorial, or caused by interactions of many environmental and genetic factors. Several genes have been proposed and studied in conjunction with these manifestations. This study focuses on one of these genes, apolipoprotein H (APOH gene, β_2-GPI protein). The effects of several polymorphisms within the coding region in the APOH gene have been studied; however, possible effects of the single nucleotide polymorphisms (SNPs) within the promoter region have not been characterized. In this study, 6 SNPs in the APOH promoter were genotyped in 381 SLE women and 497 healthy women controls. This study aimed to determine the association of these polymorphisms with the occurrence of SLE, with the plasma levels of β_2-GPI, and with the presence of antiphospholipid antibodies (APA). It was hypothesized that the genetic variation in the promoter region of the APOH gene may affect the risk of SLE and may do so through an effect on plasma β_2-GPI levels or through its influence on APA. Among whites, the risk of SLE was modestly affected by the -1219 SNP ($p = 0.057$). While in blacks, the -759 SNP ($p = 0.022$) and the -700 SNP ($p = 0.035$) showed association with SLE. The haplotype pattern in whites was associated with SLE risk ($p = 0.00015$). The -643 SNP showed a modest effect on plasma β_2-GPI levels in white SLE

cases ($p = 0.096$) and black controls ($p = 0.081$). Significant differences were seen between antibody negative and all antibody positive groups in whites for the -1284 SNP ($p = 0.02$) and the -759 SNP ($p = 0.046$). These results suggest that genetic variation in the APOH promoter may affect SLE risk, β_2-GPI levels, and the occurrence of antiphospholipid antibodies.

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1. BACKGROUND AND SIGNIFICANCE

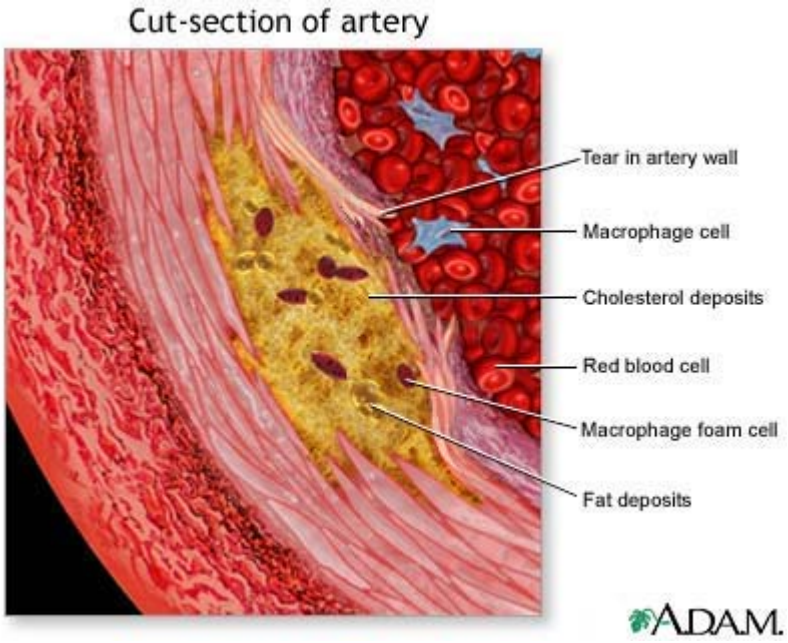
1.1. Introduction

Systemic lupus erythematosus is an autoimmune disease in which autoantibodies are produced against nuclear antigens, cytoplasmic antigens, and blood cell surface antigens. The binding of these autoantibodies to their antigens causes the build up of immune complexes in blood vessel walls. This leads to the manifestations of SLE depending on which organs and cell types are involved (Trethewey, 2004). SLE has an incidence of 1.8-7.6 per 100,000 and a prevalence of 14.6-50.8 per 100,000 in the United Stated. All ethnic groups are susceptible to SLE; however, the incidence in blacks, 1:250, is greater than in whites, 1:1000. It does not affect the sexes equally with a 1:9 male to female ratio, and 80% of cases occur in women in their child-bearing years (Lamont, 2005).

The rates of cardiovascular disease in young women with SLE are very high; women in the 35 to 44 year old age group have a 50-fold increase in risk of myocardial infarction. SLE targets the vacular system with manifestations such as vasculitis, vasculopathy, vasospasm, thromboembolism, and, now that SLE patients are living longer, premature atherosclerotic vascular disease (Manzi et al. 1997).

An early event in atherosclerosis is the formation of cholesterol-filled foam cells. Low density lipoproteins (LDL) play an important role in foam cell formation. High levels of LDL particles

can lead to accumulation and modification of LDL into acetylated LDL and oxidized LDL (ox-LDL) in the subendothelial space. These modified LDL particles recruit monocytes which become macrophages that take up modified LDL and generate foam cells (Fisher et al. 1972). Figure 1 provides a picture of an enlarged view of atherosclerosis.



Keller S, Atherosclerosis. *MedlinePlus Medical Encyclopedia.* Updated 2004.

Figure 1. Atherosclerosis

Apolipoproteins associate with lipoproteins and help make up the lipoprotein particles, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons (CM). This study focuses on one specific apolipoprotein called apolipoprotein H, or β_2-glycoprotein I (APOH gene; β_2-GPI protein). β_2-GPI plays a role in the lipoprotein

metabolic pathway, influences the development of atherosclerosis, and plays a role in the development of other aspects of SLE.

1.2. Role of apoH in Antiphospholipid syndrome

Another aspect of SLE that causes problems for many women is Antiphospholipid syndrome (APS). Antiphospholipid syndrome occurs when a specific type of autoantibody, anti-phospholipid antibodies, are present and cause at least one clinical manifestation. Clinical manifestions often include venous or arterial thrombosis or recurrent pregnancy loss. APS can be isolated, called primary antiphospholipid syndrome (PAPS) or it can be a part of another disease, particulary SLE (Hanly 2003).

Antiphospholipid antibodies are seen in about 1-5% of healthy individuals in the population; in contrast, about 50% of people with SLE have these antibodies. Approximately 34-42% of people with SLE actually develop APS (Carsons 2004). The major antiphospholipid antibodies that have been tested for are anticardiolipin (aCL) and Lupus anticoagulant (LAC). LACs are a family of antiphospholipid-plasma antibodies that interfere with phospholipids dependent coagulation reactions. aCL antibodies are antiphosipholipid antibodies that bind to negatively charged phospholipids; they are named for the representative antigen, cardiolipin, which is used in the immunoassay to test for aCLs (Kumar et al. 2002). It is now known that phospholipids are not actually the antigens for most of the antibodies seen in APS. Most of these antiphospholipid antibodies actually recognize the protein β_2-GPI (Roubey 2004). Antibodies that directly

recognize β_2-GPI, called anti- β_2-GPI antibodies are also tested for when considering APS in addition to aCL and LAC.

1.3. Role of β_2-GPI in Lipid Metabolism and Atherosclerosis

β_2-GPI has been shown to play a role in phospholipid-dependent reactions, cholesterol transport, foam cell formation, and ultimately, atherosclerosis. As mentioned previously, cholesterol accumulation in the cells of vessel walls is a characteristic of atherosclerosis. Data from a study by Lin et al (2001) has shown that β_2-GPI has some control over cholesterol transport. It reduced the influx of [^{3}H]CE-labeled oxidized LDL (ox-LDL) and slightly increased the efflux of [^{3}H] cholesterol. Therefore, it increased the net efflux of cholesterol. The same study also suggested that β_2-GPI may inhibit oxidation of LDL particles. Oxidized LDL has an increased electrophoretic mobility in the presence of copper as compared to native LDL. When ox-LDL particles were treated with β_2-GPI the relative electrophoretic mobility (REM) was reduced in a dose-dependent manner. This inhibitory effect was diminished when treatment with anti- β_2-GPI antibodies was added. This study has shown that β_2-GPI may work to inhibit LDL oxidation and may also reduce cholesterol accumulation in macrophages. Both of these steps are thought to be important factors in atherosclerosis; thus, β_2-GPI may play a role in prevention of atherosclerosis. Additionally this prevention is compromised in the presence of anti- β_2-GPI antibodies (Lin et al. 2001).

Hasunuma et al (1997) showed that β_2-GPI binds to all oxidized plasma lipoproteins in vitro. They found that the binding of β_2-GPI to oxidized LDL (ox-LDL) partially inhibited uptake by

macrophages of ox-LDL in vitro. This effect was dose dependent and was only partial, even at the highest β_2-GPI level (1200 μg/ml). About 50% of ox-LDL binding to macrophages was inhibited when 400 μg/ml of β_2-GPI was used; this is twice the average concentration of this protein. This inhibition of ox-LDL uptake is anti-atherogenic. However, when aCL or anti- β_2-GPI antibodies were added along with β_2-GPI the binding of ox-LDL to macrophages was significantly increased, up to 211% and 208% respectively (Hasunuma et al. 1997).

β_2-GPI normally binds weakly to membranes with anionic phospholipids, but does not inhibit binding of anticoagulant proteins or coagulation factors. However, the presence of anti- β_2-GPI antibodies creates crosslinked complexes that bind more tightly to membranes and inhibit the binding of other phospholipids binding proteins (Takeya et al. 1997). This may play a role in harmful processes such as thrombosis. For example, a recent study has shown that antiphospholipid antibodies inhibit the binding of annexin V to endothelial cells and trophoblasts in the presence of β_2-GPI (Rand et al. 1997). Annexin V is thought to have antithrombotic function. These antibodies are seen more frequently in the SLE population than they are in the general population; likewise, thrombosis is also seen more frequently in those with SLE and APS then people in the general population.

1.4. Molecular Aspects of ApoH

The APOH gene is 18 kb long with 8 exons and has been mapped to chromosomal location 17q23-17q24. It codes for 326 amino acids which make up a single chain glycoprotein with a molecular weight of about 50 kDa (Lozier et al. 1984). Of 8 exons, exons 2 through 7 code for

short consensus repeats (SCRs). Four SCRs are encoded by 4 separate exons and the fifth SCR is encoded by 2 exons. Exon 1 encodes the 5' UTR and the single peptide sequences; the transcription initiations site is deemed +1 and is located 31 bp upstream from the translational start codon. Exon 8 encodes the 3'UTR, the stop codon, and the C-terminus (Okkels et al. 1999).

This protein is made up of 5 domains, four complement control protein (CCP) modules and a fifth domain. The fifth domain is positively charged and provides a binding region for negatively charged substances such as phospholipids (Schwarzenbacher et al. 1999). Sanghera et al (1996) found two structural mutations in the fifth domain of β_2-GPI. Homozygotes for these mutations as well as compound heterozygotes lack the ability to bind to negatively charged phosphatidylserine (PS), indicating that the fifth domain is the location for the lipid binding region. Wang et al (2001) have provided evidence that β_2-GPI binds to the lipid layer in two different orientations. It has an upright orientation in the liquid phase region and a flat orientation in the condensed domain region. The different types of lipoproteins are each partially composed of β_2-GPI. It makes up 17% of HDLs, 16% of CMs and VLDLs, and 2% of LDLs (Lozier et al. 1984).

ApoH was originally thought to be produced in only the liver. It has since been shown, using reverse transcriptase-polymerase chain reaction (RT-PCR) and southern analysis to analyze expression levels, that it is also expressed at the mRNA level in the small and large intestines (Averna et al. 1997). Similarly, Caronti et al (1999) demonstrated that β_2-GPI mRNA is expressed in many cell types which are involved in anti-phospholipid syndrome (APS) using RT-PCR. They observed expression in endothelial cells, which are targeted by autoantibodies in

APS, astrocytes and neurons which are involved in the central nervous system stymptoms of APS, and in lymphocytes which are targeted by a large number of antibodies that are seen in patients with SLE.

1.5. APOH Promoter Polymorphisms

Several polymorphisms within the coding region of the APOH gene have been found to have functional significance. Mutations at codons 306 and 316 have been shown to inhibit apoH binding to cardiolipin (Mehdi et al. 2000). A specific SNP in the APOH gene at codon 247 has been found to interact with another defective allele to increase plasma triglycerides and LDL cholesterol in familial hypercholesterolemia families (Takada et al. 2002).

Kamboh et al (2004) have explained the molecular basis for the β_2-GPI protein polymorphism by examining SNPs in the codong region of the APOH gene. It had previously been found by isoelectric focusing that the APOH gene had a structural polymorphism with 4 alleles. These alleles were termed APOH*1, APOH*2 (which is the most common allele), APOH*3 (which has been split into 3^w and 3^B base on reactivity with a monoclonal antibody), and APOH*4 (which was only observed in the African American population (Kamboh et al. 1988). The molecular basis of the APOH*1 allele, a missense mutation at codon 88, and the molecular basis of the APOH*3^w a missense mutation at codon 316, was determined in 1997 (Sanghera et al). Kamboh et al (2004) further characterized this structural polymorphism by explaining the remaining alleles. They examined 5 additional SNPs at codon 122 (T>C), codon 135 (G>A), codon 140 (A>G), codon 141 (C>A), and a -10 A>C mutation at the intron 4- exon 5 junction. They found

that all people with the APOH*3^{B} allele carried the codon 122 mutation and the codon 135 mutation. The APOH*3^{W} allele was found to have arisen on the APOH*3^{B} allele because it contained the codon 122 and 135 mutations plus the additional codon 316 mutation previously reported. Likewise the APOH*4 allele was found to have the codon 122 and 135 mutations plus an additional codon 141 mutation also providing evidence that it arose from the APOH*3^{B} allele. For these reasons new nomenclature was proposed changing the name of original APOH*3^{B} allele to APOH*3 and changing the original APOH*4 allele to APOH*3^{B}. Thus, the β_2-GPI protein polymorphism has been explained by several SNPs within the coding region of the APOH gene. While previous studies of APOH coding polymorphisms have found that these SNPs have functional significance, polymorphisms within the promoter region of the APOH gene have not been thoroughly studied.

The promoter region is located at the 5' end of the gene and it plays a crucial role in initiation and regulation of transcription. The importance of the promoter is evident because many elements of promoters are conserved in different genes. Likewise, mutations in promoter sequences in several genes interfere with expression of the gene and contribute to genetic diseases (Nussbaum et al. 2001). For example, Hall et al (1997) studied a T>G SNP in the promoter region of the lipoprotein lipase gene at position -93. Individuals with the G allele at position -93 had lower triglyceride levels than people who were homozygous for the T allele. Also the -93G promoter had higher activity levels than the -93T promoter, 24% higher in a rat smooth muscle cell line and 18% higher in a human adrenal cell line (Hall et al. 1997). Likewise a SNP (-629 A>C) in the promoter region of the cholesteryl ester transfer protein (CETP) gene was found to have functional significance. The -629A allele was associated with higher HDL

cholesterol and lower CETP mass. The -629A promoter also showed 25% lower activity levels than the -629C promoter (Dachet et al. 2000). SNPs in the promoter regions of genes can have significant effects related to the gene or protein and can affect the activity of the promoter. Because of the importance the promoter region plays in gene transcription and eventually protein function, it is valuable to study the polymorphisms within the APOH promoter to help determine if have any effect on the function of the gene.

The APOH gene has been sequenced in both white and black subjects by the University of Washington-Fred Hutchinson Cancer Research Center group and they reported 146 single nucleotide polymorphisms (SNPs). Of these SNPs, 12 are in the promoter region. Each SNP contains two alleles appearing at different frequencies (Table 1.). Five of these SNPs are present only in the African American population. This study focuses of six of the seven promoter polymorphisms that are present in the Caucasian population. The seventh polymorphism is and insertion polymorphism that was not characterized due to difficulties caused by the surrounding sequence.

The -1284 polymorphism is present only in the white population. It is a C>G SNP with a reported minor allele frequency being only .02 in European descendents (.01 in the total population). The -1219 polymorphism is a G>A SNP present in both whites and blacks. The reported minor allele frequency is .02 in blacks, .04 in whites, and .03 overall. The -1190 G>C SNP has a reported minor allele frequency of .43 in blacks, .28 in whites, and .35 in the total population. The -759 polymorphism, also present in both blacks and whites, is an A>G SNP. The minor allele frequency that has been reported is .12 in African descendents, .20 in European

descendents, and .16 overall. The -700 C>A SNP has a minor allele frequency of .11 in blacks, .20 in whites, and .15 overall. The final SNP analyzed in this study is a T>C polymorphism at position -643. It has a reported minor allele frequency of .17 in people of both European and African descent (http://pga/gs.washington.edu/data/apoh/).

Table 1. Frequency of polymorphisms in the APOH promoter region according to the University of Washington website: http://pga/gs.washington.edu/data/apoh/

SNP	Allele	African frequency	European frequency	Allele	African frequency	European frequency
-1284*	**G**	**0.00**	**0.02**	**C**	**1.00**	**0.98**
-1219*	**A**	**0.02**	**0.04**	**G**	**0.98**	**0.96**
-1190*	**C**	**0.43**	**0.28**	**G**	**0.57**	**0.72**
-1075	A	0.02	0.00	G	0.98	1.00
-1054	G	0.02	0.00	T	0.98	1.00
-759*	**G**	**0.12**	**0.20**	**A**	**0.88**	**0.80**
-742	+	0.11	0.20	-	0.89	0.80
-700*	**A**	**0.11**	**0.20**	**C**	**0.89**	**0.80**
-643*	**C**	**0.17**	**0.17**	**T**	**0.83**	**0.83**
-627	C	0.07	0.00	A	0.93	1.00
-581	C	0.02	0.00	A	0.98	1.00
-363	T	0.12	0.00	C	0.88	1.00

*The SNPs highlighted in blue are the polymorphisms analyzed in this study.

1.6. Specific Aims

The specific aims of the current study are as follows:

1) To determine the allele frequency and genotype distributions of 6 SNPs in the promoter region of APOH in whites and blacks.

Hypothesis: The distribution of APOH SNPs is significantly different between whites and blacks.

2) To determine the association of the polymorphisms in the promoter region with the occurrence of SLE.

Hypothesis: Genetic variation in the APOH gene may affect the risk of SLE.

3) To determine the association of the SNPs in the promoter region with plasma levels of β_2-GPI.

Hypothesis: genetic variation in the APOH gene may affect the risk of SLE by affecting plasma levels of β_2-GPI.

4) To determine the association of the SNPs in the promter region with the occurrence of antiphospholipid antibodies (aCL, LAC, and anti- β_2-GPI).

Hypothesis: Genetic variation in the APOH gene may affect the risk of SLE through its influence on the production of antiphospholipid antibodies.

All these pieces of information will help to characterize whether the SNPs in the promoter region may have an impact on the APOH gene or β_2-GPI protein function. This is of public health relevance because it is known that β_2-GPI plays a role in atherosclerosis, thrombosis, and antiphospholipid syndrome, among other diseases. All of these disorders are of public health concern, especially atherosclerosis which is the most common disorder of the arteries leading to coronary artery disease (Keller 2004).

MATERIALS AND METHODS

1.7. Study Populations

The population used in this study includes 381 females with SLE ages 20-75 (mean age 43.32 years), of which 335 are European Americans and 46 are African Americans, and 497 healthy control females ages 17-81 (mean age 45.25 years), of which 455 are European Americans and 42 are African Americans.

The SLE women were recruited through a study of Dr. S. Manzi which was funded by AHA, Arthritis Foundation, and National Institute of Health. The purpose of that study was to measure cardiovascular disease and risk factors in women with SLE. All the women were at least 18 years old and met the 1982 American College of Rheumatology criteria for definite or probable SLE (Tan et al. 1982). All of the SLE patients participated in the Pittsburgh Lupus Registry.
The control samples had no history of SLE and were obtained from the Central Blood Bank of Pittsburgh. The samples were from the initial baseline visit. All participants went through the informed consent process and provided written informed consent. This study was approved by the University of Pittsburgh Institutional Review Board.

1.8. DNA Isolation and PCR

Genomic DNA had been isolated from buffy coat using the QIAamp kit (QIAGEN). For each sample, 1 µg of genomic DNA was amplified in a 50 µl reaction mixture composed of 5µl of

10X PCR buffer (100mM Tris-HCl, pH=8.3, 500mM KCl), 4µL of 50 mM $MgCl_2$, 1.25mM of each dNTP (Pharmacia), .25 µL of one 20 µmol/L primer, .025 µL of othe other 20 µmol/L primer including the universal tag sequence, .25 µL of 20µmol/L biotinylated universal tag sequence, and 1.25 units of *Taq* DNA polymerase (Display Biosystems). The DNA was denatured at a temperature of 95°C for 5 minutes and then the reaction mixture was subjected to 45 cycles of the following steps: 30 seconds at 95°C (denaturation), 30 seconds at 60°C (annealing), and 30 seconds at 72°C (extension). Finally the mixture underwent a final extension step at 72°C for 5 minutes. For this study, two different PCR fragments were amplified each containing 3 SNPs. For specific primers and conditions see Table 1. The size and purity of the product was determined by gel electrophoresis by running out 5µl of the PCR product on a 2 % agarose gel.

Table 2 . PCR conditions and primers

PCR Fragment	SNPs	PCR conditions	Primers
PR.2	**-1284** **-1219** **-1190**	95°C for 5 min. - 1 cycle 95°C for 30 sec 60°C for 30 sec -> 45 cycles 72°C for 30 sec 72°C for 5 min. -1 cycle	**Biotinylated Universal Tag-** 5' GCTGCTCCGG TTCATAGATT **Forward Primer + tag-** 5' AGCGCTGCTCCGG TTCATAGATTTCTCCCTGACAGATGGAGATT **Reverse Primer-** 5' CACACCTGAAGCCTTTCC
PR.3	**-759** **-700** **-643**	95°C for 5 min. - 1 cycle 95°C for 30 sec 60°C for 30 sec -> 45 cycles 72°C for 30 sec 72°C for 5 min. -1 cycle	**Biotinylated Universal Tag-** 5' GCTGCTCCGG TTCATAGATT **Forward Primer-** 5' _GAACCCTCTCAAGCAACA **Reverse Primer + tag-** 5' AGCGCTGCTCCGG TTCATAGATTTTGCAAGCTCCTATAGCTCCA

1.9. Genotype Determination

Genotypes were determined using pyrosequencing, a technology that can be used to analyze bi-, tri-, and tetra-allelic polymorphisms, multiple SNPs at once, and insertions/deletions. The 6 SNPs analyzed in this study were done in 3 duplex assays on the pyrosequencing machine. See Table 2 for sequencing primers and conditions for each assay.

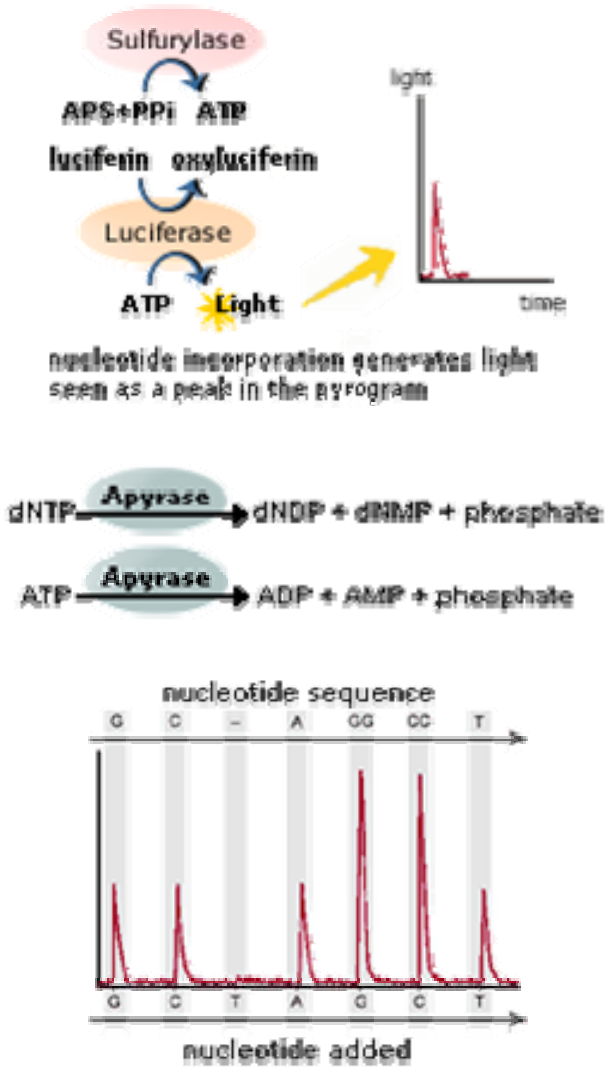
Table 3. Pyrosequencing Assay information

Pyrosequencing Assays	
-1284 & -1190	
dispensation order	GCTCGCGATCACGCGT
- 1283 sequencing primer	CTAAAATTCCCTGAGACAC
-1190 sequencing primer	TTAATGGCTGCCTCC
-1285 & -759	
dispensation order	GCTCGATAGACTCG
- 1218 sequencing primer	AGCTCTGAATAAATAACCTC
-759 sequencing primer	TCAGCACTGGCCC
-700 & -643	
dispensation order	GCTGAGATACACTCA
- 700 sequencing primer	CCCAAGTTGTTAATTTCA
-643 sequencing primer	CTAGACAGATCCAAGACATA

Each PCR amplified sample is converted to a single stranded DNA template to which a specific sequencing primer is annealed in a single well on a 96 well plate. The pyrosequencing machine first dispenses the enzymes, DNA polymerase, ATP sulfurylase, luciferase, and apyrase and the substrates, adenosine 5' phosphosulfate (APS) and luciferin into each well containing a sample. Next the dNTPs are added to the reaction mixture one at a time in a given sequence. If the dNTP that is added is complementary to the template strand, then DNA polymerase incorporates it into the DNA strand. Each time a dNTP is incorporated and equimolar amount of pyrophosphate

(PPi) is released and subsequently converted to ATP by ATP sulfurylase and APS. The ATP and luciferase convert luciferin to oxyluciferin, which produces visible light in an amount proportional to the amount of ATP and therefore proportional to the number of nucleotides incorporated. The light produced is detected by the charge coupled device (CCD) camera within the machine, and is displayed on the computer as a peak with in the pyrogram. Finally, apyrase continuously degrades unused dNTPs and excess ATP. The peak heights seen are proportional to the number of nucleotides incorporated, and if a nucleotide is dispensed that is not complimentary to the next nucleotide on the template strand, then no light is produced and no peak is seen. As the complementary strand is formed, the nucleotide sequence is determined by the peaks in the pyrogram.

Software that is used with the PSQ-96MA automatically analyzes the data, and the data can also be manually analyzed by the user on the computer (Biotage, 2005). Figures 2, 3, and 4 show example pyrograms and theoretical histograms for each genotype of each SNP analyzed.



"Principle of pyrosequencing", www.pyrosequencing.com, Biotage, 2005

Figure 2. Diagram of the principle of pyrosequecing

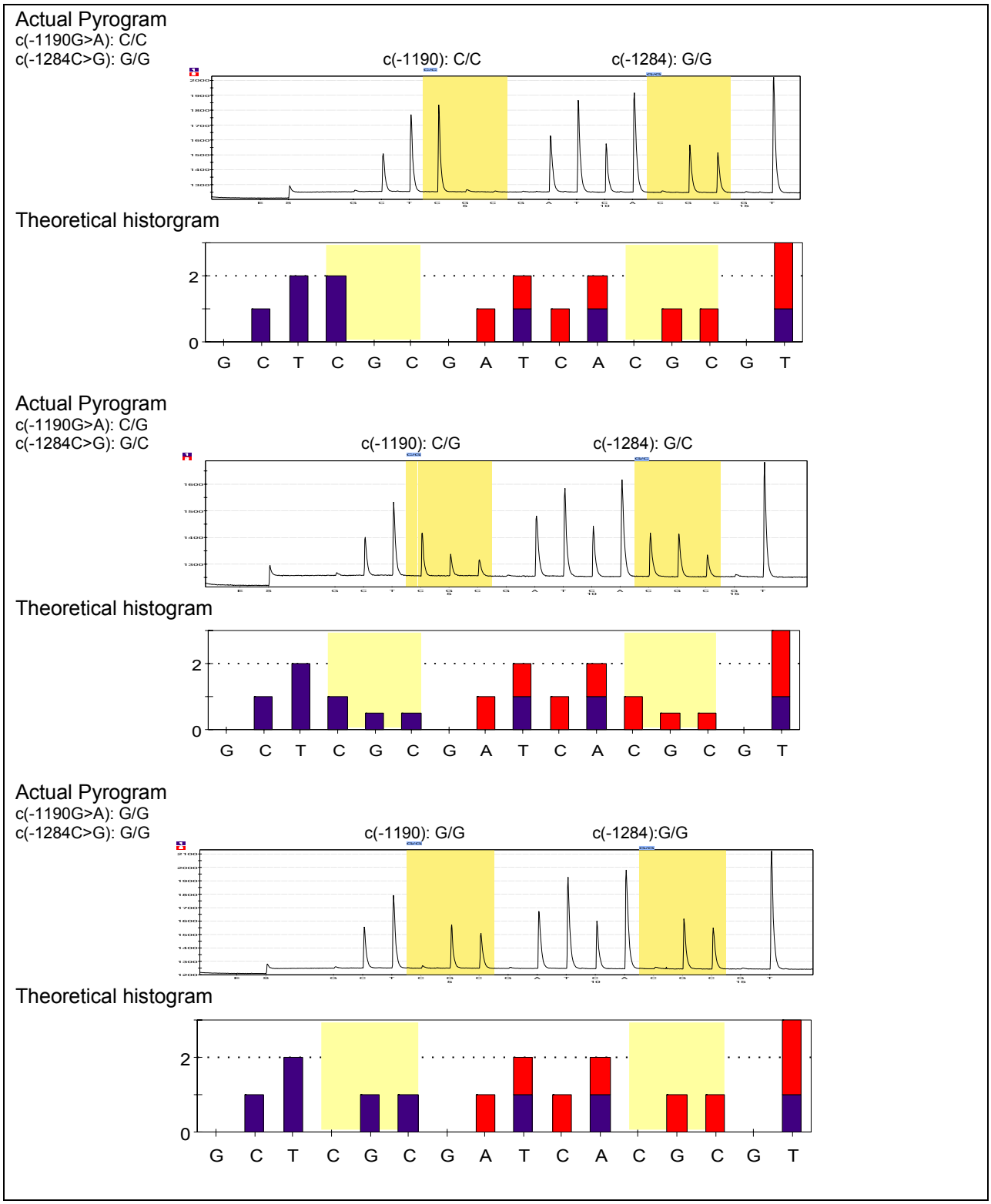
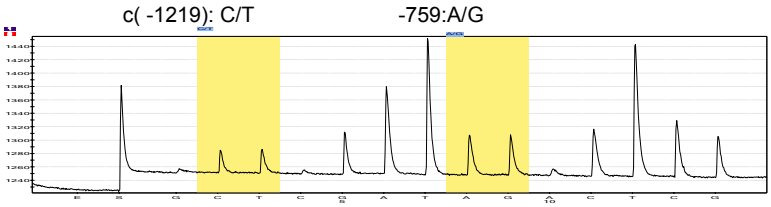
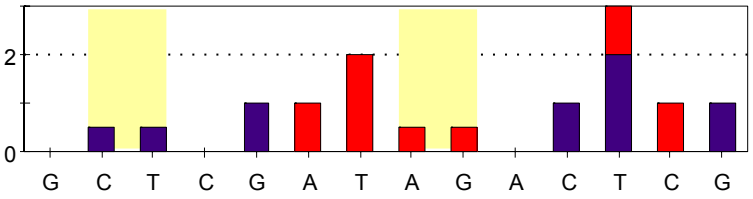


Figure 3. Sample pyrograms and theoretical histograms from -1284 & -1190 Pyrosequencing assay

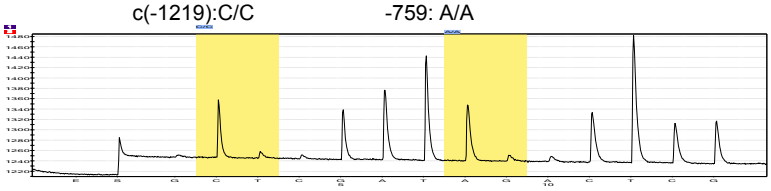
Actual Pyrogram
c(-1219G>A): C/T
-759A>G: A/G



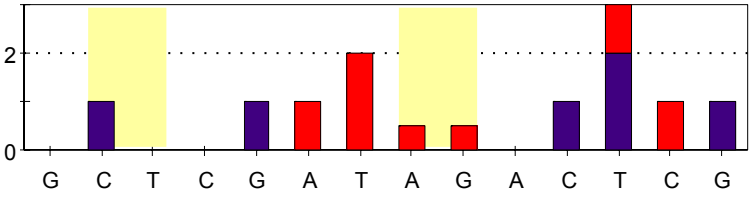
Theoretical histogram



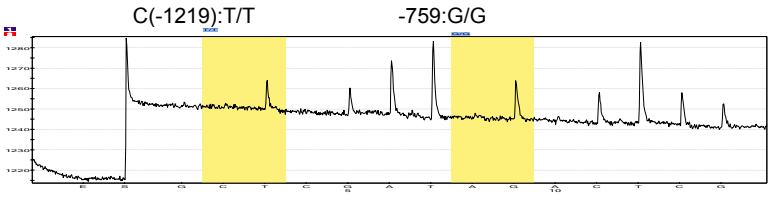
Actual Pyrogram
c(-1219G>A): C/C
-759A>G: A/A



Theoretical Histogram



Actual program
c(-1219G>A): T/T
-759A>G: G/G



Theoretical histogram

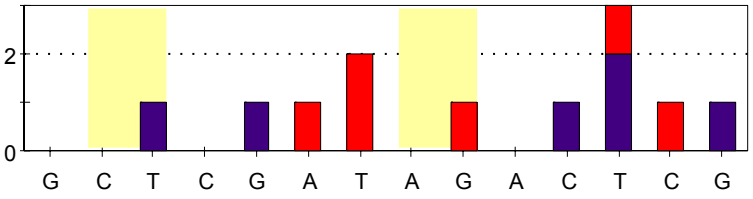


Figure 4. Sample pyrograms and theoretical histograms from -1219 & -759 Pyrosequencing assay

Actual Pyrogram

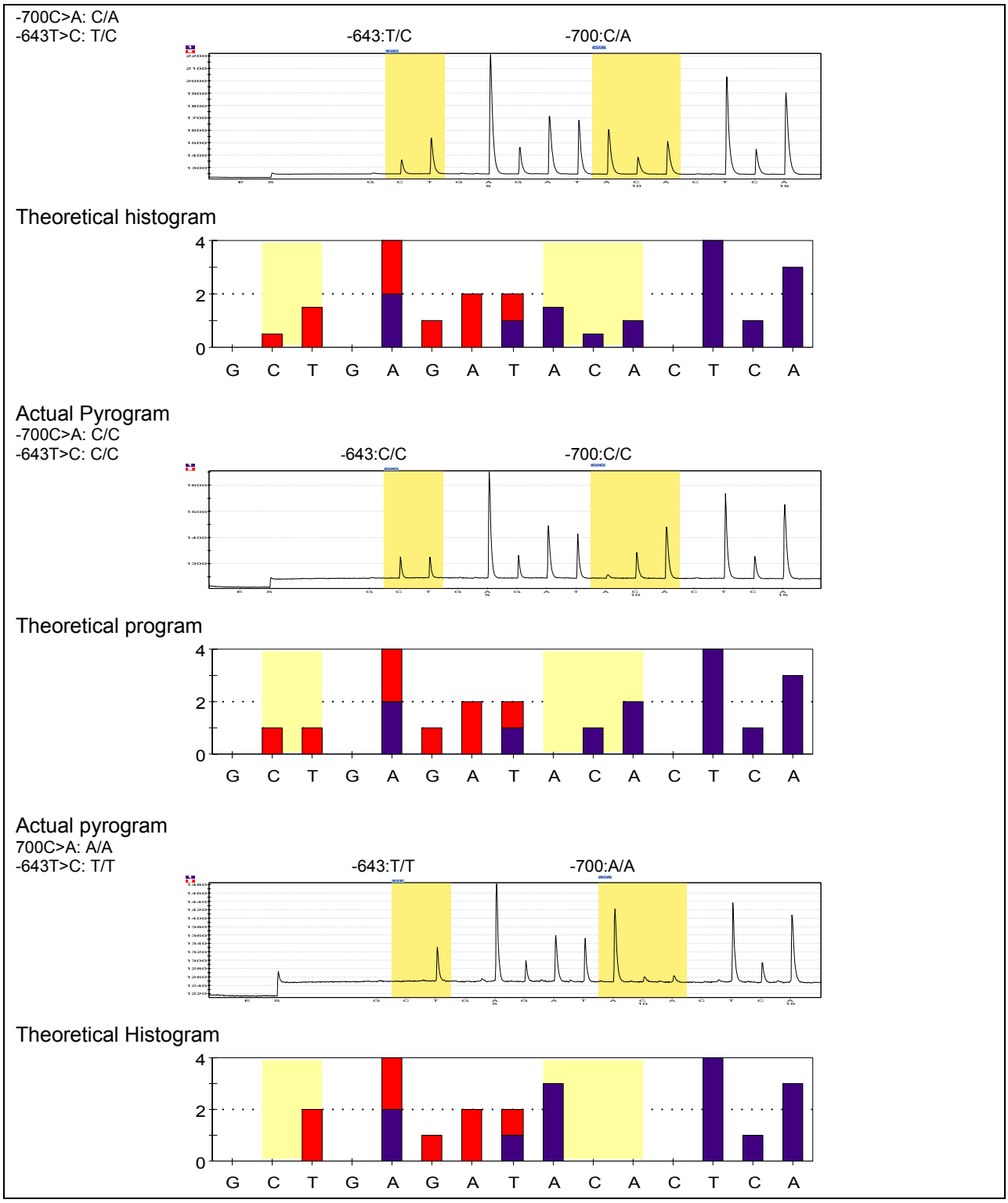


Figure 5. Sample pyrograms and theoretical histograms from -700 & -643 Pyrosequencing assay

1.10. Antiphospholipid antibodies and β_2-glycoprotein I quantitation

All patients and controls were previously tested for the presence of antiphospholipid antibodies (APA) as described (Sanghera et al. 2004). Briefly, the presence of three APAs was measured including anti-cardiolipin antibodies (ACL; IgG>15 GPL units; IgM>10 MPL units; Incstar, Stillwater, MN, USA), lupus anticoagulant (LAC; partial thromboplastin time or Russell's viper venom time with mix), and anti-β2-glycoprotein I (a β2GPI; Quantilite, β2GPI screen, INOVA Diagnostics, San Diego, CA, USA). Each sample was screen in duplicate and any positive IgG or IgM for ACL or LAC is considered positive. β_2-glycoprotein I quantitation was also previously done as described by Kamboh et al. (1999).

1.11. Statistical Methods

Allele frequencies were calculated by allele counting. The significance of deviations of the observed genotype frequencies from Hardy-Weinberg equilibrium were tested by X^2 goodness-of-fit test. X^2 analysis was used to determine genotype differences between races and Z-tests were used to determine allele frequency differences. All further testing was carried out separately within the white population and within the black population. Linkage disequilibrium was determined for each pair of SNPs using the R statistical program version 2.1.0. Genotype differences between cases and controls were determined using X^2 analysis, and Z-tests were used to determine allele frequency differences between cases and controls. EH software was utilized to determine the frequency of all possible haplotypes. This program uses the E-M (expectation maximization) algorithm to determine maximum likelihood estimates of the haplotype

frequencies. Z-tests were used to compare haplotype frequencies between cases and controls and the T5 statistic, $2[\ln(L)_{cases}+\ln(L)_{controls}-\ln(L)_{total}]$, was used with 63 df and the X^2 distribution to determine the p-value for total haplotype difference between cases and controls. β_2GPI levels were analyzed for each SNP by analysis of variance (ANOVA) adjusted for significant variables as determined by stepwise linear regression using the R statistical program. Analysis of antibody carriers was done for the following groups: ACL positive, LAC positive, aβ_2GPI positive, samples positive for any antibody and samples positive for all three antibodies compared to samples that were negative for all three antibodies as controls. This analysis was done using X^2 analysis to determine genotype differences and Z-tests to determine allele frequency differences within the SLE population, within the control population, and within the total population.

RESULTS

1.12. Distribution of the APOH promoter polymorphisms in Blacks and Whites

The genotype and allele frequency distributions for each APOH SNP were compared between black and white subjects (Tables 4-9). While the distribution of two SNPs, -1284 and -1190, was similar between blacks and whites, the other 4 SNPs showed significant difference between the two groups. The genotype distribution for the -1219 SNP was not different between blacks and whites ($p = 0.247$); however, the allele frequencies were significantly different. The frequency of the A allele was 5.6% in blacks and 9.6% in whites ($p = .042$) (Table 5). The frequencies of the less common alleles at the other 3 polymorphic sites were significantly higher in whites than blacks (SNP- 759, 24.8% vs. 12.7%, $p < 0.001$; SNP -700, 25.3% vs. 12%, $p < 0.001$; and SNP -643, 14.9% vs. 9.1%, $p = 0.017$).

Table 4. Distribution of the -1284 polymorphism between blacks and whites.

	BLACKS		WHITES	
Genotype	n	%	n	%
CC	81	98.8	758	99.6
CG	1	1.2	3	0.4
GG	0	0	0	0
Total	82		761	
		$\chi^2 = 1.07$; p= 0.302		
	BLACKS		**WHITES**	
Allele Frequency				
C	0.994		0.998	
G	0.006		0.002	
		p= 0.515		

Table 5. Distribution of the -1219 polymorphism between blacks and whites.

	BLACKS		WHITES	
Genotype	n	%	n	%
AA	0	0	11	1.5
AG	9	11.1	118	16.2
GG	71	88.9	600	82.3
Total	81		729	
	$\chi^2 = 2.80$; p= 0.247			

	BLACKS	WHITES
Allele Frequency		
A	0.056	0.096
G	0.994	0.904
	p= 0.042	

Table 6. Distribution of the -1190 polymorphism between blacks and whites.

	BLACKS		WHITES	
Genotype	n	%	n	%
CC	12	14.5	92	12.2
CG	39	47.0	375	49.7
GG	32	38.6	288	38.1
Total	83		755	
	$\chi^2 = 0.423$; p= 0.809			

	BLACKS	WHITES
Allele Frequency		
C	0.38	0.37
G	0.62	0.63
	p= 0.801	

Table 7. Distribution of the -759 polymorphism between blacks and whites.

Genotype	BLACKS n	BLACKS %	WHITES n	WHITES %
AA	60	75.9	404	56.2
AG	18	22.8	273	38.0
GG	1	1.3	42	5.8
Total	79		719	
	$\chi^2 = 12.05$; p= 0.002			

Allele Frequency	BLACKS	WHITES
A	.873	.752
G	.127	.248
	p= <0.001	

Table 8. Distribution of the -700 polymorphism between blacks and whites.

Genotype	BLACKS n	BLACKS %	WHITES n	WHITES %
AA	1	1.3	46	6.4
AC	16	21.3	274	37.8
CC	58	77.3	404	55.8
Total	75		724	
	$\chi^2 = 13.47$; p= 0.001			

Allele Frequency	BLACKS	WHITES
A	0.12	0.253
C	0.88	0.747
	p= <0.001	

Table 9. Distribution of the -643 polymorphism between blacks and whites.

Genotype	BLACKS n	BLACKS %	WHITES n	WHITES %
CC	0	0.0	18	2.4
TC	15	18.3	185	24.9
TT	67	81.7	541	72.7
Total	82	744		
		$\chi^2 = 4.11$; p= 0.128		

Allele Frequency	BLACKS	WHITES
C	0.091	0.149
T	0.909	0.851
	p= 0.017	

1.13. Pairwise Linkage Disequalibrium

The pairwise linkage disequilibrium (LD) data for 6 APOH promoter SNPs are presented in Table 11 for whites and Table 12 for blacks. Both D' and p-values are present to represent the extent of LD between SNPs. Among whites, the -1284 SNP was in significant LD with the adjacent two SNPs, -1219 and -1190, but not with the farthest 3 SNPs. Likewise the -1219 SNP was in significant LD with the adjacent 4 SNPs but not with the farthest SNP, -643.

In the black population several SNP pairs seem to be in nearly complete linkage disequilibrium; however, this is not statistically significant due to the small sample size and minor allele frequency; this includes -643 paired with -1219, -759, and -700 as well as -1190 paired with -1284. The -759 SNP is nearly in complete LD the -1284 SNP in blacks (D' = .985, p = .012);

however, the minor allele frequency of the -1284 SNP is very low. Also, the -759 SNP is in close LD with the -700 SNP in the black population (D' = .933, p <.001).

Table 10. Pairwise measure of Linkage Disequalibrium (D') of APOH promoter polymorphisms in whites.

	-1284	-1219	-1190	-759	-700	-643
		D'	D'	D'	D'	D'
-1284		0.385	0.945	0.050	0.067	0.767
-1219	p= 0.048		0.582	0.620	0.635	0.010
-1190	p= 0.034	p= <.001		0.846	0.926	0.812
-759	p= 0.961	p= <.001	p= <.001		0.923	0.713
-700	p= 0.948	p= <.001	p= <.001	p= <.001		0.954
-643	p= 0.589	p= 0.815	p= <.001	p= <.001	p= <.001	

Table 11. Pairwise measure of Linkage Disequalibrium (D') of APOH promoter polymorphisms in blacks.

	-1284	-1219	-1190	-759	-700	-643
		D'	D'	D'	D'	D'
-1284		0.763	0.965	0.985	0.890	0.856
-1219	p= 0.857		0.681	0.352	0.528	0.987
-1190	p= 0.449	p= 0.008		0.771	0.880	0.766
-759	p= 0.012	p=0.006	p= <.001		0.933	0.994
-700	p= 0.757	p= <.001	p= <.001	p= <.001		0.994
-643	p= 0.789	p= 0.346	p= 0.016	p= 0.134	p= 0.154	

1.14. Association of the APOH polymorphisms with SLE

Association studies for SNPs have found that some SNPs alone alter gene function and disease risk. Therefore, association studies were performed with SLE for each SNP individually. However, there is increasing evidence that in genes that are very polymorphic, like APOH, haplotype structure may be more important in influencing phenotypes rather than individual SNPs (McDonald et al. 2002). Likewise, the linkage disequilibrium studies showed that all the SNPs in the APOH promoter are not completely unlinked. Thus, association between haplotype structure and SLE was also performed.

1.14.1. Individual SNP associations with SLE

The association of each SNP with SLE was determined using a case-control model for whites and blacks separately. χ^2 tests were used to determine genotype differences between cases and controls and Z-tests were used to determine allele frequency differences. This data is displayed in Tables 13-19.

The -1284 polymorphism did not show association with SLE. The minor allele is rare and was only seen in 3 white individuals and 1 black individual, 3 cases and 1 control (Table 13). The -1219 polymorphism was not significantly associated with SLE in blacks. The distribution of genotypes (blacks: $\chi^2 = 0.659$, $p = 0.637$) and allele frequencies (blacks $p = 0.659$) were comparable between SLE cases and healthy controls in blacks. In whites, there was not a significant difference in genotype frequencies; however, when genotypes AA + AG were

compared to GG there was a borderline significant association with SLE (χ^2 = 3.61.; p= 0.057). There was a slight difference in allele frequencies (p = 0.089). The frequency of the G allele was greater in cases (0.919) than in controls (0.893) (Table 14).

The -1190 SNP was not significantly different between cases and controls in whites or blacks.
The -759 polymorphism did not show association with SLE risk in whites; however, there was a significant association in blacks (Table 16). The genotype distribution differed between cases and controls χ^2 = 7.61, p = 0.022 and the G allele was more frequent in cases (0.175) than in controls (0.077) (p = 0.06).

Similarly, the -700 allele was also associated with SLE risk in blacks, but showed no association in whites (Table 17). The genotype distribution was significantly different between black cases and black controls (χ^2 = 6.72, p = 0.035). The allele frequency also differed slightly between cases and controls (p = 0.098).

The -643 genotype frequency did not significantly differ between cases and controls in whites (χ^2 = 0.75, p = 0.688) or blacks (χ^2 = 0.36, p = 0.548). Similarly there was no difference in allele frequencies in either whites (p = 0.593) or blacks (p = 0.583) (Table 18).

Table 12. Distribution of the APOH -1284 Polymorphism between cases and controls in blacks and whites.

GENOTYPE	CASES		CONTROLS	
whites	n	%	n	%
CC	323	99.4	436	99.8
CG	2	0.6	1	0.2
GG	0	0	0	0
Total	325		437	
		$\chi^2 = 0.71$; p= 0.399		
blacks				
CC	41	97.6	40	100
CG	1	2.4	0	0
GG	0	0	0	0
Total	42		40	
		$\chi^2 = 0.96$; p= 0.326		
Allele Frequency				
whites				
C	0.997		0.999	
G	0.003		0.001	
		p= 0.404		
blacks				
C	0.998		1	
G	0.012		0	
		p= 0.313		

Table 13. Distribution of the APOH -1219 Polymorphism between cases and controls in blacks and whites.

GENOTYPE	CASES		CONTROLS	
whites	n	%	n	%
AA	5	1.6	6	1.4
AG	40	13	79	18.6
GG	263	85.4	339	80
Total	308		424	
	χ^2 = .42; p= 0.123 GG vs. AA+AG: χ^2 = 3.61.; p= 0.057			
blacks				
AA	0	0	0	0
AG	4	9.5	5	12.8
GG	38	90.5	34	87.2
Total	42		39	
	χ^2 = 0.22; p=0.637			
Allele Frequency				
whites				
A	0.081		0.107	
G	0.919		0.893	
	p= 0.089			
blacks				
A	0.048		0.064	
G	0.952		0.936	
	p= 0.659			

Table 14. Distribution of the APOH -1190 Polymorphism between cases and controls in blacks and whites.

GENOTYPE	CASES		CONTROLS	
whites	n	%	n	%
CC	40	0.12	52	12
CG	156	48.3	219	50.5
GG	127	39.3	163	37.6
Total	323		434	
		$\chi^2 = 0.35$; p= 0.840		
blacks				
CC	8	18.6	4	10
CG	20	46.5	19	47.5
GG	15	34.9	17	42.5
Total	43		40	
		$\chi^2 = 1.38$; p= 0.502		
Allele Frequency				
whites				
C	0.365		0.372	
G	0.635		0.628	
		p= 0.78		
blacks				
C	0.419		0.338	
G	0.581		0.663	
		p= 0.28		

Table 15. Distribution of the APOH -759 Polymorphism between cases and controls in blacks and whites.

GENOTYPE	CASES		CONTROLS	
whites	n	%	n	%
CC	158	54.3	247	57
CG	116	39.9	158	36.7
GG	17	5.8	25	5.8
Total	291		430	
		χ^2 = .75; p= 0.687		
blacks				
CC	26	65	34	87.2
CG	14	35	4	10.3
GG	0	0	1	2.6
Total	40		39	
		χ^2 = 7.61; p= 0.022		
Allele Frequency				
whites				
C	0.742		0.758	
G	0.258		0.242	
		p= 0.492		
blacks				
C	0.825		0.923	
G	0.175		0.077	
		p= 0.06		

Table 16. Distribution of the APOH -700 Polymorphism between cases and controls in blacks and whites.

GENOTYPE	CASES		CONTROLS	
whites	n	%	n	%
AA	21	6.9	25	5.9
AC	117	38.2	159	37.8
CC	168	54.9	237	56.3
Total	306		421	
		$\chi^2 = 0.311$; p= 0.856		
blacks				
AA	0	0	1	2.9
AC	13	31.7	3	8.8
CC	28	68.3	30	88.2
Total	41		34	
		$\chi^2 = 6.72$; p= 0.035		
Allele Frequency				
whites				
A	0.26		0.248	
C	0.74		0.752	
		p= 0.864		
blacks				
A	0.159		0.074	
C	0.841		0.926	
		p= 0.098		

Table 17. Distribution of the APOH -643 Polymorphism between cases and controls in blacks and whites.

GENOTYPE	CASES		CONTROLS	
whites	n	%	n	%
CC	7	2.2	11	2.6
CG	83	26.3	102	23.7
GG	225	71.4	318	73.8
Total	315		431	
		$\chi^2 = 0.75$; p= 0.688		
blacks				
CC	0	0	0	0
CG	7	15.9	8	21
GG	37	84.1	30	78.9
Total	44		38	
		$\chi^2 = 0.36$; p= 0.548		
Allele Frequency				
whites				
C	0.154		0.144	
G	0.846		0.856	
		p= 0.593		
blacks				
C	0.08		0.105	
G	0.92		0.895	
		p= 0.583		

1.14.2. Haplotype associations with SLE

Six-site haplotype analysis was performed to determine their association with SLE risk in the white case-control sample. The haplotype frequencies were determined in both SLE cases and healthy controls. Z tests were performed to identify associations of haplotypes with SLE risk.
26 different haplotypes were present in the white subjects. Eight of these haplotypes were significantly different between cases and controls (highlighted in Table 18). Six haplotypes were significantly more frequent in cases than controls, indicating that they are associated with a greater risk of SLE. Conversely, 2 haplotypes were significantly more frequent in controls than cases, indicating that they may be protective. There are 4 common haplotypes that are present in greater than 10% of the population. There was no significant difference in the frequencies of these haplotypes between cases and controls; therefore these haplotypes do not seem to be associated with the risk of SLE.

The T5 test statistic was calculated to determine whether there was an overall difference in haplotypes seen in cases and controls. This difference was detected and was significant ($p = .00015$).

1.15. Association of the APOH polymorphisms with plasma β_2-GPI levels

Associations of each of promoter SNPS with plasma levels of β_2-GPI were carried out using analysis of varience (ANOVA) in both whites and blacks. Linear regression was used to determine which covariates significantly affect β_2-GPI levels. BMI ($p = .07$) and smoking ($p =$

Table 18. Six-site haplotype distribution of APOH promoter polymorphisms in white cases and controls.

Haplotype distribution						Total	Cases	Controls	
-1284	-1219	-1190	-759	-700	-643	n=641	n=259	n=382	p'
C	A	C	G	A	C	0.002	0.003	<.001	0.230
C	G	C	G	A	C	<.001	<.001	0.000	0.899
C	A	G	G	A	C	0.001	0.004	0.000	0.174
C	G	G	G	A	C	<.001	0.002	0.000	0.300
C	A	C	A	C	C	0.001	0.000	0.002	0.181
C	G	C	A	C	C	0.006	0.010	0.003	0.121
C	A	G	A	C	C	0.005	0.005	0.004	0.783
C	G	G	A	C	C	0.131	0.127	0.135	0.679
C	G	C	G	C	C	0.001	0.002	0.000	0.299
C	A	G	G	C	C	<.001	0.001	0.000	0.592
C	G	G	G	C	C	0.006	0.017	0.000	0.003
C	A	C	A	A	T	0.001	0.001	0.000	0.457
C	G	C	A	A	T	0.014	0.034	0.001	<.001
C	A	C	G	A	T	0.061	0.032	0.081	<.001
C	G	C	G	A	T	0.158	0.146	0.165	0.343
G	G	C	G	A	T	0.001	0.002	0.000	0.317
C	A	G	G	A	T	0.003	0.003	0.003	0.931
C	G	G	G	A	T	0.005	0.010	<.001	0.026
C	A	C	A	C	T	0.005	0.000	0.010	0.006
G	A	C	A	C	T	0.001	0.000	0.001	0.317
C	G	C	A	C	T	0.111	0.109	0.111	0.880
C	A	G	A	C	T	0.005	0.013	0.000	0.009
C	G	G	A	C	T	0.471	0.453	0.482	0.299
C	G	C	G	C	T	0.004	0.009	0.000	0.027
C	A	G	G	C	T	0.001	0.003	0.000	0.229
C	G	G	G	C	T	0.007	0.017	0.000	0.003
Association studies									
ln(L)						-1783.160	-783.960	-943.270	
χ^2						1399.620	399.530	1102.850	p=0.00015 **

p':p values were calculated using Z-tests to compare haplotype frequencies between cases and controls.

** Calculated from the T5 statistic: 2[ln(L)cases+ ln(L)controls- ln(L)total]

.02) both impacted β_2-GPI levels; therefore, they were included as covariates in the ANOVA. No significant association was found between β_2-GPI levels and the genotypes at the -1284, -1219, -1190, -759, or -700 positions (Tables 19-23). The distribution of β_2-GPI levels within white cases was slightly associated with the genotypes at position -643 ($p = .096$). Mean β_2-GPI levels were 21.07 +/- 1.54 within the CC genotype, 18.25 +/- 3.19 within the TC genotype and 17.61 +/- 4.00 within the TT genotype. Similarly, the β_2-GPI levels also differed among the genotypes for the -643 polymorphism within black controls. The CC genotype was not present in the black subjects studies; the mean β_2-GPI level was 19.47 +/- 3.54 for the TC genotype and 17.67 +/- 2.07 for the TT genotype (Table 24).

Table 19. Distribution of mean plasma β_2-GPI levels (+/- SD) among the APOH -1284 polymorphism in blacks and whites - adjusted for BMI and smoking.

	APOH: Genotype			P-Value
Whites	**CC**	**CG**	**GG**	
Controls	N = 175 18.21 +/- 4.62	N =1 23.55	N =0	.2186
SLE Cases	N = 231 17.80 +/- 4.11	N =2 16.37 +/- 8.76	N = 0	.62057
Blacks	**CC**	**CG**	**GG**	
Controls	N = 21 18.25 +/- 2.73	N = 0	N = 0	
SLE Cases	N = 28 18.43 +/- 4.03	N = 0	N = 0	

Table 20. Distribution of plasma β_2-GPI levels among the APOH -1219 polymorphism in blacks and whites - adjusted for BMI and smoking.

	APOH: Genotype			P-Value
Whites	**AA**	**AG**	**GG**	
Controls	N = 1 23.06	N = 48 19.19 +/- 4.17	N = 122 17.98 +/- 4.73	.1959
SLE Cases	N = 1 20.48	N = 26 17.51 +/- 4.22	N = 198 17.81 +/- 3.98	.7808
Blacks	**AA**	**AG**	**GG**	
Controls	N = 0	N = 4 18.52 +/- 3.48	N = 17 18.21 +/- 2.59	.7045
SLE Cases	N = 0	N = 1 18.56	N = 26 17.85 +/- 3.00	.9315

Table 21. Distribution of plasma β_2-GPI levels among the APOH -1190 polymorphism in blacks and whites - adjusted for BMI and smoking.

	APOH: Genotype			P-Value
Whites	**CC**	**CG**	**GG**	
Controls	N = 23 18.21 +/- 4.45	N = 103 18.01 +/- 4.91	N = 47 18.69 +/- 4.26	0.6973
SLE Cases	N = 31 18.32 +/- 2.99	N = 112 17.62 +/- 4.19	N = 86 17.69 +/- 4.42	0.69887
Blacks	**CC**	**CG**	**GG**	
Controls	N = 1 13.35	N = 12 18.35 +/- 2.42	N = 8 18.68 +/- 2.88	0.1387
SLE Cases	N = 6 18.65 +/- 2.73	N = 11 17.68 +/- 3.35	N = 11 19.01 +/- 5.29	0.6593

Table 22. Distribution of plasma β_2-GPI levels among the APOH -759 polymorphism in blacks and whites - adjusted for BMI and smoking.

	APOH: Genotype			P-Value
Whites	**AA**	**AG**	**GG**	
Controls	N = 93 18.15 +/- 5.02	N =69 18.41 +/- 4.27	N = 12 18.32 +/- 3.19	0.9485
SLE Cases	N = 113 17.75 +/- 3.95	N = 84 17.81 +/- 3.76	N = 15 19.10 +/- 2.69	0.4210
Blacks	**AA**	**AG**	**GG**	
Controls	N = 17 18.23 +/- 2.53	N = 3 16.89 +/- 0.95	N = 0	0.2836
SLE Cases	N = 18 17.86 +/- 3.32	N = 7 18.04 +/- 2.35	N = 0	0.7914

Table 23. Distribution of plasma β_2-GPI levels among the APOH -700 polymorphism in blacks and whites - adjusted for BMI and smoking.

	APOH: Genotype			P-Value
Whites	**AA**	**AC**	**CC**	
Controls	N = 12 18.35 +/- 3.21	N = 70 18.42 +/- 4.25	N = 92 18.18 +/- 5.03	.9589
SLE Cases	N = 17 19.01 +/- 2.32	N = 81 18.29 +/- 3.61	N = 127 17.51 +/- 3.89	.1355
Blacks	**AA**	**AC**	**CC**	
Controls	N = 0	N = 3 16.85 +/- 0.95	N = 17 18.25 +/- 2.56	.2836
SLE Cases	N = 0	N = 6 18.41 +/- 2.24	N = 21 18.37 +/- 4.57	.8108

Table 24. Distribution of plasma β_2-GPI levels among the APOH -643 polymorphism in blacks and whites - adjusted for BMI and smoking.

	APOH: Genotype			P-Value
Whites	**CC**	**CT**	**TT**	
Controls	N = 3 19.03 +/- 1.14	N = 34 18.43 +/- 5.18	N = 137 18.23 +/- 4.51	.9300
SLE Cases	N = 5 21.07 +/- 1.54	N = 56 18.25 +/- 3.19	N = 166 17.61 +/- 4.00	.09557
Blacks	**CC**	**CT**	**TT**	
Controls	N = 0	N = 4 19.47 +/- 3.54	N = 16 17.67 +/- 2.07	.08087
SLE Cases	N = 0	N = 6 20.38 +/- 7.14	N = 22 17.85 +/- 2.74	.2198

1.16. Association of the APOH polymorphisms with anti-phospholipid antibodies

Each of the 6 APOH promoter SNPs examined in this study were analyzed for association with antiphospholipid antibodies (APA) in whites. Subjects included in this analysis were screen for the presence of 3 different antibodies, anticardiolipin antibodies (aCL), anti-beta$_2$ glycoprotein I (β_2GPI), and lupus anticoagulant (LAC). Subjects who were negative for all three antibodies were used as the control group. APA positive groups included positive for all three antibodies (ALL 3), positive for at least one antibody (ANY), positive for aCL, positive for β_2GPI, and positive for LAC. Each of these five groups were compared to the control group to look for differences in genotype distribution. This was done overall, within SLE cases, and within healthy controls for each of the 6 SNPs studied.

For the -1284 SNP a significant difference was found in the genotype distributions between the group positive for all 3 antibodies and the antibody negative group overall ($p = 0.05$) and within the SLE cases ($p = 0.02$). However, no differences were seen between the allele frequencies of these groups. No significant differences were seen within the control group between the antibody negative group and any of the other groups (Tables 25 – 27).

There were no significant associations of antibody status with the -1219 genotypes or allele frequencies seen in cases or controls for any of the antibody groups (Tables 28-30). Similarly, there were no differences in allele frequency or genotype distribution for the -1190 SNP between any of the antibody groups (Tables 31 -33).

No significant differences were seen between the genotype distributions of the antibody groups for the -759 SNP. However, within the total white population, the allele frequency of the group positive for all 3 antibodies (G allele- 0.141) differed from that of the antibody negative group (G allele- 0.243) ($p = 0.030$). Likewise a difference was seen between the same allele frequencies within the SLE cases ($p = 0.046$). No other significant differences were seen within the controls or in the remaining comparisons in the SLE cases (Tables 34 – 36).

In the total population there was one difference seen in allele frequencies for the -700 SNP. The allele frequency differed slightly ($p = 0.095$) between the group positive for all 3 antibodies (A allele- 0.156) and the group negative for all antibodies (A allele- 0.237). There were no significant differences seen among the genotype distributions in any of the anitbody groups in cases or controls (Tables 37 – 39).

Finally, no significant differences were found among the antibody groups in genotype distributions or allele frequencies of the -643 polymorphism (Tables 40 – 42).

Table 25. Distribution of the -1284 Polymorphism in antibody positive and antibody negative groups in all whites.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	CG	GG	Total	CC	CG	GG	Total	χ^2	p
aCL	201	1	0	202	314	1	0	315	0.10	0.751
LAC	134	1	0	135	314	1	0	315	0.38	0.536
β2GPI	174	2	0	176	314	1	0	315	1.25	0.264
ALL 3	32	1	0	33	314	1	0	315	3.85	0.050
ANY	365	2	0	367	314	1	0	315	0.20	0.654

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	G	C	G	Z	p
aCL	0.998	0.002	0.998	0.002	0.00	1.000
LAC	0.996	0.004	0.998	0.002	-0.47	0.637
β2GPI	0.994	0.006	0.998	0.002	-0.89	0.372
ALL 3	0.985	0.015	0.998	0.002	-0.86	0.388
ANY	0.997	0.003	0.998	0.002	-0.37	0.710

Table 26. Distribution of the -1284 Polymorphism in antibody positive and antibody negative groups in white SLE cases.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	CG	GG	Total	CC	CG	GG	Total	χ^2	p
aCL	80	1	0	81	139	0	0	139	1.72	0.189
LAC	79	1	0	80	139	0	0	139	1.75	0.186
β2GPI	99	2	0	101	139	0	0	139	2.78	0.096
ALL 3	25	1	0	26	139	0	0	139	5.38	0.020
ANY	164	2	0	166	139	0	0	139	1.69	0.194

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	G	C	G	Z	p
aCL	0.994	0.006	1	0	-0.99	0.323
LAC	0.994	0.006	1	0	-0.98	0.326
β2GPI	0.99	0.01	1	0	-1.43	0.153
ALL 3	0.981	0.019	1	0	-1.00	0.316
ANY	0.994	0.006	1	0	-1.42	0.157

Table 27. Distribution of the -1284 Polymorphism in antibody positive and antibody negative groups in white healthy controls.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	CG	GG	Total	CC	CG	GG	Total	χ^2	p
aCL	175	0	0	175	175	1	0	176	1.00	0.318
LAC	28	0	0	28	175	1	0	176	0.16	0.689
β2GPI	40	0	0	40	175	1	0	176	0.23	0.633
ALL 3	7	0	0	7	175	1	0	176	0.04	0.841
ANY	201	0	0	201	175	1	0	176	1.15	0.285

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	G	C	G	Z	p
aCL	1	0	0.997	0.003	1.03	0.303
LAC	1	0	0.997	0.003	1.03	0.303
β2GPI	1	0	0.997	0.003	1.03	0.303
ALL 3	1	0	0.997	0.003	1.03	0.303
ANY	1	0	0.997	0.003	1.03	0.303

Table 28. Distribution of the -1219 Polymorphism in antibody positive and antibody negative groups in all white samples.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	AG	GG	Total	AA	AG	GG	Total	χ^2	p
aCL	2	24	169	195	2	50	252	304	1.77	0.412
LAC	1	22	107	130	2	50	252	304	0.03	0.984
β2GPI	1	23	151	175	2	50	252	304	0.96	0.619
ALL 3	0	4	30	34	2	50	252	304	0.75	0.688
ANY	4	51	299	354	2	50	252	304	0.89	0.640

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	G	A	G	Z	p
aCL	0.072	0.928	0.089	0.911	-0.97	0.330
LAC	0.092	0.908	0.089	0.911	0.14	0.888
β2GPI	0.071	0.929	0.089	0.911	-1.00	0.316
ALL 3	0.059	0.941	0.089	0.911	-0.97	0.330
ANY	0.083	0.917	0.089	0.911	-0.39	0.699

Table 29. Distribution of the -1219 Polymorphism in antibody positive and antibody negative groups in white SLE cases.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	AG	GG	Total	AA	AG	GG	Total	χ^2	p
aCL	1	7	73	81	0	20	113	133	3.43	0.180
LAC	1	8	69	78	0	20	113	133	2.62	0.270
β2GPI	0	10	89	99	0	20	113	133		
ALL 3	0	3	24	27	0	20	113	133		
ANY	2	15	143	160	0	20	113	133	3.77	0.152

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	G	A	G	Z	p
aCL	0.056	0.944	0.075	0.925	-0.78	0.433
LAC	0.064	0.936	0.075	0.925	-0.43	0.665
β2GPI	0.051	0.949	0.075	0.925	-1.07	0.286
ALL 3	0.056	0.944	0.075	0.925	-0.54	0.589
ANY	0.059	0.941	0.075	0.925	-0.77	0.443

Table 30. Distribution of the -1219 Polymorphism in antibody positive and antibody negative groups in white healthy controls.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	AG	GG	Total	AA	AG	GG	Total	χ^2	p
aCL	1	17	96	114	2	30	139	171	0.41	0.813
LAC	0	14	38	52	2	30	139	171	2.72	0.256
β2GPI	1	13	62	76	2	30	139	171	0.02	0.992
ALL 3	0	1	6	7	2	30	139	171	0.14	0.933
ANY	2	36	156	194	2	30	139	171	0.08	0.963

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	G	A	G	Z	p
aCL	0.083	0.917	0.099	0.901	-0.66	0.512
LAC	0.135	0.865	0.099	0.901	0.97	0.333
β2GPI	0.099	0.901	0.099	0.901	0.00	1.000
ALL 3	0.071	0.929	0.099	0.901	-0.40	0.691
ANY	0.103	0.897	0.099	0.901	0.18	0.858

Table 31. Distribution of the -1190 Polymorphism in antibody positive and antibody negative groups in all white samples.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	CG	GG	Total	CC	CG	GG	Total	χ^2	p
aCL	24	90	85	199	33	160	119	312	1.79	0.409
LAC	15	69	49	133	33	160	119	312	0.09	0.956
β2GPI	16	84	74	174	33	160	119	312	0.95	0.621
ALL 3	2	14	17	33	33	160	119	312	2.42	0.298
ANY	44	178	140	362	33	160	119	312	0.53	0.769

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	G	C	G	Z	p
aCL	0.347	0.653	0.362	0.638	-0.49	0.625
LAC	0.372	0.628	0.362	0.638	0.28	0.777
β2GPI	0.333	0.667	0.362	0.638	-0.91	0.361
ALL 3	0.273	0.727	0.362	0.638	-1.53	0.126
ANY	0.367	0.633	0.362	0.638	0.19	0.849

Table 32. Distribution of the -1190 Polymorphism in antibody positive and antibody negative groups in white SLE cases.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	CG	GG	Total	CC	CG	GG	Total	χ^2	p
aCL	12	36	30	78	14	67	57	138	1.30	0.523
LAC	10	34	35	79	14	67	57	138	0.72	0.697
β2GPI	13	43	44	100	14	67	57	138	0.90	0.637
ALL 3	2	11	13	26	14	67	57	138	0.70	0.704
ANY	25	75	63	163	14	67	57	138	1.79	0.409

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	G	C	G	Z	p
aCL	0.385	0.615	0.344	0.656	0.85	0.396
LAC	0.342	0.658	0.344	0.656	-0.04	0.966
β2GPI	0.345	0.655	0.344	0.656	0.02	0.982
ALL 3	0.288	0.712	0.344	0.656	-0.81	0.417
ANY	0.383	0.617	0.344	0.656	0.99	0.321

Table 33. Distribution of the -1190 Polymorphism in antibody positive and antibody negative groups in white healthy controls.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	CG	GG	Total	CC	CG	GG	Total	χ^2	p
aCL	12	54	55	121	19	93	62	174	2.92	0.232
LAC	5	35	14	54	19	93	62	174	2.22	0.329
β2GPI	3	41	30	74	19	93	62	174	3.13	0.209
ALL 3	0	3	4	7	19	93	62	174	1.76	0.415
ANY	19	103	77	199	19	93	62	174	0.46	0.796

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	G	C	G	Z	p
aCL	0.322	0.678	0.376	0.624	-1.36	0.174
LAC	0.417	0.583	0.376	0.624	0.76	0.448
β2GPI	0.318	0.682	0.376	0.624	-1.25	0.210
ALL 3	0.214	0.786	0.376	0.624	-1.44	0.150
ANY	0.354	0.646	0.376	0.624	-0.62	0.534

Table 34. Distribution of the -759 Polymorphism in antibody positive and antibody negative groups in all white samples.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	AG	GG	Total	AA	AG	GG	Total	χ^2	p
aCL	121	65	8	194	174	111	18	303	1.56	0.458
LAC	68	51	7	126	174	111	18	303	0.56	0.756
β2GPI	104	58	5	167	174	111	18	303	2.45	0.294
ALL 3	24	7	1	32	174	111	18	303	3.71	0.157
ANY	199	132	17	348	174	111	18	303	0.41	0.815

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	G	A	G	Z	p
aCL	0.791	0.209	0.757	0.243	1.26	0.208
LAC	0.742	0.258	0.757	0.243	-0.46	0.645
β2GPI	0.796	0.204	0.757	0.243	1.39	0.165
ALL 3	0.859	0.141	0.757	0.243	2.18	0.030
ANY	0.761	0.239	0.757	0.243	0.17	0.866

Table 35. Distribution of the -759 Polymorphism in antibody positive and antibody negative groups in white SLE cases.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	AG	GG	Total	AA	AG	GG	Total	χ^2	p
aCL	42	28	4	74	71	48	8	127	0.07	0.966
LAC	44	23	5	72	71	48	8	127	0.69	0.710
β2GPI	54	36	3	93	71	48	8	127	1.07	0.586
ALL 3	19	5	1	25	71	48	8	127	3.51	0.173
ANY	79	61	9	149	71	48	8	127	0.28	0.868

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	G	A	G	Z	p
aCL	0.757	0.243	0.748	0.252	0.20	0.840
LAC	0.771	0.229	0.748	0.252	0.52	0.604
β2GPI	0.774	0.226	0.748	0.252	0.63	0.526
ALL 3	0.86	0.14	0.748	0.252	2.00	0.046
ANY	0.735	0.265	0.748	0.252	-0.35	0.728

Table 36. Distribution of the -759 Polymorphism in antibody positive and antibody negative groups in white healthy controls.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	AG	GG	Total	AA	AG	GG	Total	χ^2	p
aCL	79	37	4	120	103	63	10	176	1.97	0.373
LAC	24	28	2	54	103	63	10	176	4.49	0.106
β2GPI	50	22	2	74	103	63	10	176	2.22	0.329
ALL 3	5	2	0	7	103	63	10	176	0.69	0.709
ANY	120	71	8	199	103	63	10	176	0.59	0.746

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	G	A	G	Z	p
aCL	0.813	0.188	0.764	0.236	1.42	0.157
LAC	0.704	0.296	0.764	0.236	-1.21	0.225
β2GPI	0.824	0.176	0.764	0.236	1.55	0.120
ALL 3	0.857	0.143	0.764	0.236	0.97	0.334
ANY	0.781	0.219	0.764	0.236	0.55	0.580

Table 37. Distribution of the -700 Polymorphism in antibody positive and antibody negative groups in all white samples.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	CA	CC	Total	AA	CA	CC	Total	χ^2	p
aCL	12	66	113	191	17	113	180	310	0.27	0.872
LAC	8	54	69	131	17	113	180	310	1.09	0.580
β2GPI	7	52	109	168	17	113	180	310	2.17	0.338
ALL 3	1	8	23	32	17	113	180	310	2.31	0.314
ANY	22	128	197	347	17	113	180	310	2.80	0.246

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	C	A	C	Z	p
aCL	0.236	0.764	0.237	0.763	-0.04	0.971
LAC	0.267	0.733	0.237	0.763	0.93	0.352
β2GPI	0.196	0.804	0.237	0.763	-1.49	0.137
ALL 3	0.156	0.844	0.237	0.763	-1.67	0.095
ANY	0.248	0.752	0.237	0.763	0.46	0.642

Table 38. Distribution of the -700 Polymorphism in antibody positive and antibody negative groups in white SLE cases.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	CA	CC	Total	AA	CA	CC	Total	χ^2	p
aCL	8	28	41	77	7	49	79	135	2.12	0.347
LAC	6	26	45	77	7	49	79	135	0.63	0.729
β2GPI	5	30	61	96	7	49	79	135	0.65	0.722
ALL 3	1	6	18	25	7	49	79	135	1.62	0.445
ANY	14	57	85	156	7	49	79	135	1.65	0.438

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	C	A	C	Z	p
aCL	0.286	0.714	0.233	0.767	1.19	0.235
LAC	0.247	0.753	0.233	0.767	0.32	0.746
β2GPI	0.208	0.792	0.233	0.767	-0.64	0.521
ALL 3	0.16	0.84	0.233	0.767	-1.26	0.207
ANY	0.272	0.728	0.233	0.767	1.08	0.279

Table 39. Distribution of the -700 Polymorphism in antibody positive and antibody negative groups in white healthy controls.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	AA	CA	CC	Total	AA	CA	CC	Total	χ^2	p
aCL	4	38	72	114	10	64	101	175	1.24	0.538
LAC	2	28	24	54	10	64	101	175	4.05	0.132
β2GPI	2	22	48	72	10	64	101	175	2.11	0.348
ALL 3	0	2	5	7	10	64	101	175	0.74	0.692
ANY	8	71	112	191	10	64	101	175	0.45	0.797

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	A	C	A	C	Z	p
aCL	0.202	0.798	0.24	0.76	-1.08	0.278
LAC	0.296	0.704	0.24	0.76	1.13	0.258
β2GPI	0.181	0.819	0.24	0.76	-1.50	0.134
ALL 3	0.143	0.857	0.24	0.76	-1.01	0.314
ANY	0.228	0.772	0.24	0.76	-0.38	0.702

Table 40. Distribution of the -643 Polymorphism in antibody positive and antibody negative groups in all white samples.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	TC	TT	Total	CC	TC	TT	Total	χ^2	p
aCL	7	44	146	197	6	77	231	314	1.53	0.465
LAC	2	37	95	134	6	77	231	314	0.54	0.765
β2GPI	8	44	118	170	6	77	231	314	3.32	0.190
ALL 3	2	6	24	32	6	77	231	314	2.78	0.250
ANY	11	90	256	357	6	77	231	314	1.01	0.602

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	T	C	T	Z	p
aCL	0.147	0.853	0.142	0.858	0.22	0.825
LAC	0.153	0.847	0.142	0.858	0.42	0.673
β2GPI	0.176	0.824	0.142	0.858	1.36	0.172
ALL 3	0.156	0.844	0.142	0.858	0.30	0.768
ANY	0.157	0.843	0.142	0.858	0.77	0.441

Table 41. Distribution of the -643 Polymorphism in antibody positive and antibody negative groups in white SLE cases.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	TC	TT	Total	CC	TC	TT	Total	χ^2	p
aCL	3	17	58	78	3	36	98	137	0.95	0.622
LAC	2	24	53	79	3	36	98	137	0.47	0.790
β2GPI	4	28	65	97	3	36	98	137	1.02	0.602
ALL 3	2	3	20	25	3	36	98	137	4.31	0.116
ANY	4	44	112	160	3	36	98	137	0.10	0.953

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	T	C	T	Z	p
aCL	0.147	0.853	0.153	0.847	-0.17	0.867
LAC	0.177	0.823	0.153	0.847	0.64	0.520
β2GPI	0.186	0.814	0.153	0.847	0.93	0.351
ALL 3	0.14	0.86	0.153	0.847	-0.24	0.809
ANY	0.163	0.838	0.153	0.847	0.30	0.764

Table 42. Distribution of the -643 Polymorphism in antibody positive and antibody negative groups in white healthy controls.

	ANTIBODY POSITIVE				ANTIBODY NEGATIVE					
	GENOTYPE				GENOTYPE					
ANTIBODY	CC	TC	TT	Total	CC	TC	TT	Total	χ^2	p
aCL	4	27	88	119	3	41	133	177	0.86	0.652
LAC	0	13	42	55	3	41	133	177	0.94	0.624
β2GPI	4	16	53	73	3	41	133	177	2.72	0.256
ALL 3	0	3	4	7	3	41	133	177	1.50	0.471
ANY	7	46	144	197	3	41	133	177	1.26	0.533

	ANTIBODY POSITIVE		ANTIBODY NEGATIVE			
	ALLELE FREQUENCY		ALLELE FREQUENCY			
ANTIBODY	C	T	C	T	Z	p
aCL	0.147	0.853	0.133	0.867	0.48	0.632
LAC	0.118	0.882	0.133	0.867	-0.42	0.674
β2GPI	0.164	0.836	0.133	0.867	0.87	0.383
ALL 3	0.214	0.786	0.133	0.867	0.73	0.466
ANY	0.152	0.848	0.133	0.867	0.74	0.457

2. DISCUSSION

Cardiovascular disease (CVD) is a leading cause of death in the United States, accounting for about 500,000-700,000 deaths each year. It is a significant worldwide public health problem, causing about 12 million deaths per year. In the US there are approximately 1.5 million cases of myocardial infarction (MI) each year (Garas et al. 2004). People with SLE are at an increased risk for CVD. Women with SLE between the ages of 35 to 44 have a 50-fold increased risk of MI. While traditional risk factors, such as diabetes and hypertension, are important in CVD risk in SLE patients, they are insufficient to account for the amount of increased risk seen in SLE (Al-Herz et al. 2003). CVD and SLE are both complex, multifactorial diseases caused by both environmental factors and genetics factors. Several candidate genes have been identified as playing a possible role in CVD. Because people suffering from SLE have a greater risk of CVD, it is possible that the genetic factors playing a role in CVD may also play a role in SLE or be present at a greater frequency in SLE populations.

β_2-GPI is one protein that is known to play a role in lipid metabolism and thought to play a role in atherosclerosis and aspects of SLE. Thus, characterization of the affects of the APOH gene is important in piecing together the causes for CVD and SLE. This study provides information about the associations of SNPs in the promoter region of the APOH gene with SLE, plasma β_2-GPI levels, and antiphospholipid antibodies.

2.1. Distribution of the APOH promoter polymorphisms in Blacks and Whites

Each promoter SNP was analyzed to determine its distribution in black and white subjects. The -1284 SNP had a very small minor allele frequency (0.006 in blacks, 0.002 in whites); therefore there was no difference seen between the races. The presence of the G allele in this SNP was previously reported only in whites in a small sample 24 white and 23 black individuals sequenced (http://pga/gs.washington.edu/data/apoh/). The present study found one black individual out of 82 genotyped for this SNP who was CG. This subject may have some white ancestry or this could be evidence that the G allele does exist in the black population at a very low frequency.

There was a significant difference in the allele frequency of the -1219 SNP between blacks and white ($p = 0.042$). The minor allele (A) was almost twice as common in the whites (0.096) as in blacks (0.056). The allele frequencies reported by the University of Washington group for this SNP also showed that the frequency was twice as great in whites as in blacks; however, the frequencies were lower (whites- 0.04, blacks-0.02) than reported in this study. There was no significant difference seen in the genotype distribution or allele frequencies of the -1190 SNP between blacks and whites ($p = .801$).

The -759 SNP differed in both genotype distribution ($p = 0.002$) and allele frequency ($p < 0.001$) between black subjects and white subjects. Similarly, the genotype distribution ($p = 0.001$) and allele frequencies ($p < 0.001$) significantly differed between races for the -700 SNP. The differences seen in the allele frequencies for the -759 and -700 SNPs are similar to the allele frequencies reported by the University of Washington group.

That group found no difference in allele frequency in the -643 SNP between whites and blacks; however, the present study did detect a significant allele frequency difference of 0.091 in blacks and 0.149 in whites ($p = 0.017$). The differences may be due to the small sample size of black subjects analyzed in both their sequencing efforts and the present study. Because there are significant differences in the distributions of many of the APOH promoter SNPs between blacks and whites, further analysis was done in the races separately

2.2. Association of the APOH promoter polymorphisms with SLE

Association of each SNP with SLE was first analyzed individually in whites and blacks. In the white subjects, the -1219 SNP was the only one to show a borderline significant association in allele frequencies ($p = 0.089$). The frequency of the AA+AG genotypes was lower in SLE cases than in controls compared to the GG genotype ($p = 0.057$). This association needs to be explored further to determine the significance of this difference. Because the significance levels of the results in this study are borderline, further testing would be useful in determining whether this SNP actually influences the risk of SLE.

In the African American samples, the -759 SNP ($p = 0.022$) and the -700 SNP ($p = 0.035$) showed significant differences in genotype distributions between cases and controls. For the -759 SNP, the frequency of the G allele was higher in cases then controls, but the difference was not statistically significant (0.175 vs. 0.077, $p = 0.06$). The same trend was observed for the -700 SNP, where the genotype difference was statistically significant between cases and controls ($p =$

0.035) but not the allele frequency (p = 0.098). This is possibly due to the small number of black cases and black controls.

Since SNPs in a gene are often in linkage disequilibrium (LD) with each other, the extent of LD in the promoter of APOH was tested. Several pairs of SNPs were in significant linkage disequilibrium with one another, and while none of the SNPs were in complete disequalibrium, several pairs showed high levels of didequalibrium (D' >.90) (See Tables 10 and 11). For these reasons, it is useful to determine the haplotype frequencies in this population and look for associations of the haplotypes with SLE.

The T5 test statistic, which was used to determine if the haplotype pattern of 6 APOH SNPs, was significantly different between cases and controls. The overall distribution of the haplotypes between cases and controls was significantly different (p = .00015). This indicates that the haplotype analysis is more informative than individual SNP analysis. However, the 4 most common haplotypes were not associated with SLE. Individually, there were 8 haplotypes that differed significantly in frequency between cases and controls. One of these haplotypes is relatively common, (6.1% in the total white sample) and its frequency was higher in controls than in cases (8.17 % vs. 3.2%, p < 0.001). This suggests that this haplotype may have a protective effect against the risk of SLE. One other haplotype also seems to have a protective effect; it was present in 1% of controls but was absent in cases (p = 0.006). Conversely, 6 of these 8 haplotypes seem to be associated with SLE and may confer greater risk. They are seen significantly more frequently in cases than in controls. The most frequent risk haplotype was seen in 3.4% of SLE cases and 0.1% of controls (p <.001).

2.3. Association of the APOH promoter polymorphisms with plasma β_2GPI levels

Previous studies have shown that specific APOH coding polymorphisms affect plasma β_2GPI levels (Kamboh et al. 1999). Significant gene dosage effects were seen for codon 306 ($p < .0001$) and codon 316 ($p < .0001$). β_2GPI concentrations decreased linearly with increasing number of copies of the variant alleles for each codon. It was found that codon 306 accounted for 30% of the variation in β_2GPI levels and codon 316 accounted for 13% of the β_2GPI plasma level variation (Kamboh et al. 1999).

Whether or not the 6 APOH promoter polymorphisms in the present study affect plasma β_2GPI levels has not previously been studied. In the present project, the impact of 6 of the APOH promoter SNPs on plasma β_2GPI levels was examined. Only one of the 6 promoter polymorphisms analyzed in this study was associated with β_2GPI levels. The -643 SNP was mildly associated with the plasma levels of β_2GPI in both white SLE cases ($p = .096$) and black controls ($p = .081$). The trend seen overall in all groups for the -643 SNP was a gene-dosage effect, CC homozygotes had the highest β_2GPI levels and TT homozygotes had the lowest β_2GPI levels, with the heterozygotes falling in between. This data indicated that the presence of the T allele may cause functional problems for the promoter. Perhaps that SNP is in a critical area for promoter function, such as a binding site for a transcription factor or an enhancer element. The statistical significance of this correlation is weak; therefore, more subjects should be analyzed to see if this effect persists with a larger sample size. Functional analysis of the promoter would be useful in supporting this data.

2.4. Association of the APOH promoter polymorphisms with antiphospholipid antibodies

Previous research has been done to analyze whether or not genetic variation in the coding region of the APOH gene is associated with the presence of antiphospholipid antibodies (APA). One polymorphism was reported to be associated with the presence of APA. The allele frequencies and the distribution of the genotypes for the codon 316 polymorphism differed between APA-positive subjects and APA-negative subjects (Kamboh et al. 1999). No research has been done as to the association of APOH promoter SNPs with APA.

In order to investigate whether or not the APOH promoter polymorphisms are markers for the presence of APA, the genotype and allele distributions were analyzed for each SNP between subjects negative for all APA and 4 groups of antibody positive people: positive for aCL, positive for LAC, positive for anti- β_2GPI, positive for any antibody, and positive for all three antibodies. This analysis was only done in whites due to the small sample size of the black subjects.

The only differences found were between the APA negative group and the group positive for all three antibodies. The differences seen were present in both the total white sample and the white SLE cases, but not in the white controls. This is because there were only 7 control samples that were positive for all 3 antibodies. Therefore the differences seen in the overall population for the all antibody positive group are reflective of the differences seen in the SLE cases, thus further discussion of the all antibody positive group will focus on the SLE cases only.

For the -1284 SNP, genotype distribution was significantly different between the all positive and the negative antibody groups ($p = 0.02$). None of the 139 antibody negative SLE cases genotyped for the -1284 SNP were of the CG genotype, while 1 out of the 25 cases in the all antibody positive group was CG. This difference is statistically significant; however, the minor allele frequency is so low that it is not unusual to only see the G allele in 1 out of 166 subjects.

The allele frequencies for the -759 SNP and for the -700 SNP differed significantly between the all positive and the negative antibody group ($p = 0.046$, $p = 0.095$). The minor allele was seen more frequently in the antibody negative group for both SNPs. This indicates that the presence of the minor allele at these polymorphic sites may be associated with protection against production if APA. However, this difference was not noted for any of the antibodies individually or for the group of subjects positive for at least one antibody. The group of people positive for all three antibodies is small, thus it would be beneficial to replicate these results with a larger sample size.

2.5. Summary

This study has examined 6 SNPs within the promoter region of the APOH gene. Differences between blacks and whites were analyzed, as well as associations with SLE, plasma β_2GPI levels, and antiphospholipid antibodies. Significant findings from this study are as follows:

1) The distribution of 4 of the 6 SNPs was significantly different between blacks and whites.

2) The genotype distribution of the -1219 SNP showed a marginally significant association with SLE in whites (p= 0.057). On the other hand, the genotype distributions of the -759 SNP and the -700 SNP significantly differed between SLE cases and controls in blacks (p = 0.022, p = 0.035).
3) The haplotype pattern in the white sample was significantly associated with SLE risk (p = 0.00015).
4) The -643 SNP has a moderate affect on plasma β_2-GPI levels in white SLE cases (p = 0.096) and black controls (p = 0.081).
5) Significant differences were seen in the distribution of the -1284 SNP between the antibody negative group and the antibody group positive for all three antibodies in whites overall (p = 0.05) and in cases (p = 0.02)
6) Significant differences were seen in the allele frequency of the -759 SNP between the all 3 antibody positive group and the antibody negative group in whites overall (p = 0.03) and in cases (p = 0.046).

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