GENETIC VARIATION IN THE PARAOXONASE-1 GENE AND ASSOCIATION WITH SYSTEMIC LUPUS ERYTHEMATOSUS

by

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease affecting approximately one million individuals in the United States. Individuals with lupus are at an extremely increased risk (up to 50-fold) to develop coronary heart disease (CHD) compared to the general population. Traditional risk factors are insufficient to explain the increase in risk. The presence of anti-phospholipid antibodies (seen at a higher rate in SLE patients than in the general population) is suspected to play a role. CHD is the leading cause of death for both men and women of all ethnic groups in the United States. Understanding the genetic causes of CHD in high risk populations, such as individuals with SLE, can help facilitate the understanding of CHD in the general population. Due to the large public health significance of CHD, investigating the contributing factors and disease etiology could have a major impact on risk assessment and possible treatment of CHD. One gene involved in lipid metabolism, a major part of the development of atherosclerotic plaques and CHD, is paraoxonase-1 (PON1). PON1 encodes the enzyme paraoxonase, which inhibits the oxidation of low density lipoprotein (LDL) to help prevent its uptake by macrophages, thereby reducing the incidence of atherosclerotic plaques. Eight genetic variants in the PON1 gene were examined to determine their impact on SLE disease status, the presence of anti-phospholipid antibodies (APA), and PON activity. Polymorphisms in PON1 were not found to have a significant impact on SLE disease status or the presence of APA, however three of the polymorphisms studied were found to have a

significant impact on PON activity. While SLE and CHD are complex diseases, likely resulting from gene-gene and gene-environment interactions, the identification of these associations between PON1 polymorphisms and PON activity may help to clarify the role of PON in CHD development and possibly lead to more accurate risk assessment and/or the investigation of treatment options for this common disease.

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1. BACKGROUND AND SIGNIFICANCE

1.1. INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects 40 to 50 per 100,000 individuals in the U.S. population (Lawrence et al., 1998). SLE affects primarily women, with female-to-male prevalence ratios estimated at 10-15:1 (Lahita, 1999). All ethnic groups are susceptible to SLE; however the incidence in individuals of African descent (9.2/100,000) is greater than in whites (3.5/100,000) (McCarty et al., 1995). Patients with SLE have increased morbidity and mortality from coronary heart disease (CHD), with risks up to 50 times that of the general population (Manzi, 1997). Clinical atherosclerotic events have been documented in 6 – 12% of SLE patients, while up to 40% of SLE patients are estimated to have sub-clinical plaque formation (reviewed in Urowitz and Gladman, 2000). Atherosclerosis and the development of CHD result from genetic and environmental factors combined. The formation of atherosclerotic lesions is thought to be initiated by the oxidation of low density lipoprotein (LDL) and subsequent development of foam cells, formed when monocyte-derived macrophages take up oxidized LDL. These foam cells then accumulate in the arterial wall, creating an atherosclerotic lesion (Steinberg et al., 1989; Witztum and Steinberg, 1991; Witztum, 1994). High density lipoprotein (HDL), on the other hand, has a strong inverse correlation with atherosclerosis and CHD. Its proposed role, in addition to reverse cholesterol metabolism, is to protect LDL from oxidative modification, with the involvement of several HDL-associated enzymes (Mackness and Durrington, 1995a).

Established risk factors for CHD in the general population are insufficient to explain the increased risk for atherosclerosis and CHD in SLE patients. One possible explanation is the presence of anti-phospholipid antibodies (APA) in up to 50% of SLE patients vs. 1 – 5 % of the general U.S. Caucasian population. APA are antibodies against phospholipids or plasma proteins bound to anionic phospholipids. Several studies have demonstrated a relationship between the LDL oxidation and APA in SLE patients. It has been proposed that immune response by APA to oxidized LDL may play a role in the pathogenesis of atherosclerosis in SLE patients. In fact, it has been shown that *in vitro*, these antibodies appear to enhance uptake of oxidized LDL by macrophages, supporting foam-cell formation (Vaarala et al., 1993; Craig et al., 1999; George et al., 1999; Wu et al., 1999; Hayem et al., 2001; Svenungsson et al., 2001).

One factor which may play a role in both LDL oxidation and APA production is paraoxonase (PON). PON, when bound to HDL, has been shown to inhibit LDL oxidation, and low PON activity has been associated with increased risk for CHD (Mackness and Durrington, 1995; Watson et al., 1995; Durrington et al., 2001). Three PON genes, PON1, PON2, and PON3, have been identified and are linked on chromosome 7. Genetic variation in these genes has been shown to affect PON activity, the extent of LDL oxidation, and the risk for CHD in non-SLE patients (Aviram et al., 1998; Mackness et al., 1997, 1998; Durrington et al., 2001; Sanghera et al., 1997, 1998). In the present study, several polymorphisms in the promoter region and coding region of PON1 were examined to determine their effects on paraoxonase activity, the presence of APA and their association with SLE risk in a cohort of SLE patients and non-SLE controls.

1.2. ROLE OF PARAOXONASE (PON) IN LIPID METABOLISM

Until the 1980s, paraoxonase had been primarily investigated in the field of toxicology due to its ability to detoxify by hydrolysis a vast number of organophosphate compounds used as insecticides and nerve gases. The crystal structure of PON1 was recently obtained. It consists of a β-propeller with a unique active site and a catabolic mechanism based on a His-His dyad (Harel et al., 2004). A variety of physiological roles for PON have been proposed, primarily related to the anti-atherosclerotic activity of PON. It is thought that PON protects against the action of oxidized LDL by protecting LDL and HDL from oxidation, participating in the destruction of oxidized phospholipids, and decreasing macrophage uptake of oxidized LDL (Watson et al., 1995; Aviram, 2004; Aviram and Rosenblat, 2004). Secondary effects of this activity include reduction of monocyte adhesion to endothelial cells and macrophage chemotaxis that would normally occur due to oxidized phospholipids. PON1 is found almost exclusively on plasma HDL and is expressed in liver. One study suggests that optimal PON activity requires the co-assembly of PON1 and apoA-I on nascent HDL (Oda et al., 2001). It has been suggested that the hydrophobic N-terminus of PON1 binds it to HDL, but the exact mechanism by which PON binds to HDL is unknown. Proposed mechanisms for the influence of PON activity on the formation of atherosclerotic lesions include phospholipase A2 action (hydrolysis of platelet activating factor and oxidized lipids) and hydrolysis and inactivation of homocysteine thiolactone, a risk factor for atherosclerotic vascular disease (Aviram and Rosenblat, 2004). In association with HDL, PON1 is thought to play a role in several HDL functions, such as mediation of cholesterol trafficking and limiting the oxidation of LDL (Aviram and Rosenblat, 2004; Harel et al., 2004). A proposed mechanism for PON activity is shown in Figure 1. HDL-associated PON1 can inhibit foam cell formation via 1) hydrolysis of macrophage oxidized

lipids, 2) reduction of macrophage-mediated oxidation of LDL, 3) reduction of oxidized LDL via hydrolysis of oxidized lipids in oxidized LDL, and 4) reduction of oxidized LDL uptake via the macrophage CD-36 scavenger receptor secondary to cellular oxidized lipid hydrolysis in the receptor area (Aviram and Rosenblat, 2004).

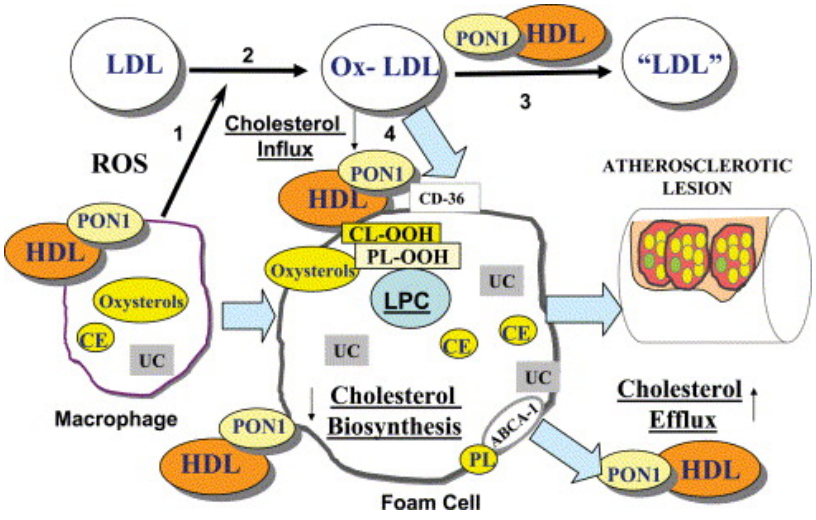

Figure 1: Role of Paraoxonase in Macrophage Foam Cell Formation (From Aviram and Rosenblat, 2004)

1.3. PON ACTIVITY

In 1986, McElveen et al. reported that serum PON enzyme activity was lower in patients who had a myocardial infarction than in control subjects. Further study showed that PON activity was lower in populations with familial hypercholesterolemia, diabetes mellitus, and renal disease than in matched controls (both groups have increased risks to develop atherosclerosis). This difference was not due to decreased amounts of PON protein, but lower activity of the PON per

unit protein (Mackness et al., 1991; Mackness and Durrington, 1995b; Mackness and Mackness, 2004). PON1 activity may differ between individuals by as much as 13-fold, and is known to be influenced by many environmental and genetic factors. These include statins, inflammatory factors, infection, growth hormone and homocysteinemia (decrease PON activity) and dexamethasone, aspirin, vitamins C and E, and exercise (increase PON activity) (reviewed in Getz and Reardon, 2004).

Traditionally, PON activity has been measured in vitro using synthetic substrates, including paraoxon, phenylacetate, and diazinon. The relationship of PON activity against these substrates to PON's physiological activity is unclear. It has been shown that the active site requirements for arylesterase/paraoxonase hydrolysis are different from those for the inhibition of LDL oxidation (Aviram et al., 1998). It has been reported that PON activity, rather than haplotype analysis is a predictor of vascular disease (Jarvik et al., 2003b).

1.4. MOLECULAR ASPECTS OF PON1

The PON genes (PON1, PON2 and PON3) are linked on chromosome 7q21.3-22.1 and are homologous (Primo-Parmo et al., 1996). It is thought that the three genes arose by gene duplication of a common evolutionary ancestor (Aviram and Rosenblat, 2004). PON1 is located at chromosome 7q21.3. The PON1 gene encodes a 355 amino acid protein, from which the amino-terminal methionine residue is removed during secretion and maturation. There are several known single nucleotide polymorphisms (SNPs) in PON1. The complete sequence of the PON1 gene has been determined from 23 Caucasians and 24 African Americans. This analysis identified about 200 SNPs in PON1. A complete list of PON1 SNPs can be found in the SeattleSNPs Variation Discovery Resource, at http://pga.gs.washington.edu/data/pon1/. Two

well-studied coding-region polymorphisms are Q192R (exon 6) and L55M (exon 3). Polymorphisms in the promoter region of PON1 have also been studied in recent years.

1.4.1. Q192R Polymorphism

At codon 192 in PON1, there is a well-studied polymorphism, Q (glutamine) to R (arginine). The Q genotype predominates in populations of European descent, while the R genotype predominates in populations of Asian and African descent (LaDu, 1992). In vitro, the 192Q isoform has a higher rate of hydrolysis of diazoxon, sarin and soman, while the 192R isoform hydrolyzes paraoxon and chlorpyrifos oxon at a higher rate. Both have been shown to hydrolyze phenylacetate at approximately the same rate (reviewed in Nakanishi, 2003). It has been suggested that the Q allele offers a protective effect against LDL oxidation, while the R allele is related to risk for CHD. Mackness et al. (1998) reported that the 192QQ genotype is most effective at protecting LDL against oxidative modification in vitro, while the 192RR was least efficient. This was supported by Aviram et al. (1998) in a later study. However, case-control studies have been conflicting. Some have found the 192R allele to be associated with increased risk for CHD (Ruiz et al., 1995; Odawara et al., 1997; Sanghera et al., 1997; Zama et al., 1997; Chen et al., 2003), while others have found no association (Ko et al., 1998; Ombres et al., 1998; Aynacioglu and Kepecki, 2000; Gardemann et al., 2000; and Imai et al., 2000). Furthermore, a recent study of PON1 polymorphisms and longevity in a combined cohort form Northern Ireland and Italy found that in individuals 80 years of age and older, the R allele conferred a small but significant survival advantage ($p=0.02$) (Rea et al., 2004). Clearly, evidence is conflicting. Recently, a large cohort study and meta-analysis of prior studies found that the Q192R distribution between British women with heart disease was no different from those without CHD.

In addition, meta-analysis of 39 published studies (10,738 cases and 17, 068 controls) found no robust evidence that the PON1 192 polymorphism is associated with CHD risk in Caucasian males or females (Lawlor et al., 2004). Further case-control studies, such as that presented here, may help to clarify the role of the PON1 192 polymorphism in CHD risk.

1.4.2. W194X Polymorphism

While investigating PON activity relative to the Q192R polymorphism, Jarvik et al. (2003a) found a few samples for which observed PON activity did not correlate with expected PON activity based on genotype at codon 192. Five individuals known to have a 192QR genotype had PON activity more consistent with either the 192QQ or 192RR genotype. They hypothesized that a known coding polymorphism near the 192 polymorphism (W194X; tryptophan to a stop codon at codon 194) caused the unexpected, altered PON activity. The five individuals with discordant activity were screened for the W194X polymorphism. A single Caucasian individual (with PON activity consistent with the 192QQ genotype) was found to have the 194stop allele associated with the 192R allele. Thus, the group concluded that the 194stop resulted in a loss of function of the 192R allele (Jarvik et al., 2003a). This SNP has not been studied in other published literature, and was investigated in the present study.

1.4.3. L55M Polymorphism

At codon 55 in PON1, there is a well-studied polymorphism, L (leucine) to M (methionine). The L55M polymorphism seems to determine PON1 concentration via interaction with another polymorphism in the promoter region (C-107T) (Brophy et al., 2001). This SNP has not been

found to have an effect on the catalytic properties of PON1, but has been associated with variation in PON1 levels, with the M allele associated with lower levels. The 55M allele has been reported to be less stable than the 55L allele (reviewed in Costa et al., 2003). Oliveira et al. (2004) found that the PON1 L55M polymorphism was an independent marker for CHD, while the Q192R polymorphism and two PON2 polymorphisms (G148A and C311S) were not. This study found that the MM genotype was protective against CHD (Odds Ratio=0.59, 95% confidence interval 0.42 – 0.82; p = 0.002). On the other hand, Mackness et al. (1998) reported that in vitro, HDL associated with the 55LL isoform protected LDL from oxidation twice as well as HDL associated with either 55LM or 55MM. In addition, the Helsinki Sudden Death study recently conducted an association study of L55M genotype and alcohol consumption with CHD. Compared to the 55LL homozygotes, carriers of the 55M allele tended to have larger areas of atherosclerotic lesions, the size of which decreased dose-dependently by reported alcohol consumption (Rontu et al., 2004). Mackness et al. (2001) found no relationship between the L55M polymorphism and paraoxonase activity and plasma PON1 concentrations. Like the Q192R data, studies examining the effects of the L55M SNP have provided conflicting results, and further investigation is needed to determine the role of L55M in CHD development.

1.4.4. Promoter Polymorphisms

The first published investigation of polymorphisms in the promoter region of PON1 was reported by Leviev and James (2000). Their hypothesis was that variations in serum PON levels may be attributable to polymorphism in the promoter. Three polymorphisms were identified at that time (T-107C, G-824A, and G-907C) and characterized with respect to their influence on promoter activity. This study found a dominant effect of the -107 polymorphism on PON expression, with

a minor contribution from the -907 polymorphism. The SNP at position -107 lies within the GG*C*GGG consensus sequence of the binding site for the transcription factor Sp1 (polymorphic site is italicized). Variations within the Sp1-binding site have been shown to affect the promoter activity of other genes. A second study by Suehiro et al. (2000) looked at the C-107T polymorphism and also identified two other SNPs in the promoter region (G-126C and G-160A). This study found that the SNPs at positions -126 and -160 (as well as the Q192R SNP) had no effect on serum PON1 concentration. However, the C-107T SNP was associated with partial regulation of the transcription of PON1 in HepG2 cells (liver cell line). In addition, it was found that the G-126C and G-160A polymorphisms were in almost complete linkage. Further study by Leviev, Righetti and James (2001) showed that the C-107T SNP was an independent risk factor for CHD in a study of 897 participants, 699 cases and 198 controls. The higher expresser -107C allele was associated with decreased risk in participants age 60 or under ($\chi^2 = 4.42$, $p = 0.034$), but did not have an effect on participants age 61 or older.

In a study of 376 white individuals, Brophy et al. (2001) found that while the C-107T SNP had a significant effect on PON activity, the G-160A SNP had a lesser effect. The G-907C SNP had no effect on PON activity. Further study by Deakin et al. (2003) found that the SNPs at positions -107, and -824, but not -907, had an impact on promoter activity *in vitro*. The C-107T and G-824A SNPs each independently influenced promoter activity, each causing a two-fold increase in activity when the high-expresser alleles (-107C and -824A) were present, and a four-fold increase when they were both present.

The SNP at position -160 was genotyped in the present study. Additional PON1 promoter polymorphisms (A-1739G, C-1432G, T-1439C, and A-1074G) were also genotyped as part of the present study. Studies of the latter four SNPs have not been published previously.

1.5. SPECIFIC AIMS

Specific aims of this study were to:

1. To determine the allele frequency and genotype distributions of three SNPs in the coding region of PON1 and five SNPs in the promoter region of PON1 in white and black subjects.

Hypothesis: The distribution of PON1 SNPs is significantly different between whites and blacks.

2. To determine the association of the studied SNPs in PON1 with SLE disease.

Hypothesis: Genetic variation in the PON1 gene may affect the risk of SLE.

3. To determine the association of the studied SNPs in PON1 with the occurrence of anti-phospholipid antibodies (APA).

Hypothesis: Genetic variation in the PON1 gene may affect the risk of SLE through its influence on the production of APA.

4. To determine the association of the studied SNPs in PON1 with PON activity.

Hypothesis: Genetic variation in the PON1 gene may affect the risk of SLE through its influence on PON activity.

2. MATERIALS AND METHODS

2.1. CASE-CONTROL SAMPLES

DNA samples from 380 women with SLE (334 European Americans and 46 African Americans) and 497 healthy control women (455 European Americans and 42 African Americans) were used in the present study. The SLE cases were recruited by Dr. S. Manzi for a study designed to determine the prevalence of cardiovascular disease and associated risk factors in women with SLE (funded by AHA, Arthritis Foundation, and the National Institutes of Health). Cases were 18 years of age or older (mean age 44.03±11.31 years), and met the 1982 American College of Rheumatology criteria for definite or probable SLE (Tan et al., 1982). All of the women with SLE were participants in the Pittsburgh Lupus Registry, which included 983 living participants seen at the University of Pittsburgh Medical Center or by practicing rheumatologists in the Pittsburgh metropolitan area. Demographic and clinical details of the population have been described elsewhere (Manzi and Wasco 2000; Selzer et al., 2001). Controls with no apparent history of SLE were obtained from the Central Blood Bank of Pittsburgh (mean age 42.73±12.53 years). Blood samples were obtained from the baseline visit. All participants provided written informed consent. The study was approved by the University of Pittsburgh Institutional Review Board.

2.2. ANTIPHOSPHOLIPID ANTIBODY (APA) MEASUREMENTS

All patients and controls were screened for the presence of anti-cardiolipin antibodies (ACL antibodies; IgG>15 GPL units, IgM>10 MPL units; Incstar, Stillwater, Minn., USA), lupus anticoagulant (LAC; partial thromboplastic time or Russel's viper venom time with mix), and

anti-β_2-glycoprotein I (aβ_2GPI; Quantalite, β_2GPI screen, INOVA Diagnostics, San Diego, California, USA). All samples were screened in duplicate. The presence or absence of the antibody was determined by comparing the optical density (OD) with the decision point calibrator provided in the kit, as described elsewhere (Kamboh et al., 1999; Sanghera et al., 2001). Participants positive for any of the antibodies (ACL/IgG, ACL/IgM, aβ_2GPI, or LAC) were considered positive for APA.

2.3. PON ACTIVITY MEASUREMENTS

Paraoxonase activity was determined using the method described by Furlong et al. (1989) and Richter and Furlong (1999) to measure hydrolysis of paraoxonase. Briefly, for each sample, 20 μL of plasma was diluted in 180 μL dilution buffer (containing 9mM Tris pH 8.0 and 1mM $CaCl_2$) and mixed. Substrate solution (containing 2M NaCl, 0.1M Tris HCl pH 8.5, 2mM $CaCl_2$ and 1.2mM paraoxon) was added to the plasma, transferred to the plate reader, mixed for 5 seconds at 37°C, and read at 405 nm, one measurement every 15 seconds for three minutes. Output was in optical density (OD)/minute. PON activity (in micromoles of substrate hydrolyzed per minute per liter of plasma) was determined using the equation mOD/min x 6. Samples were run in triplicate and a mean activity was determined and used in PON activity analysis.

2.4. GENETIC VARIATION IN PON1 INCLUDED IN PRESENT STUDY

Five variants in the promoter region of PON1 and three variants in the coding region of PON1 were genotyped in the present study. These variants are shown in the map in Figure 2.

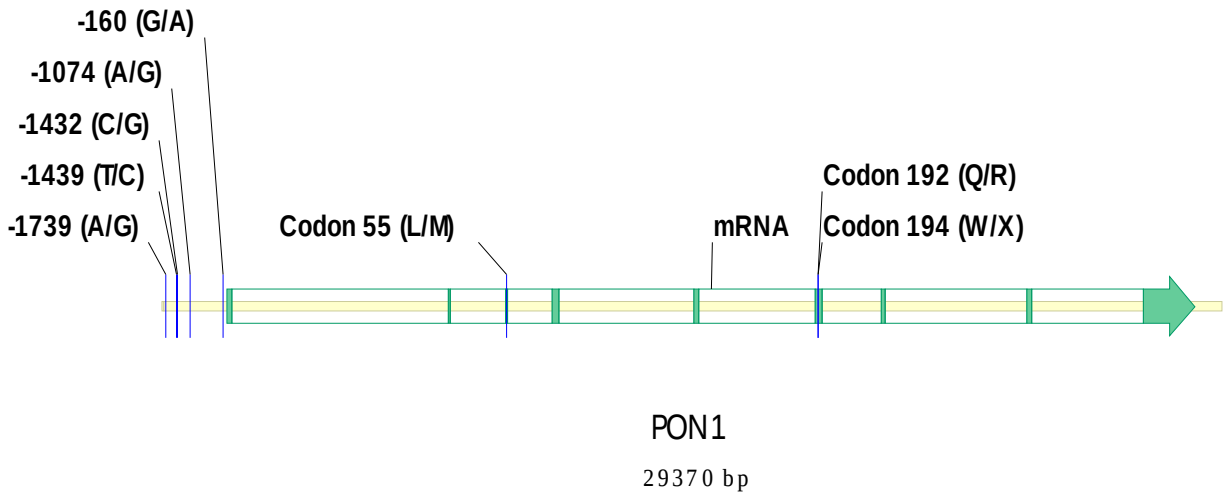

Figure 2: Map of PON1 Variants Examined in Present Study

2.5. GENOTYPING

Genomic DNA was isolated from buffy coat using the QIAamp kit (QIAGEN). Target fragments were amplified using polymerase chain reaction (PCR). Briefly, 1-5 μg of genomic DNA was amplified in a 50 μl reaction mixture consisting of 5 μl of 10X PCR buffer (100 mM Tris-HCl, pH = 8.3, 500 mM KCl), 1.0–2.5 mM $MgCl_2$, 0–2.5 mM dimethylsulfoxide (DMSO), 1.25 mM each dNTP (Pharmacia), 0.2 μM each primer, and 1.25 units of *Taq* DNA polymerase (Invitrogen). After initial denaturation at 95°C, the reaction mixture was subjected to multiple cycles of denaturation, annealing, and extension. The final step was an extension step at 72°C. Temperatures and times for each step were variable based on the fragment. PCR conditions for amplifying DNA in the present study are summarized in Table 1. The correct size and purity of amplified PCR product was verified by running 5 ml of PCR product on a 2% agarose gel.

Table 1: PCR Conditions for Amplifying Fragments Containing SNPs

SNP	Thermocycler Conditions	Primer Sequence 5'→3' (B = biotin)
G-1739A	95°C x 5' → (94°C x 30" → 56°C x 30" → 72°C x 30") x 35 cycles → 72°C x 5'	F: GGGGGATTAAGAGTTTTCCTTT R: TAGCTGCTAAACCAAACAATCA
G-1074A	95°C x 5' → (94°C x 30" → 56°C x 30" → 72°C x 30") x 35 cycles → 72°C x 5'	F: GGCTTAAGAGCAAGTGTTCAGA R: ATTGCCAGCCAATACCAAC
T-1439C & C-1432G	95°C x 5' → (95°C x 30" → 59°C x 30" → 72°C x 30") x 45 cycles → 72°C x 5'	F: ATAGCCACATTGGACACAGATCA R: B-GGCCACCAACTGAATACCACT
G-160A	95°C x 5' → (94°C x 30" → 56°C x 30" → 72°C x 30") x 40 cycles → 72°C x 5'	F: AAATGGGACTTTTGGCTGAA R:GGGGATAGACAAAGGGATCG
L55M	95°C x 9' → (95°C x 1' → 60°C x 1' → 72°C x 1') x 35 cycles → 72°C x 10'	F: GAAGAGTGATGTATAGCCCCAG R: TTTAATCCAGAGCTAAAAGCC
Q192R & W194X	95°C x 5' → (94°C x 30"→ 61°C x 30" → 72°C x 30") x 40 cycles → 72°C x 5'	F: TATTGTTGCTGTGGGACCTGAG R: CACGCTAAACCCAAATACATCTC

Genotyping of the eight SNPs was determined by restriction digestion (four SNPs), fluorescence polarization (two SNPs), and pyrosequencing (two SNPs).

2.5.1. Genotyping by Restriction Fragment Length Polymorphism (RFLP)

Four SNPs were genotyped using restriction fragment length polymorphism (RFLP); G-160A, L55M, Q192R, and W194X. Restriction endonucleases were used when the genetic variant creates or abolishes a restriction site. Fragments containing polymorphic sites were amplified using PCR (see Table 1), then digested with a particular restriction endonuclease. The endonuclease cut the fragment at both alleles (homozygote for restriction site polymorphism), cut the fragment at one allele (heterozygote), or did not cut either allele (homozygote for polymorphism without the restriction site). Digested products were run out on a 3% NuSieve agarose gel. Analysis of the number of fragments present as well as the size of these fragments allowed for genotype determination. Digestion conditions are summarized in Table 2.

Table 2: RFLP Endonucleases and Digestion Conditions

SNP	Endonuclease	Restriction Site	Manufacturer	Digestion Conditions
G-160A	*BstUI*	5'-CG▼CG-3' 3'-GC▲GC-5'	New England Biolabs	60°C x 16 hours
L55M	*Hsp92II*	5'-CATG▼-3' 3'-▲GTAC-5'	Promega	37°C x 16 hours
Q192R	*AlwI*	5'-GGATC $(N)_4$▼-3' 3'-CCTAG $(N)_5$▲-5'	New England Biolabs	37°C x 16 hours
W194X	*BstNI*	5'-CC▼WGG-3' 3'-GGW▲CC-5'	New England Biolabs	60°C x 16 hours

Genotypes of the Q192R SNP were determined by scoring the bands visualized on the agarose gel following restriction digestion with *AlwI*. An example of each genotype result is shown in Figure 3.

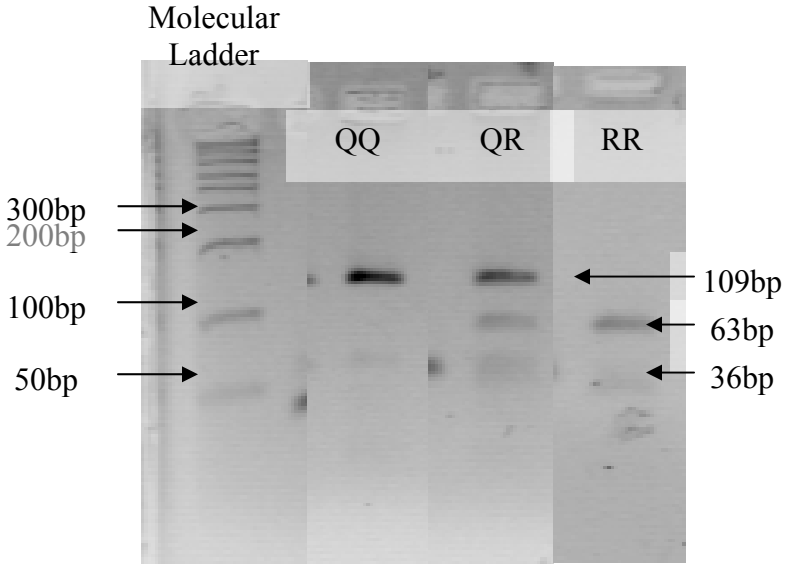

Figure 3: RFLP Result for Q192R SNP

The uncut, 109bp PCR product represents the Q allele. The *AlwI* endonuclease cut the 109bp fragment into a 63bp fragment and a 36bp fragment, when the R allele was present.

Genotypes of the W194X SNP were determined by scoring the bands visualized on the agarose gel following digestion with *BstNI*. The stop codon was not detected in a sample of 753 samples (282 white SLE cases, 400 white SLE controls, 38 black cases and 33 black controls). Since all samples analyzed had the same genotype, no further analysis of this data was performed. An example of the RFLP result is shown in Figure 4.

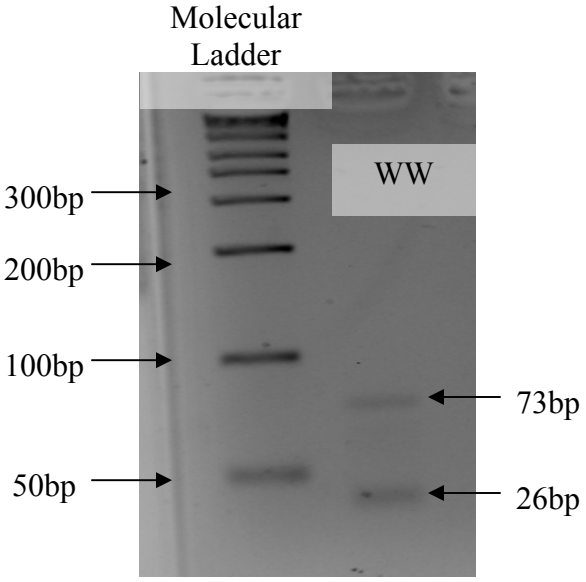

Figure 4: RFLP Result for W194X SNP

The original PCR product is 109 bp in length. The *BstNI* endonuclease cut the fragment into 73 bp and 26 bp fragments when the W allele was present. An uncut 109 bp fragment would represent the stop codon, however this allele was not detected in the population studied.

Genotypes of the L55M SNP were determined by scoring the bands visualized on the agarose gel following digestion with *Hsp92II*. An example of each genotype result is shown in Figure 5.

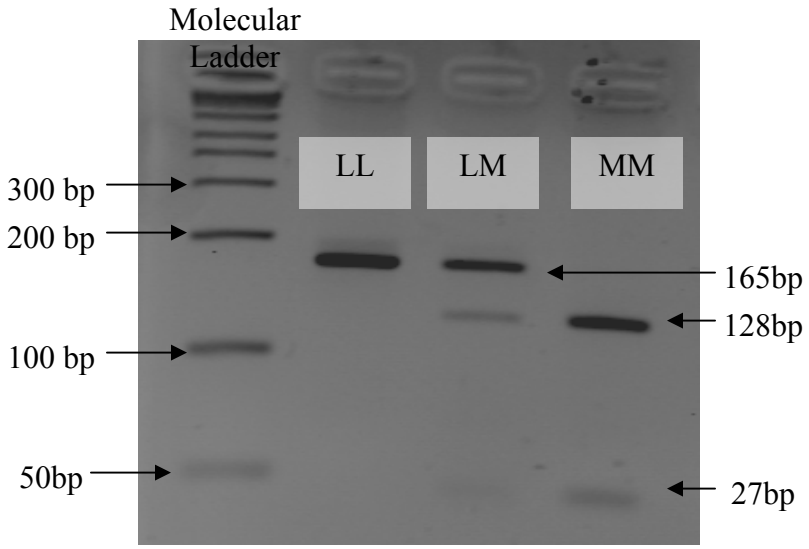

Figure 5: RFLP Result for L55M SNP

The uncut PCR product is 165 bp in length, which represents the L allele. The *Hsp92II* endonuclease cut the fragment into 128 bp and 27 bp fragments when the M allele was present.

Genotypes of the G-160A SNP were determined by scoring the bands visualized on the agarose gel when digested samples were electrophoresed. An example of each genotype result is shown in Figure 6.

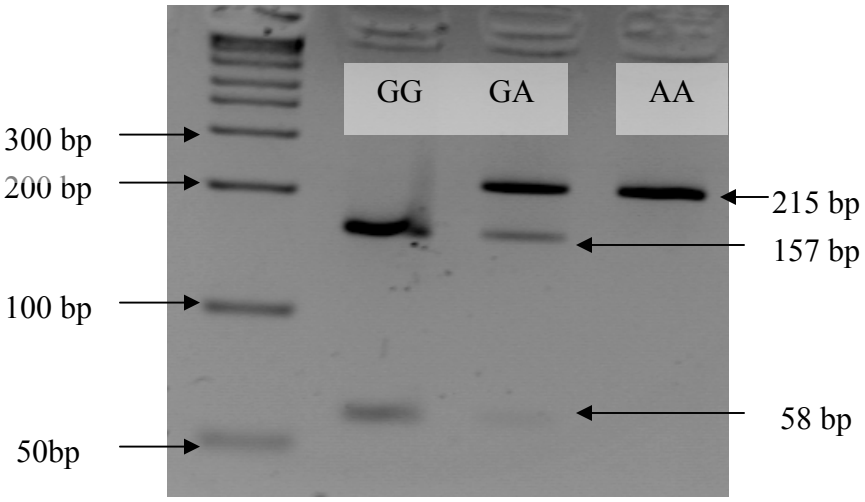

Figure 6: RFLP Result for G-160A SNP

The uncut PCR product was 215 bp in length, representing the A allele. The BstU1 endonuclease cut the fragment into 157 bp and 58 bp fragments when the G allele was present.

2.5.2. Genotyping with Fluorescence Polarization (FP)

The G-1739A and G-1074A SNPs were genotyped using fluorescence polarization (FP). FP is based on the premise that when a fluorescent molecule is excited by plane polarize light, it emits polarized fluorescent light into a fixed plane relative to the molecule itself. The FP of a molecule is proportionate to the molecule's rotational relaxation time (the time it takes to rotate through an angle of 68.5°). The rotational relaxation time is affected by the viscosity of the solvent, absolute temperature, molecular volume and gas constant. When viscosity and temperature are held constant, FP is directly proportional to the molecular volume. Large molecules rotate more slowly through space (FP is preserved) while small molecules rotate more quickly (FP is lost, the molecule becomes more depolarized). The assay used in this study (HEFP-SNP Genotyping Assay, LJL Biosystems, US) uses this premise to facilitate high-throughput genotyping. In summary, fluorescently labeled ddNTPs are added to amplified fragments of DNA containing

the SNP of interest. The ddNTPs are added with a primer upstream of the SNP site in a terminator base extension reaction. The specific fluorescent ddNTP incorporated at the polymorphic site alters the molecular weight of ddNTP labels by at least 10-fold, causing increased FP. The genotype can then be determined by exciting the dye on the terminator to determine if FP is observed (Chen et al., 1999; Kwok, 2002; Chen, 2003). A schematic illustrating these steps is shown in Figure 7.

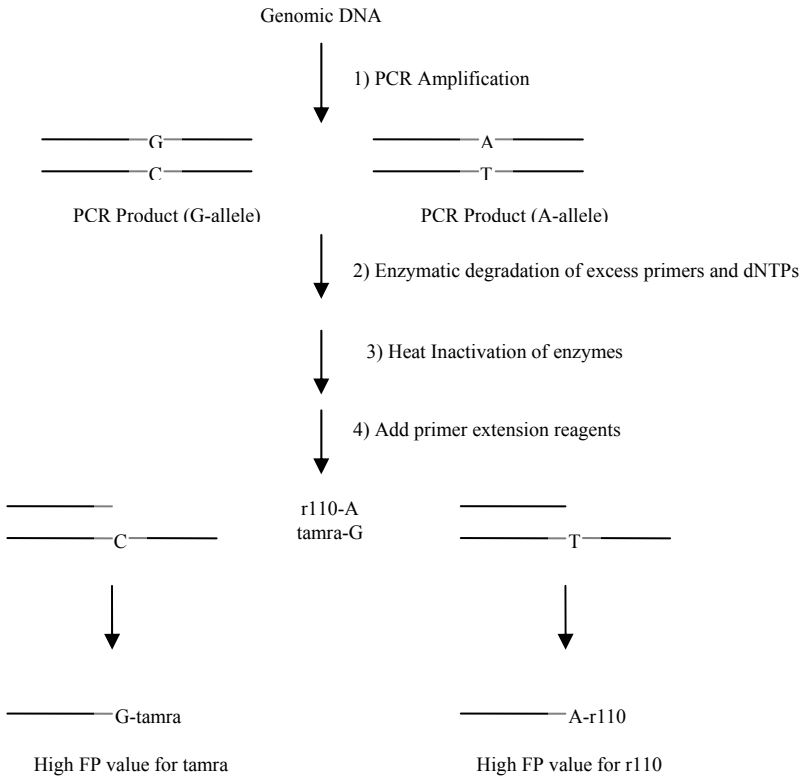

Figure 7: FP Genotype Detection Schematic (adapted from Kwok, 2002)

As illustrated in Figure 3, genomic DNA is amplified in PCR reaction (Step 1). Excess primers and dNTPs from PCR reaction are degraded using Shrimp-Alkaline Phosphatase (SAP) and Exonuclease I (ExoI) (Step 2). SAP and ExoI enzymes are inactivated with heat (Step 3). Primer and thermosequenase extension reagents are added with fluorescent dyes (Step 4). This is followed by excitation and emission studies on a fluorescence plate reader to determine the FP value of each sample. Homozygote for the G allele will have high FP for the tamra dye. Homozygotes for the A allele will have high FP for the r110 dye. Heterozygotes will have intermediate FP.

In the present study, Step 2 (degradation of excess primers and dNTPs) was performed using 0.1μL (1 unit) of ExoI (in 20 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 5 mM 2-mercaptoethanol and 50% glycerol), 1 μL (1 unit) of SAP (in 25 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 50% glycerol), and 1 μL of 10X Buffer (200 mM Tris-HCl, pH 8.0 and 100 mM $MgCl_2$). This mixture was incubated at 37°C for 90 minutes. Step 3 (inactivation of ExoI and SAP) was performed by incubating at 95°C for 15 minutes and held at 4°C. Step 4 (template directed dye incorporation) was performed by adding 1 μL (1 μM) sequencing primer (shown in Table 3), 0.025 μL (0.8 units) of Thermosequenase (in 20 mM Tris-HCl, pH 8.5, 0.1 mM EDTA, 0.5% Tween™ 20, 0.5% Nonidet P-40, 1 mM DTT, 100 mM KCl, 50% glycerol), 1 μL Reaction Buffer (25 mM TAPS buffer, pH 9.3 at 25 °C, 50 mM KCl, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 200 μm dATP, dGTP, dTTP, 100 μM [a-^{32}P]-dCTP (0.05-0.1 Ci/mmol), and 400 μg/ml activated DNA), and 0.05 μL of a mixture of 3μM tamra-ddGTP and 3μM r110-ddATP fluorescent dyes. This mixture was incubated at 94°C for 1 minute, then cycled 35 times at 94°C for 10 seconds then 55°C for 30 seconds, and finally held at 4°C. ExoI, SAP, 10X Buffer, Thermosequenase, Reaction Buffer, and dyes were obtained from Amersham Biosciences, Piscataway, NJ. All steps were performed in black skirted 96-well microtiter plates from MJ Research, Waltham, MA. Fluorescence polarization was measured using the Analyst plate reader and Criterion Host software version 2.0 (LJL Biosystems, Sunnyvale, CA). Alleles were called automatically from raw FP values using Allele Caller™ software (LJL Biosystems, Sunnyvale, CA).

Table 3: Primers used for FP Genotyping

SNP	FP Primer
G-1739A	5'-CTTTGGACACATTTTGAAAAGACT-3'
G-1074A	5'-GCCAAAGAAGCTTCCCCCCAGAAA-3'

Examples of the graphic output generated by the Allele Caller software are shown in Figure 8 and Figure 9.

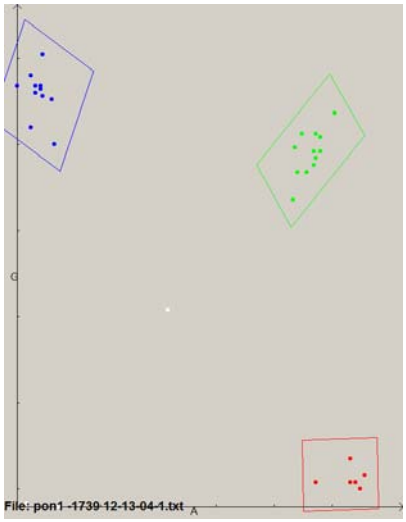

Figure 8: Graphic Output of FP Genotype Analysis for A-1739G

The vertical axis represents the G allele while the horizontal axis represents the A allele. Samples clustering along the vertical axis (blue) have high FP for the tamra dye and are classified as GG homozygotes, samples clustering along the horizontal axis (red) have high FP for the r110 dye and are classified as AA homozygotes, and samples with intermediate FP (green) are classified as AG heterozygotes. Samples unable to be genotyped (including negative control) are shown in white.

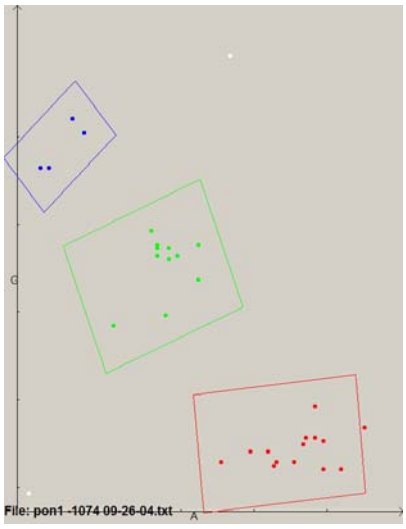

Figure 9: Graphic Output of FP Genotype Analysis for A-1074G

The vertical axis represents the G allele while the horizontal axis represents the A allele. Samples clustering along the vertical axis (blue) have high FP for the tamra dye and are classified as GG homozygotes, samples clustering along the horizontal axis (red) have high FP for the r110 dye and are classified as AA homozygotes, and samples with intermediate FP (green) are classified as AG heterozygotes. Samples unable to be genotyped (including negative control) are shown in white.

2.5.3. Genotyping with Pyrosequencing (PSQ)

Pyrosequencing (PSQ) is a method for obtaining genotype information using the principle of sequencing by synthesis. PSQ is capable of analyzing all types of genetic variations, such as bi-, tri-, and tetra-allelic polymorphisms, multiple SNPs, mutations, and insertion/deletions. PCR products (with bound biotin) in all wells of a 96-well plate are converted to single stranded templates. The template is isolated with a vacuum prep tool and a sequencing primer is then annealed onto these templates. Analysis begins with the dispensation of the enzyme-substrate reagents into the wells containing test samples. Light is produced when a nucleotide forms a base pair with its complement. The light and base are registered by a charge coupled device (CCD) camera, and is interpreted as a peak. If the next nucleotide added to the template is not

complimentary to the nucleotide base, no light is detected by the CCD. The height of the peak is proportional to the number of bases that have been integrated. Software used with the PSQ-96MA automatically analyzes the quantitative data. The data can be evaluated by the user as well. The software allows for multiplex genotyping of up to three polymorphisms in a single well. The T-1439C and C-1432 G SNPs were genotyped using the PSQ system in a single, simplex assay.

Isolation of the biotinylated, single stranded template was performed by first mixing 20 μL of PCR product with 40 μL binding buffer (10 mM Tris-HCl pH 7.6, 2M NaCl, 1 mM EDTA, 0.1% Tween 20), 2 μL Streptavidin Sepharose (Amersham Biosciences, Piscataway, NJ) and 18 μL high purity water. The mixture was shaken by vortexing for a minimum of 10 minutes. The beads containing the immobilized templates were captured onto the filter probes after applying vacuum, and then washed with 70% ethanol for 5 sec, denaturation solution (0.2M NaOH) for 5 sec and washing buffer (10 mM Tris-Acetate pH 7.6) for 10 sec. The vacuum was then released and the beads were released into a PSQ 96 Plate Low containing 9.5 annealing buffer μL (20 mM Tris-Acetate, 2 mM $MgAc_2$ pH 7.6) and 0.5 μM sequencing primer in each well. The sequencing primer for this assay is shown in Table 4. The annealing reaction was performed by incubating the plate on a heating block with lid at 90°C for 2 minutes, removing the entire block including the plate and incubating on the bench for 5 minutes, then removing the plate from the block and incubating on the bench at room temperature for 5 minutes. Pyrosequencing reactions were performed according to the manufacturer's instructions using the PSQ 96 SNP Reagent Kit which contained the enzyme and substrate mixture and nucleotides. The assay was performed using the nucleotide dispensation order GCTCTCGCATACT.

Table 4: Primer used for PSQ Genotyping

SNP	PSQ Sequencing Primer
T-1439C/ C-1432G	5'-CACAGATCATCATTCCC-3'

Genotypes of the T-1439C and C-1432G SNPs were determined using PSQ. The C allele at position -1439 was not detected in a sample of 822 samples (313 white SLE cases, 430 white SLE controls, 39 black cases and 40 black controls). Since all samples analyzed had the same genotype, no further analysis of this data was performed. Examples of pyrograms generated by the PSQ system software while analyzing the T-1439C and C-1432G SNPs are shown in Figure 10.

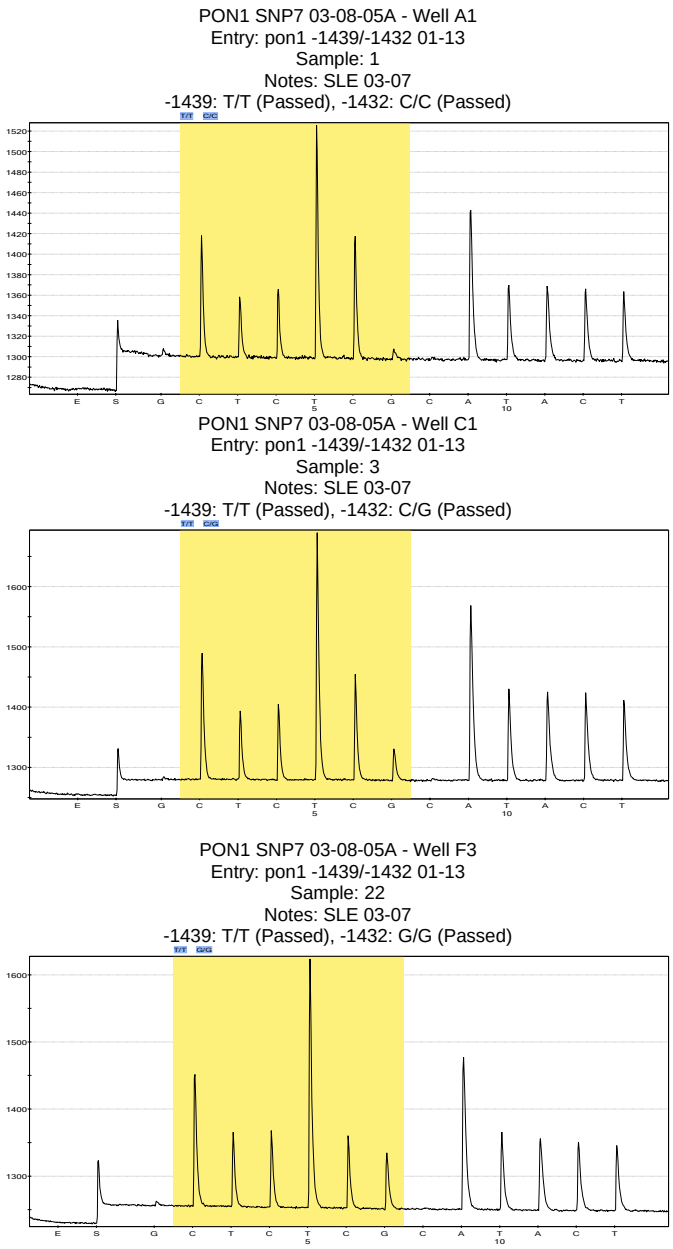

Figure 10: Pyrogram Output of PSQ Genotype Analysis for T-1439C and G-1432C

Dispensation order of nuleotides and time intervals are shown along the horizontal axis. Light output read by the CCD is shown along the vertical axis. The yellow shaded area indicates the critical analysis region necessary to determine genotype. High C peak height at the sixth nucleotide dispensation and no G peak at the seventh position indicates a CC genotype (top figure). High C peak height at the sixth nucleotide dispensation and a G peak 1/3 the height of the C peak at the seventh position indicates a CG genotype (middle figure). Equal C peak and G peak heights at the sixth and seventh positions indicates a GG genotype (bottom figure).

2.6. STATISTICAL METHODS

2.6.1. General Statistical Calculations

Allele frequency for each polymorphism was calculated by allele counting. Observed genotype frequencies were compared to the Hardy-Weinberg equilibrium, and the significance of deviations was tested by the χ^2 goodness-of-fit test. Comparison of allele frequencies between cases and controls within race (white or black) and between racial groups were calculated using a standard Z-test of two binomial proportions.

2.6.2. SNP Association Studies

The relationships between each SNP and SLE disease status and antibody status were determined by using the χ^2 goodness-of-fit test and standard Z-test of two binomial proportions. Due to small African American sample size, antibody association studies were performed only on white samples. Association studies of continuous variables (PON activity) were performed by first transforming the data by taking the square root to ensure normal distribution. All analyses were performed separately for blacks and whites. Logistic regressions were performed using the R statistical software package (version 2.0.1, http://www.r-project.org) (Ihaka and Gentleman, 1996).

2.6.3. Linkage Disequilibrium and Haplotype Analysis

Linkage disequilibrium between markers was estimated by both |D'| calculation (Lewontin, 1964) and associated p-value using the R statistical software package (version 2.0.1,

http://www.r-project.org/) (Ihaka and Gentleman, 1996). Haplotype analysis was performed using EH (Estimate Haplotype-frequencies, version 1.2). The EH program estimates allele frequencies for each marker, then estimates haplotype frequencies with allelic association (H_1) and without (H_0). The EH program then provides log likelihood, chi-square and the number of degrees of freedom under both hypotheses (H_0 and H_1) (Xie and Ott, 1993).

3. RESULTS

3.1. DISTRIBUTION OF PON1 POLYMORPHISMS IN WHITES AND BLACKS

3.1.1. Distribution of the Q192R Polymorphism in Whites and Blacks

The allele frequency of the Q192R polymorphism was significantly different between white and black samples, with a higher frequency of the R allele in blacks (0.481) than whites (0.259; Z-test, $p<0.0001$). The overall genotype distribution was also significantly different between races. The frequency of the QQ genotype was significantly higher in the white samples than in the black samples (54.1% vs. 24.7%; χ^2-test, $p<0.0001$). These results are summarized in Table 5.

Table 5: Distribution of the Q192R SNP in Whites and Blacks

	WHITES		BLACKS	
GENOTYPE	**N**	**%**	**N**	**%**
QQ	418	54.1	20	24.7
QR	310	40.1	44	54.3
RR	45	5.8	17	21.0
TOTAL	773		81	
$\chi^2=39.15$, $p<0.0001$				
ALLELE FREQUENCY	**WHITES**		**BLACKS**	
Q	0.741		0.519	
R	0.259		0.481	
$Z = -5.440$, $p<0.0001$				

3.1.2. Distribution of the L55M Polymorphism in Whites and Blacks

The allele frequency of the L55M polymorphism was significantly different between white and black samples, with a higher frequency of the M allele in whites (0.358) than blacks (0.210; Z-test, $p<0.0001$). The overall genotype distribution was also significantly different between races.

The frequency of the LL genotype was significantly higher in the black samples than in the white samples (63.0% vs. 41.4%; χ^2-test, p=0.0007). These results are summarized in Table 6.

Table 6: Distribution of the L55M SNP in Whites and Blacks

	WHITES		BLACKS	
GENOTYPE	**N**	**%**	**N**	**%**
LL	307	41.4	51	63.0
LM	338	45.6	26	32.1
MM	96	13.0	4	4.9
TOTAL	741		81	
χ^2=14.643, p=0.0007				
ALLELE FREQUENCY	**WHITES**		**BLACKS**	
L	0.642		0.790	
M	0.358		0.210	
Z=4.310, p<0.0001				

3.1.3. Distribution of the A-1739G Polymorphism in Whites and Blacks

The allele frequency of the A-1739G polymorphism was significantly different between white and black samples, with a higher frequency of the G allele in whites (0.564) than blacks (0.201; Z-test, p<0.0001). The overall genotype distribution was also significantly different between races. The frequency of the AA genotype was significantly higher in the black samples than in the white samples (65.9% vs. 21.0%; χ^2-test, p<0.0001). These results are summarized in Table 7.

Table 7: Distribution of the A-1739G Polymorphism in Whites and Blacks

	WHITES		BLACKS	
GENOTYPE	**N**	**%**	**N**	**%**
AA	161	21.0	54	65.9
AG	347	45.2	23	28.0
GG	259	33.8	5	6.1
TOTAL	767		82	
χ^2=82.1417, p<0.0001				
ALLELE FREQUENCY	**WHITES**		**BLACKS**	
A	0.564		0.799	
G	0.436		0.201	
Z=10.753, p<0.0001				

3.1.4. Distribution of the C-1432G Polymorphism in Whites and Blacks

The allele frequency of the C-1432G polymorphism was significantly different between white and black samples, with a higher frequency of the G allele in blacks (0.646) than whites (0.383; Z-test, p<0.0001). The overall genotype distribution was also significantly different between races. The frequency of the CC genotype was significantly higher in the white samples than in the black samples (36.7% vs. 14.6%; χ^2-test, p<0.0001). These results are summarized in Table 8.

Table 8: Distribution of C-1432G SNP in Whites and Blacks

	WHITES		BLACKS	
GENOTYPE	**N**	**%**	**N**	**%**
CC	272	36.7	13	14.6
CG	371	50.0	37	41.6
GG	99	13.3	39	43.8
TOTAL	742		89	
χ^2=33.542, p<0.0001				
ALLELE FREQUENCY	**WHITES**		**BLACKS**	
C	0.617		0.354	
G	0.383		0.646	
Z=-6.561, p<0.0001				

3.1.5. Distribution of the A-1074G Polymorphism in Whites and Blacks

The allele frequency of the A-1074G polymorphism was significantly different between white and black samples, with a higher frequency of the G allele in blacks (0.394) than whites (0.272; Z-test, p=0.0025). The overall genotype distribution was also significantly different between races. The frequency of the GG genotype was significantly higher in the black samples than in the white samples (12.5% vs. 6.2%; χ^2-test, p=0.0036). These results are summarized in Table 9.

Table 9: Distribution of the A-1074G SNP in Whites and Blacks

	WHITES		BLACKS	
GENOTYPE	**N**	**%**	**N**	**%**
AA	377	51.9	27	33.8
AG	305	42.0	43	53.8
GG	45	6.2	10	12.5
TOTAL	727		80	
χ^2=11.256, p=0.0036				
ALLELE FREQUENCY	**WHITES**		**BLACKS**	
A	0.728		0.606	
G	0.272		0.394	
Z=-3.023, p=0.0025				

3.1.6. Distribution of the G-160A Polymorphism in Whites and Blacks

The allele frequency of the G-160A polymorphism was significantly different between white and black samples, with a higher frequency of the A allele in blacks (0.380) than whites (0.264; Z-test, p=0.0032). The overall genotype distribution was also significantly different between races. The frequency of the AA genotype was significantly higher in the black samples than in the white samples (13.3% vs. 6.8%; χ^2-test, p=0.0049). These results are summarized in Table 10.

Table 10: Distribution of the G-160A SNP in Whites and Blacks

	WHITES		BLACKS	
GENOTYPE	**N**	**%**	**N**	**%**
GG	414	54.0	31	37.3
GA	300	39.2	41	49.4
AA	52	6.8	11	13.3
TOTAL	766		83	
χ^2=10.628, p=0.0049				
ALLELE FREQUENCY	**WHITES**		**BLACKS**	
G	0.736		0.620	
A	0.264		0.380	
Z=-2.950, p=0.0031				

3.2. LINKAGE DISEQUILIBRIUM BETWEEN PON1 POLYMORPHISMS

All SNPs studied in the white samples (Table 11) and black samples (Table 12) showed significant linkage disequilibrium. P-values are shown on the left and D-values are shown on the right. Significant p-values are highlighted.

Table 11: Linkage Disequilibrium between PON1 Polymorphisms in Whites

WHITE	-1739	-1432	-1074	-160	55	192
-1739	-	0.7004	0.613	0.5641	0.6039	0.1545
-1432	<0.0001	-	0.836	0.8323	0.6986	0.1426
-1074	<0.0001	<0.0001	-	0.9339	0.7484	0.3213
-160	<0.0001	<0.0001	<0.0001	-	0.7227	0.3009
55	<0.0001	<0.0001	<0.0001	<0.0001	-	0.8926
192	<0.0001	0.0126	<0.0001	<0.0001	<0.0001	-

Table 12: Linkage Disequilibrium between PON1 Polymorphisms in Blacks

BLACK	-1739	-1432	-1074	-160	55	192
-1739	-	0.8001	0.8769	0.7236	0.341	0.7662
-1432	<0.0001	-	0.8375	0.6818	0.5124	0.3101
-1074	<0.0001	<0.0001	-	0.7442	0.7397	0.1266
-160	0.0003	<0.0001	<0.0001	-	0.8771	0.0411
55	<0.0001	<0.0001	0.0002	<0.0001	-	0.4686
192	<0.0001	0.0029	0.1917	0.6787	0.0044	-

3.3. DISTRIBUTION OF PON1 POLYMORPHISMS BETWEEN SLE CASES AND CONTROLS

3.3.1. Distribution of the Q192R Polymorphism

No significant associations were found between the Q192R SNP and SLE disease status. Due to the previously observed effect of R allele on PON activity, the R-carriers (QR+RR) were grouped for analysis. The difference in distribution between the R-carriers and QQ homozygotes was not significantly different between cases and controls in the white population or the black population. The allele frequency was not significantly different between cases and controls in white or black samples. These results are summarized in Table 13.

Table 13: Distribution of the Q192R SNP in SLE Cases and Controls

GENOTYPE	CASES		CONTROLS	
WHITES	**N**	**%**	**N**	**%**
QQ	165	50.6	252	56.6
QR	140	42.9	170	38.2
RR	21	6.4	23	5.2
TOTAL	326		445	
χ^2=2.85, p=0.24				
QQ vs. QR+RR: χ^2=2.74, p=0.098				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
Q	0.721		0.757	
R	0.279		0.243	
Z=-1.586, p=0.113				
	CASES		**CONTROLS**	
BLACKS	**N**	**%**	**N**	**%**
QQ	11	25.6	9	23.7
QR	22	51.2	22	57.9
RR	10	23.3	7	18.4
TOTAL	43		38	
χ^2=0.42, p=0.81				
QQ vs. QR+RR: χ^2=0.422, p=0.843				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
Q	0.512		0.526	
R	0.488		0.474	
Z=-0.178, p=0.859				

3.3.2. Distribution of the L55M Polymorphism

No significant associations were found between the L or M allele or the LM or MM genotypes and SLE disease status. A borderline significant association was found between the LL and LM + MM genotypes and the presence of SLE disease in the white samples (χ^2=4.00, p=0.046). The allele frequency was not significantly different between cases and controls in white or black samples. These results are summarized in Table 14.

Table 14: Distribution of the L55M SNP in SLE Cases and Controls

GENOTYPE	CASES		CONTROLS	
WHITES	**N**	**%**	**N**	**%**
LL	142	45.7	164	38.3
LM	132	42.4	205	47.9
MM	37	11.9	59	13.8
TOTAL	311		428	
χ^2=4.01, p=0.13				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
L	0.669		0.623	
M	0.331		0.377	
Z=-1.832, p=0.067				
	CASES		**CONTROLS**	
BLACKS	**N**	**%**	**N**	**%**
LL	29	67.4	22	57.9
LM	12	27.9	14	36.8
MM	2	4.7	2	5.3
TOTAL	43		38	
χ^2=0.81, p=0.67				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
L	0.814		0.763	
M	0.186		0.237	
Z=0.793, p=0.428				

3.3.3. Distribution of the A-1739G Polymorphism

No significant associations were found between the A or G allele and SLE disease status. Genotype distribution between the cases and controls was significantly different in the white samples (χ^2=6.43, p=0.040). The allele frequency was not significantly different between cases and controls in white or black samples. These results are summarized in Table 15.

Table 15: Distribution of the A-1739G SNP in SLE Cases and Controls

GENOTYPE	CASES		CONTROLS	
WHITES	**N**	**%**	**N**	**%**
AA	78	23.4	83	18.9
AG	134	40.2	216	49.3
GG	121	36.3	139	31.7
TOTAL	333		438	
χ^2=6.43, p=0.040				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
A	0.435		0.436	
G	0.565		0.564	
Z=-0.039, p=0.969				
	CASES		**CONTROLS**	
BLACKS	**N**	**%**	**N**	**%**
AA	29	67.4	25	64.1
AG	12	27.9	11	28.2
GG	2	4.7	3	7.7
TOTAL	43		39	
χ^2=0.35, p=0.841				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
A	0.814		0.782	
G	0.186		0.218	
Z=0.509, p=0.610				

3.3.4. Distribution of the C-1432G Polymorphism

No significant associations were found between the C or G allele or any of the genotypes and SLE disease status. The allele frequency was not significantly different between cases and controls in white or black samples. These results are summarized in Table 16.

Table 16: Distribution of the C-1432G SNP in SLE Cases and Controls

GENOTYPE	CASES		CONTROLS	
WHITES	**N**	**%**	**N**	**%**
CC	111	35.6	161	37.4
CG	157	50.3	214	49.8
GG	54	14.1	55	12.8
TOTAL	312		430	
χ^2=0.42, p=0.812				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
C	0.607		0.623	
G	0.393		0.377	
Z=-0.625, p=0.532				
	CASES		**CONTROLS**	
BLACKS	**N**	**%**	**N**	**%**
CC	4	10.3	9	22.5
CG	20	51.3	14	35.0
GG	15	38.5	17	42.5
TOTAL	39		40	
χ^2=2.19, p=0.335				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
C	0.359		0.400	
G	0.641		0.600	
Z=-0.532, p=0.595				

3.3.5. Distribution of the A-1074G Polymorphism

No significant associations were found between the A or G allele or the different genotypes and SLE disease status. The allele frequency was not significantly different between cases and controls in white or black samples. These results are summarized in Table 17.

Table 17: Distribution of A-1074G SNP in SLE Cases and Controls

GENOTYPE	CASES		CONTROLS	
WHITES	**N**	**%**	**N**	**%**
AA	155	50.0	220	53.0
AG	132	42.6	173	41.7
GG	23	7.4	22	5.3
TOTAL	310		415	
χ^2=1.63, p=0.443				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
A	0.726		0.744	
G	0.274		0.256	
Z=-0.546, p=0.585				
	CASES		**CONTROLS**	
BLACKS	**N**	**%**	**N**	**%**
AA	11	26.2	16	42.1
AG	25	59.5	18	47.4
GG	6	14.3	4	10.5
TOTAL	42		38	
χ^2=2.27, p=0.321				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
A	0.560		0.658	
G	0.440		0.352	
Z=-1.276, p=0.202				

3.3.6. Distribution of the G-160A Polymorphism

In whites, no significant associations were found between the A or G allele or the different genotypes and SLE disease status. In blacks, a borderline significant difference between the genotype distribution in cases and controls was found (χ^2=5.98, p=0.050). In addition, the black SLE cases were significantly more likely to be carriers of the A allele than controls (χ^2=5.28, p=0.022). The allele frequency was significantly different, with the A allele more frequent in the cases than controls (Z=-2.396, p=0.017). These results are summarized in Table 18.

Table 18: Distribution of the G-160A SNP in SLE Cases and Controls

GENOTYPE	CASES		CONTROLS	
WHITES	**N**	**%**	**N**	**%**
GG	167	51.7	247	55.8
GA	132	40.9	168	37.9
AA	24	7.4	28	6.3
TOTAL	323		443	
χ^2=1.32, p=0.517				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
G	0.721		0.747	
A	0.279		0.253	
Z=-1.135, p=0.256				
	CASES		**CONTROLS**	
BLACKS	**N**	**%**	**N**	**%**
GG	11	25.6	20	50.0
GA	24	55.8	17	42.5
AA	8	18.6	3	7.5
TOTAL	43		40	
χ^2=5.98, p=0.050				
ALLELE FREQUENCY	**CASES**		**CONTROLS**	
G	0.535		0.713	
A	0.465		0.288	
Z=-2.396, p=0.017				

3.4. HAPLOTYPE DISTRIBUTION IN SLE CASES AND CONTROLS

3.4.1. Haplotype Distribution in Whites

There are 64 possible haplotypes for the six SNPs analyzed in the present study. The G-160A SNP was not included in the analysis due to its strong linkage disequilibrium with the A-1074G SNP (D'=0.93). This decreased the degrees of freedom to increase the ability to detect significance. With this SNP excluded, there are 32 possible haplotypes for the 5 SNPs. Fourteen of these haplotypes were seen at frequencies of 1% or greater (these haplotypes are shown in Table 17). Of these fourteen haplotypes, four had significantly different frequencies in white

SLE cases and controls (highlighted in Table 17). The overall distribution of haplotypes was different between white SLE cases and controls, with borderline significance (p=0.051).

3.4.2. Haplotype Distribution in Blacks

There are 64 possible haplotypes for the six SNPs analyzed in the present study. The G-160A SNP was not included in the analysis due to its strong linkage disequilibrium with the A-1074G SNP. This decreased the degrees of freedom to increase the ability to detect significance. With this SNP excluded, there are 32 possible haplotypes for the 5 SNPs. Fifteen haplotypes had frequencies of 1% or greater (these haplotypes are shown in Table 18). One of the haplotypes (highlighted in Table 17) had a significantly different frequency in black SLE cases and controls. The overall distribution of haplotypes was not significantly different between black SLE cases and controls.

Table 19: Haplotype Distribution in Whites

Haplotype Distribution								
Allele at -1739	**Allele at -1432**	**Allele at -1074**	**Allele at 55**	**Allele at 192**	**Total n=633**	**Case n=257**	**Control n=376**	**p-value***
A	C	A	L	Q	0.0289	0.0346	0.0260	0.3866
G	C	A	L	Q	0.0927	0.0979	0.0883	0.5653
A	G	A	L	Q	0.0639	0.0609	0.0629	0.8839
A	C	G	L	Q	0.0126	0.0043	0.0158	0.0327
A	G	G	L	Q	0.1384	0.1393	0.1427	0.8663
G	G	G	L	Q	0.0405	0.0619	0.0233	0.0013
A	C	A	M	Q	0.0301	0.0179	0.0393	0.0199
G	C	A	M	Q	0.2677	0.2464	0.2868	0.1089
G	G	A	M	Q	0.0144	0.0035	0.0216	0.0022
A	G	G	M	Q	0.0152	0.0168	0.0129	0.5863
A	C	A	L	R	0.0452	0.0450	0.0427	0.8422
G	C	A	L	R	0.1096	0.1212	0.0992	0.2219
A	G	A	L	R	0.0464	0.0411	0.0496	0.4703
A	G	G	L	R	0.0501	0.0593	0.0449	0.2622
Association Studies								
ln(L)					-2518.93	-1012.98	-1483.51	
χ^2					1002.65	468.65	571.62	p**=0.051

*p-value calculated using Z-test

**p-value calculated using T5 statistic; $2[\ln(L)_{cases} + \ln(L)_{controls} - \ln(L)_{cases+controls}]$; $\chi 2$-distribution, df=31

Table 20: Haplotype Distribution in Blacks

Haplotype Distribution								
Allele at -1739	**Allele at -1432**	**Allele at -1074**	**Allele at 55**	**Allele at 192**	**Total n=68**	**Case n=35**	**Control n=33**	**p-value***
A	C	A	L	Q	0.0299	0.0316	0.0638	0.3801
G	C	A	L	Q	0.0764	0.0446	0.0903	0.2888
A	G	A	L	Q	0.0928	0.0543	0.0712	0.6852
A	C	G	L	Q	0.0265	0.0209	0.0223	0.9542
A	G	G	L	Q	0.1087	0.1797	0.1008	0.1812
G	G	G	L	Q	0.0124	0.0177	0.0000	0.2615
A	C	A	M	Q	0.0247	0.0251	0.0000	0.1791
G	C	A	M	Q	0.1101	0.1114	0.1212	0.8587
A	G	G	M	Q	0.0405	0.0000	0.0466	0.0726
A	C	A	L	R	0.0950	0.0494	0.1115	0.1824
A	G	A	L	R	0.1145	0.1224	0.1334	0.8477
G	G	A	L	R	0.0216	0.0000	0.0308	0.1477
A	G	G	L	R	0.2090	0.2246	0.1788	0.5047
A	G	A	M	R	0.0272	0.0621	0.0000	0.0313
A	G	A	M	R	0.0107	0.0152	0.0000	0.2986
Association Studies								
ln(L)					-268.64	-133.49	-127.43	
χ^2					101.83	58.49	56.44	p**=0.991

*p-value calculated using Z-test

**p-value calculated using T5 statistic; $2[\ln(L)_{cases} + \ln(L)_{controls} - \ln(L)_{cases+controls}]$; $\chi 2$-distribution, df=63

3.5. IMPACT OF PON1 POLYMORPHISMS ON ANTIBODY STATUS

3.5.1. Association of the Q192R Polymorphism with Antibody Status

The allele and genotype frequencies of the Q192R SNP were not significantly different between individuals with positive antibody status (one or more antibodies positive) and individuals with negative antibody status (all antibodies negative). These results are summarized in Table 21. Antibody association studies also were performed separately in SLE cases and controls (Table 22 and Table 23). No significant associations between the Q192R polymorphism and antibody status were found.

Table 21: Distribution of the Q192R SNP in Antibody Positive and Antibody Negative Individuals

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	QQ	QR	RR	Total	QQ	QR	RR	Total		
ACL	111	82	14	207	163	137	19	319	0.62	0.734
LAC	74	54	10	138	163	137	19	319	0.71	0.702
β_2GPI	86	84	12	182	163	137	19	319	0.69	0.708
ALL 3	17	13	4	34	163	137	19	319	1.76	0.415
ANY	199	152	24	375	163	137	19	319	0.42	0.809
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY		ANTIBODY NEGATIVE ALLELE FREQUENCY		Z	p
	Q	R	Q	R		
ACL	0.734	0.266	0.726	0.274	0.29	0.775
LAC	0.732	0.268	0.726	0.274	0.19	0.851
β_2GPI	0.703	0.297	0.726	0.274	-0.77	0.44
ALL 3	0.691	0.309	0.726	0.274	-0.60	0.55
ANY	0.733	0.267	0.726	0.274	0.29	0.77

Table 22: Distribution of the Q192R SNP in Antibody Positive and Antibody Negative SLE Cases

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	QQ	QR	RR	Total	QQ	QR	RR	Total		
ACL	37	39	9	85	72	58	6	136	4.00	0.135
LAC	40	36	7	83	72	58	6	136	1.64	0.441
β_2GPI	49	48	9	106	72	58	6	136	2.23	0.328
ALL 3	13	11	3	27	72	58	6	136	1.95	0.377
ANY	79	79	15	173	72	58	6	136	3.01	0.222
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	Q		R		Q		R			
ACL	0.665		0.335		0.743		0.257		-1.74	0.082
LAC	0.699		0.301		0.743		0.257		-0.99	0.322
β_2GPI	0.689		0.311		0.743		0.257		-1.31	0.192
ALL 3	0.685		0.315		0.743		0.257		-0.85	0.397
ANY	0.685		0.315		0.743		0.257		-1.59	0.111

Table 23: Distribution of the Q192R SNP in Antibody Positive and Antibody Negative Controls

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	QQ	QR	RR	Total	QQ	QR	RR	Total		
ACL	73	43	5	121	91	79	12	182	3.34	0.189
LAC	34	18	3	55	91	79	12	182	2.38	0.304
β_2GPI	37	36	3	76	91	79	12	182	0.85	0.653
ALL 3	4	2	1	7	91	79	12	182	0.99	0.608
ANY	120	73	9	202	91	79	12	182	3.01	0.222
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	Q		R		Q		R			
ACL	0.781		0.219		0.717		0.283		1.800	0.072
LAC	0.782		0.218		0.717		0.283		1.416	0.157
β_2GPI	0.724		0.276		0.717		0.283		0.162	0.872
ALL 3	0.714		0.286		0.717		0.283		-0.024	0.981
ANY	0.775		0.225		0.717		0.283		1.844	0.065

3.5.2. Association of the L55M Polymorphism with Antibody Status

The allele and genotype frequencies of the L55M SNP were not significantly different between individuals with positive antibody status for LAC, β_2GPI, all three or any antibodies and individuals with negative antibody status (all antibodies negative). The allele frequency of the L55M SNP was significantly different between individuals positive for the ACL antibody and individuals with negative antibody status (p=0.0303). These results are summarized in Table 24. Antibody association studies also were performed separately in SLE cases and controls (Table 25 and Table 26). No significant associations between the L55M polymorphism and antibody status were found.

Table 24: Distribution of the L55M SNP in Antibody Positive and Antibody Negative Individuals

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	LL	LM	MM	Total	LL	LM	MM	Total		
ACL	73	95	32	200	140	135	35	310	4.66	0.097
LAC	48	67	17	132	140	135	35	310	2.94	0.230
β_2GPI	68	78	19	165	140	135	35	310	0.72	0.697
ALL 3	13	14	3	30	140	135	35	310	0.12	0.941
ANY	136	170	50	356	140	135	35	310	3.56	0.169
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	L		M		L		M			
ACL	0.603		0.398		0.669		0.331		-2.17	0.030
LAC	0.617		0.383		0.669		0.331		-1.47	0.142
β_2GPI	0.648		0.352		0.669		0.331		-0.65	0.517
ALL 3	0.667		0.333		0.669		0.331		-0.03	0.975
ANY	0.621		0.379		0.669		0.331		-1.83	0.067

Table 25: Distribution of the L55M SNP in Antibody Positive and Antibody Negative SLE Cases

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	LL	LM	MM	Total	LL	LM	MM	Total		
ACL	30	38	11	79	63	55	15	133	1.79	0.408
LAC	33	37	9	79	63	55	15	133	0.69	0.709
β_2GPI	42	49	11	92	63	55	15	133	0.07	0.966
ALL 3	12	9	2	23	63	55	15	133	0.24	0.888
ANY	67	72	21	160	63	55	15	133	0.91	0.632
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	L		M		L		M			
ACL	0.620		0.380		0.680		0.320		-1.25	0.212
LAC	0.652		0.348		0.680		0.320		-0.59	0.555
β_2GPI	0.668		0.332		0.680		0.320		-0.27	0.790
ALL 3	0.717		0.283		0.680		0.320		0.51	0.609
ANY	0.644		0.356		0.680		0.320		-0.92	0.358

Table 26: Distribution of the L55M SNP in Antibody Positive and Antibody Negative Controls

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	LL	LM	MM	Total	LL	LM	MM	Total		
ACL	43	56	21	120	76	80	20	176	2.92	0.232
LAC	15	30	8	53	76	80	20	176	3.79	0.151
β_2GPI	26	39	8	73	76	80	20	176	1.41	0.493
ALL 3	1	5	1	7	76	80	20	176	2.36	0.307
ANY	69	98	29	196	76	80	20	176	2.74	0.254
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	L		M		L		M			
ACL	0.592		0.408		0.659		0.341		-1.65	0.099
LAC	0.566		0.434		0.659		0.341		-1.71	0.087
β_2GPI	0.623		0.377		0.659		0.341		-0.76	0.448
ALL 3	0.500		0.500		0.659		0.341		-1.17	0.242
ANY	0.602		0.398		0.659		0.341		-1.61	0.107

3.5.3. Association of the A-1739G Polymorphism with Antibody Status

The allele and genotype frequencies of the A-1739G SNP were not significantly different between individuals with positive antibody status (one or more antibodies positive) and individuals with negative antibody status (all antibodies negative). These results are summarized in Table 27. Antibody association studies also were performed separately in SLE cases and controls (Table 28 and Table 29). No significant associations between the A-1739G polymorphism and antibody status were found.

Table 27: Distribution of the A-1739G SNP in Antibody Positive and Antibody Negative Individuals

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	AA	AG	GG	Total	AA	AG	GG	Total		
ACL	41	85	76	202	64	154	96	314	3.12	0.210
LAC	28	60	50	138	64	154	96	314	1.58	0.454
β_2GPI	43	75	59	177	64	154	96	314	2.15	0.342
ALL 3	6	16	10	32	64	154	96	314	0.05	0.976
ANY	80	159	133	372	64	154	96	314	2.95	0.228
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	A		G		A		G			
ACL	0.413		0.587		0.449		0.551		-1.14	0.254
LAC	0.423		0.577		0.449		0.551		-0.73	0.467
β_2GPI	0.455		0.545		0.449		0.551		0.18	0.856
ALL 3	0.438		0.563		0.449		0.551		-0.18	0.854
ANY	0.429		0.571		0.449		0.551		-0.74	0.457

Table 28: Distribution of the A-1739G SNP in Antibody Positive and Antibody Negative SLE Cases

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	AA	AG	GG	Total	AA	AG	GG	Total		
ACL	17	34	32	83	32	53	49	134	0.34	0.843
LAC	17	36	31	84	32	53	49	134	0.44	0.801
β_2GPI	30	39	34	103	32	53	49	134	0.87	0.649
ALL 3	5	13	7	25	32	53	49	134	1.36	0.506
ANY	40	69	64	173	32	53	49	134	0.02	0.988
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	A		G		A		G			
ACL	0.410		0.590		0.437		0.563		-0.55	0.580
LAC	0.417		0.583		0.437		0.563		-0.41	0.681
β_2GPI	0.481		0.519		0.437		0.563		0.95	0.340
ALL 3	0.460		0.540		0.437		0.563		0.30	0.764
ANY	0.431		0.569		0.437		0.563		-0.15	0.882

Table 29: Distribution of the A-1739G SNP in Antibody Positive and Antibody Negative Controls

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	AA	AG	GG	Total	AA	AG	GG	Total		
ACL	24	51	44	119	32	101	47	180	5.47	0.065
LAC	11	24	19	54	32	101	47	180	2.42	0.298
β_2GPI	13	36	25	74	32	101	47	180	1.63	0.442
ALL 3	1	3	3	7	32	101	47	180	0.97	0.617
ANY	40	90	69	199	32	101	47	180	4.75	0.093
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	A		G		A		G			
ACL	0.416		0.584		0.458		0.542		-1.02	0.310
LAC	0.426		0.574		0.458		0.542		-0.59	0.556
β_2GPI	0.419		0.581		0.458		0.542		-0.81	0.420
ALL 3	0.357		0.643		0.458		0.542		-0.77	0.440
ANY	0.427		0.573		0.458		0.542		-0.86	0.391

3.5.4. Association of the C-1432G Polymorphism with Antibody Status

The allele and genotype frequencies of the C-1432G SNP were not significantly different between individuals with positive antibody status (one or more antibodies positive) and individuals with negative antibody status (all antibodies negative). These results are summarized in Table 30. Antibody association studies also were performed separately in SLE cases and controls (Table 31 and Table 32). In SLE cases, no significant associations between the C-1432G polymorphism and antibody status were found. In controls, the genotype distribution was significantly different between individuals positive for all three antibodies and those negative for all three antibodies (χ^2=7.0461, p=0.0295). Also in controls, the allele frequency of the C-1432G SNP was significantly different between individuals positive for the β_2GPI antibody and individuals negative for all three antibodies (Z=1.983, p=0.0473). In addition, the allele frequency of the C-1432G SNP was significantly different between individuals positive for all three antibodies and individuals negative for all three antibodies (Z=3.847, p=0.0001). No other significant associations were found.

Table 30: Distribution of the C-1432G SNP in Antibody Positive and Antibody Negative Individuals

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	CC	CG	GG	Total	CC	CG	GG	Total		
ACL	80	94	24	198	98	157	41	296	2.75	0.253
LAC	49	69	14	132	98	157	41	296	1.19	0.553
β_2GPI	65	80	22	167	98	157	41	296	1.61	0.447
ALL 3	13	15	4	32	98	157	41	296	0.73	0.694
ANY	141	173	42	356	98	157	41	296	3.03	0.220
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	C		G		C		G			
ACL	0.641		0.359		0.596		0.404		1.43	0.152
LAC	0.633		0.367		0.596		0.404		1.03	0.302
β_2GPI	0.629		0.371		0.596		0.404		0.99	0.321
ALL 3	0.641		0.359		0.596		0.404		0.71	0.477
ANY	0.639		0.361		0.596		0.404		1.59	0.112

Table 31: Distribution of the C-1432G SNP in Antibody Positive and Antibody Negative SLE Cases

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	CC	CG	GG	Total	CC	CG	GG	Total		
ACL	26	42	9	77	39	59	18	116	0.61	0.739
LAC	29	41	8	78	39	59	18	116	1.16	0.561
β_2GPI	32	45	15	92	39	59	18	116	0.08	0.961
ALL 3	8	14	4	26	39	59	18	116	0.09	0.956
ANY	57	81	20	158	39	59	18	116	0.51	0.774
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	C		G		C		G			
ACL	0.610		0.390		0.591		0.409		0.37	0.709
LAC	0.635		0.365		0.591		0.409		0.88	0.382
β_2GPI	0.592		0.408		0.591		0.409		0.02	0.984
ALL 3	0.577		0.423		0.591		0.409		-0.19	0.853
ANY	0.617		0.383		0.591		0.409		0.62	0.539

Table 32: Distribution of the C-1432G SNP in Antibody Positive and Antibody Negative Controls

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	CC	CG	GG	Total	CC	CG	GG	Total		
ACL	54	51	15	120	54	95	22	171	5.83	0.054
LAC	20	28	6	54	54	95	22	171	0.58	0.750
β_2GPI	33	33	6	72	54	95	22	171	4.69	0.096
ALL 3	5	1	0	6	54	95	22	171	7.05	0.030
ANY	84	92	22	198	54	95	22	171	4.62	0.099
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY		ANTIBODY NEGATIVE ALLELE FREQUENCY		Z	p
	C	G	C	G		
ACL	0.663	0.338	0.594	0.406	1.68	0.093
LAC	0.630	0.370	0.594	0.406	0.67	0.501
β_2GPI	0.688	0.313	0.594	0.406	1.98	0.047
ALL 3	0.917	0.083	0.594	0.406	3.84	0.000
ANY	0.657	0.343	0.594	0.406	1.77	0.077

3.5.5. Association of the A-1074G Polymorphism with Antibody Status

The allele frequency of the A-1074G SNP was not significantly different between individuals with positive antibody status (one or more antibodies positive) and individuals with negative antibody status (all antibodies negative). These results are summarized in Table 33. Antibody association studies also were performed separately in SLE cases and controls (Table 34 and Table 35). In SLE cases, no significant associations between the A-1074G polymorphism and antibody status were found. In controls, the allele frequency was significantly different between individuals positive for the LAC antibody and those negative for all three antibodies (Z=-2.053, p=0.0401). No other significant associations were found.

Table 33: Distribution of the A-1074G SNP in Antibody Positive and Antibody Negative Individuals

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	AA	AG	GG	Total	AA	AG	GG	Total		
ACL	108	71	13	192	155	132	16	303	2.26	0.323
LAC	60	54	13	127	155	132	16	303	3.56	0.169
β_2GPI	83	67	12	162	155	132	16	303	0.91	0.633
ALL 3	14	11	3	28	155	132	16	303	1.43	0.488
ANY	180	140	26	346	155	132	16	303	1.64	0.440
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	A		G		A		G			
ACL	0.747		0.253		0.729		0.271		0.63	0.529
LAC	0.685		0.315		0.729		0.271		-1.28	0.199
β_2GPI	0.719		0.281		0.729		0.271		-0.33	0.746
ALL 3	0.696		0.304		0.729		0.271		-0.52	0.607
ANY	0.723		0.277		0.729		0.271		-0.24	0.809

Table 34: Distribution of the A-1074G SNP in Antibody Positive and Antibody Negative SLE Cases

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	AA	AG	GG	Total	AA	AG	GG	Total		
ACL	40	33	4	77	62	60	8	130	0.37	0.831
LAC	41	30	7	78	62	60	8	130	1.44	0.487
β_2GPI	47	38	8	93	62	60	8	130	0.89	0.641
ALL 3	11	9	2	22	62	60	8	130	0.38	0.826
ANY	83	64	13	160	62	60	8	130	1.27	0.530
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	A		G		A		G			
ACL	0.734		0.266		0.708		0.292		0.57	0.567
LAC	0.718		0.282		0.708		0.292		0.22	0.827
β_2GPI	0.710		0.290		0.708		0.292		0.05	0.963
ALL 3	0.705		0.295		0.708		0.292		-0.04	0.968
ANY	0.719		0.281		0.708		0.292		0.29	0.771

Table 35: Distribution of the A-1074G SNP in Antibody Positive and Antibody Negative Controls

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	AA	AG	GG	Total	AA	AG	GG	Total		
ACL	67	38	9	114	92	72	8	172	2.85	0.240
LAC	19	24	6	49	92	72	8	172	5.56	0.062
β_2GPI	36	29	4	69	92	72	8	172	0.15	0.930
ALL 3	3	2	1	6	92	72	8	172	1.77	0.413
ANY	97	76	13	186	92	72	8	172	0.88	0.643
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	A		G		A		G			
ACL	0.754		0.246		0.744		0.256		0.27	0.787
LAC	0.633		0.367		0.744		0.256		-2.05	0.040
β_2GPI	0.732		0.268		0.744		0.256		-0.27	0.787
ALL 3	0.667		0.333		0.744		0.256		-0.56	0.577
ANY	0.726		0.274		0.744		0.256		-0.55	0.585

3.5.6. Association of the G-160A Polymorphism with Antibody Status

The allele frequency of the G-160A SNP was not significantly different between individuals with positive antibody status (one or more antibodies positive) and individuals with negative antibody status (all antibodies negative). These results are summarized in Table 36. Antibody association studies also were performed separately in SLE cases and controls (Table 37 and Table 38). No significant associations between the G-160A polymorphism and antibody status were found.

Table 36: Distribution of G-160A SNP in Antibody Positive and Antibody Negative Individuals

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	GG	GA	AA	Total	GG	GA	AA	Total		
ACL	118	69	16	203	167	129	18	314	3.03	0.220
LAC	68	58	12	138	167	129	18	314	1.57	0.456
β_2GPI	96	66	13	175	167	129	18	314	0.89	0.641
ALL 3	18	12	3	33	167	129	18	314	0.73	0.694
ANY	201	139	29	369	167	129	18	314	1.67	0.434
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	G		A		G		A			
ACL	0.751		0.249		0.737		0.263		0.51	0.614
LAC	0.703		0.297		0.737		0.263		-1.04	0.298
β_2GPI	0.737		0.263		0.737		0.263		0.00	1
ALL 3	0.727		0.273		0.737		0.263		-0.17	0.862
ANY	0.733		0.267		0.737		0.263		-0.17	0.867

Table 37: Distribution of the G-160A SNP in Antibody Positive and Antibody Negative SLE Cases

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	GG	GA	AA	Total	GG	GA	AA	Total		
ACL	44	32	5	81	68	58	8	134	0.299	0.861
LAC	43	33	7	83	68	58	8	134	0.613	0.736
β_2GPI	52	42	8	102	68	58	8	134	0.361	0.835
ALL 3	13	11	2	26	68	58	8	134	0.111	0.946
ANY	89	66	14	169	68	58	8	134	0.931	0.628
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	G		A		G		A			
ACL	0.741		0.259		0.724		0.276		0.387	0.699
LAC	0.717		0.283		0.724		0.276		-0.158	0.875
β_2GPI	0.716		0.284		0.724		0.276		-0.192	0.848
ALL 3	0.712		0.288		0.724		0.276		-0.175	0.861
ANY	0.722		0.278		0.724		0.276		-0.055	0.956

Table 38: Distribution of the G-160A SNP in Antibody Positive and Antibody Negative Controls

ANTIBODY +	ANTIBODY POSITIVE GENOTYPE				ANTIBODY NEGATIVE GENOTYPE				χ^2	p
	GG	GA	AA	Total	GG	GA	AA	Total		
ACL	74	37	11	122	99	71	10	180	3.35	0.188
LAC	25	25	5	55	99	71	10	180	1.92	0.382
β_2GPI	44	24	5	73	99	71	10	180	1.00	0.607
ALL 3	5	1	1	7	99	71	10	180	2.31	0.315
ANY	112	73	15	200	99	71	10	180	0.78	0.678
ANTIBODY +	ANTIBODY POSITIVE ALLELE FREQUENCY				ANTIBODY NEGATIVE ALLELE FREQUENCY				Z	p
	G		A		G		A			
ACL	0.758		0.242		0.747		0.253		0.308	0.758
LAC	0.682		0.318		0.747		0.253		-1.301	0.193
β_2GPI	0.767		0.233		0.747		0.253		0.478	0.633
ALL 3	0.786		0.214		0.747		0.253		0.348	0.728
ANY	0.743		0.258		0.747		0.253		-0.158	0.875

3.6. IMPACT OF PON1 POLYMORPHISMS ON PARAOXONASE ACTIVITY

3.6.1. PON Activity Data Analysis

PON activity data was transformed by taking the square root to ensure normal distribution. Covariates possibly affecting PON activity (age, BMI and smoking) were investigated, but were not found to significantly affect PON activity ($p>0.05$ for each). Further analysis was therefore not adjusted for these covariates. ANOVA analyses were performed on the transformed data to determine the significance of PON1 SNPs' effects on PON activity. Within each genotype, PON activity was consistently lower in cases than in controls in whites and blacks.

3.6.2. Association of the Q192R Polymorphism with PON Activity

PON activity was significantly different across genotypes for the Q192R SNP, with the RR genotype having the highest PON activity in all groups ($p<0.0001$ in white SLE cases, white controls, black SLE cases and black controls). This is summarized in Table 39. In addition, multiple linear regression analysis showed that the Q192R SNP had the most significant impact on PON activity of all the SNPs studied.

Table 39: Impact of Q192R SNP on PON Activity (+/-SD)

	PON1 192 GENOTYPE			
WHITE SUBJECTS	**QQ**	**QR**	**RR**	**p-value***
SLE CASES	n=146 352.30+/-249.82	n=133 821.55+/-323.46	n=18 1306.46+/-478.29	<0.0001
CONTROLS	n=246 457.03+/-342.80	n=161 992.28+/-415.50	n=20 1695.89+/-704.50	<0.0001
	PON1 192 GENOTYPE			
BLACK SUBJECTS	**QQ**	**QR**	**RR**	**p-value***
SLE CASES	n=7 430.75+/-560.11	n=21 966.18+/-418.60	n=10 1311.78+/-308.28	<0.0001
CONTROLS	n=9 753.77+/-764.74	n=22 1021.04+/-393.11	n=7 2055.61+/-514.55	<0.0001

*p-value determined from ANOVA analysis

3.6.3. Association of the L55M Polymorphism with PON Activity

PON activity was significantly different across genotypes for the L55M SNP, with the LL genotype having the highest PON activity in white SLE cases ($p<0.0001$), white controls ($p<0.0001$), and black SLE cases ($p=0.0009$). PON activity was not significantly different across L55M genotypes in black controls. This is summarized in Table 40.

Table 40: Impact of the L55M SNP on PON Activity (+/-SD)

	PON1 55 GENOTYPE			
WHITE SUBJECTS	**LL**	**LM**	**MM**	**p-value***
SLE CASES	n=124 789.97+/-452.20	n=120 563.51+/-341.75	n=34 238.78+/-118.48	<0.0001
CONTROLS	n=157 933.78+/-574.19	n=195 683.16+/-445.09	n=58 281.05+/-140.79	<0.0001
	PON1 55 GENOTYPE			
BLACK SUBJECTS	**LL**	**LM**	**MM**	**p-value***
SLE CASES	n=24 1189.45+/-466.48	n=10 591.87+/-466.10	n=2 436.01+/-14.03	0.0009
CONTROLS	n=22 1284.98+/-698.99	n=14 1020.76+/-683.21	n=2 710.72+/-499.89	0.3001

*p-value determined from ANOVA analysis of transformed (square root) PON activity

3.6.4. Association of the A-1739G Polymorphism with PON Activity

PON activity was significantly different across genotypes for the A-1739G SNP, with the AA genotype having the highest PON activity in white SLE cases ($p<0.0001$), white controls ($p<0.0001$), and black controls ($p=0.0155$). PON activity was not significantly different across L55M genotypes in black SLE cases. This is summarized in Table 41.

Table 41: Impact of the A-1739G SNP on PON Activity (+/-SD)

	PON1 -1739 GENOTYPE			
WHITE SUBJECTS	**AA**	**AG**	**GG**	**p-value***
SLE CASES	n=68 792.62+/-482.30	n=121 639.66+/-392.40	n=103 481.22+/-341.52	<0.0001
CONTROLS	n=78 974.36+/-635.22	n=209 734.84+/-482.26	n=133 517.91+/-390.49	<0.0001
	PON1 -1739 GENOTYPE			
BLACK SUBJECTS	**AA**	**AG**	**GG**	**p-value***
SLE CASES	n=25 1068.44+/-488.48	n=11 840.33+/-571.23	n=1 275.12	0.1347
CONTROLS	n=25 1375.83+/-729.10	n=11 840.41+/-301.75	n=3 461.09+/-261.32	0.0155

*p-value determined from ANOVA analysis of transformed (square root) PON activity

3.6.5. Association of the C-1432G Polymorphism with PON Activity

PON activity was not significantly different across genotypes for the C-1432G SNP, with the exception of the white controls (p=0.0203). In this group, the GG genotype had the highest PON activity. PON activity was not significantly different across C-1432G genotypes in white SLE cases, black SLE cases or black SLE cases. This is summarized in Table 42.

Table 42: Impact of the C-1432G SNP on PON Activity (+/-SD)

	PON1 -1432 GENOTYPE			
WHITE SUBJECTS	**CC**	**CG**	**GG**	**p-value***
SLE CASES	n=90 586.67+/-427.37	n=134 617.73+/-397.76	n=36 649.74+/-431.88	0.5100
CONTROLS	n=153 642.24+/-481.67	n=208 758.46+/-500.39	n=52 805.36+/-654.87	0.0203
	PON1 -1432 GENOTYPE			
BLACK SUBJECTS	**CC**	**CG**	**GG**	**p-value***
SLE CASES	n=4 700.96+/-514.69	n=16 817.98+/-459.85	n=14 1058.77+/-416.44	0.2603
CONTROLS	n=9 874.48+/-370.05	n=17 1133.07+/-536.67	n=14 1375.57+/-898.77	0.3946

*p-value determined from ANOVA analysis of transformed (square root) PON activity

3.6.6. Association of the A-1074G Polymorphism with PON Activity

PON activity was not significantly different across genotypes for the A-1074G SNP. This is summarized in Table 43.

Table 43: Impact of the A-1074G SNP on PON Activity (+/-SD)

	PON1 -1074 GENOTYPE			
WHITE SUBJECTS	**AA**	**AG**	**GG**	**p-value***
SLE CASES	n=139 613.76+/-413.36	n=117 613.34+/-377.45	n=20 738.18+/-583.91	0.5243
CONTROLS	n=209 716.15+/-499.57	n=169 733.23+/-533.96	n=19 744.84+/-433.66	0.8213
	PON1 -1074 GENOTYPE			
BLACK SUBJECTS	**AA**	**AG**	**GG**	**p-value***
SLE CASES	n=10 901.77+/-476.65	n=20 965.12+/-488.12	n=6 866.57+/-402.98	0.9826
CONTROLS	n=16 891.51+/-387.38	n=18 1359.23+/-826.58	n=4 1178.77+/-646.34	0.2564

*p-value determined from ANOVA analysis of transformed (square root) PON activity

3.6.7. Association of the G-160A Polymorphism with PON Activity

PON activity was not significantly different across genotypes for the G-160A SNP. This is summarized in Table 44.

Table 44: Impact of the G-160A SNP on PON Activity (+/- SD)

	PON1 -160 GENOTYPE			
WHITE SUBJECTS	**GG**	**GA**	**AA**	**p-value***
SLE CASES	n=150 605.41+/-425.00	n=118 608.15+/-372.87	n=21 765.45+/-582.56	0.3003
CONTROLS	n=235 688.82+/-496.39	n=166 757.25+/-559.59	n=24 695.38+/-397.68	0.3058
	PON1 -160 GENOTYPE			
BLACK SUBJECTS	**GG**	**GA**	**AA**	**p-value***
SLE CASES	n=10 844.07+/-386.83	n=20 1074.96+/-626.91	n=7 979.11+/-424.27	0.8033
CONTROLS	n=20 1070.69+/-563.76	n=17 1126.42+/-751.58	n=3 2028.88+/-286.07	0.0869

*p-value determined from ANOVA analysis of transformed (square root) PON activity

3.6.8. Interactions between and Independence of SNPs and Effects on PON Activity

Interaction studies were performed between coding SNPs (Q192R and L55M) and promoter SNPs to determine the effects of interactions on PON activity. In white controls, the Q192R and C-1432G SNPs showed significant interaction (p=0.017). In white cases, the Q192R SNP showed significant interaction with both the C-1432G SNP (p=0.028), the A-1074G SNP (p=0.004) and the G-160A SNP (p=0.008). No significant interactions were seen between the L55M SNP and the promoter SNPs in the white SLE cases or white controls. In black samples, no significant interactions were seen between the Q192R SNP and the promoter SNPs. In black cases, the L55M and G-160A SNPs showed significant interaction (p=0.037). P-values from generated by ANOVA interaction analysis are summarized in Table 45, with significant values highlighted.

Table 45: Interaction between Coding SNPs and Promoter SNPs and PON Activity

WHITE CONTROLS	A-1739G	C-1432G	A-1074G	G-160A
Q192R	0.092	0.017	0.076	0.065
L55M	0.460	0.818	0.483	0.409
WHITE CASES	A-1739G	C-1432G	A-1074G	G-160A
Q192R	0.460	0.028	0.004	0.008
L55M	0.650	0.282	0.064	0.058
BLACK CONTROLS	A-1739G	C-1432G	A-1074G	G-160A
Q192R	0.773	0.164	0.528	0.459
L55M	0.189	0.908	0.446	0.835
BLACK CASES	A-1739G	C-1432G	A-1074G	G-160A
Q192R	0.080	0.314	0.504	0.872
L55M	0.643	0.452	0.231	0.037

Multiple linear regression was performed to determine whether the significant effects of SNPs on PON activity were independent of one another. The Q192R SNP had an independent, significant impact on PON activity in all groups studied. It accounted for the greatest portion of the variation on PON activity (approximately 41% - 50%). The L55M SNP had an independent, significant impact on PON activity in white cases, white controls, and black cases. This SNP had accounted for less of the variation in PON activity than the Q192R SNP (approximately 20% in whites and 35% in black cases). The A-1432G SNP was not independently significant in white controls. The A-1739 SNP had an independent, significant impact on PON activity in white cases and white controls. The A-1739G SNP accounted for approximately 10% of the variation in PON activity in white cases and white controls.

Table 46: Multiple Linear Regression Analysis

	WHITE CONTROLS		WHITE CASES		BLACK CONTROLS		BLACK CASES	
SNP	**p-value**	**R^2x100**	**p-value**	**R^2x100**	**p-value**	**R^2x100**	**p-value**	**R^2x100**
A-1739G	<0.0001	11.01	0.0019	8.67	0.2938	20.66	0.3125	11.12
C-1432G	0.1867	1.88	-	-	-	-	-	-
L55M	0.0002	20.99	<0.0001	21.98	0.9571	6.65	0.0123	34.58
Q192R	<0.0001	44.49	<0.0001	50.43	0.0031	41.33	0.0001	48.85

4. DISCUSSION AND SUMMARY

Coronary heart disease (CHD) is a leading cause of death in the United States. According to the American Heart Association, cardiovascular diseases are the number one cause of death in males and females of all ethnic groups, with CHD accounting for 53% of those deaths. Since the 1970s, numbers of people affected with CHD have continued to rise universally, resulting in major public health concerns (American Heart Association, 2004). In addition to environmental and biological risk factors known to play a role in CHD, genetics have a significant impact on CHD risk and development. The importance of understanding the genetics of CHD has become evident as more and more individuals develop the disease. Studying the genetics of common complex diseases such as CHD can facilitate the understanding of disease etiology, leading to the opportunity for research into appropriate treatments. Association studies such as the one presented here will allow for a better understanding of the risks associated with individual genes and the impact of common genetic variation on disease risk. Identification of candidate genes for CHD, particularly those directly involved in lipid metabolism (like PON1) has allowed for a wealth of research and many opportunities for future study.

Case-control cohorts play an important role in understanding the effects of candidate genes on disease risk through association studies. Due to the markedly increased risk of CHD in SLE, a cohort of SLE cases was selected to investigate PON1 as a candidate gene for CHD. The Pittsburgh SLE population used in this study has been well-studied, and a wealth of clinical and biological data are available on these patients. Prior to beginning association analyses, homogeneity tests were performed to determine the genotype distribution in individuals of European descent and individuals of African descent. Since genotype distributions and allele

frequencies were significantly different between these populations, all further analyses were performed separately in white and black samples.

In this study, we examined eight SNPs in PON1 (three in the coding region and five in the promoter). Six of these SNPs were informative because they were commonly distributed. No significant associations were seen between the Q192R, C-1432G, or A-1074G SNPs and SLE disease in white or black samples.

Some borderline significant associations were seen between PON1 SNPs and SLE disease in the white population. A borderline significant association was found between the LL and LM+MM genotypes and the presence of SLE disease in white samples (χ^2=4.00, p=0.046). These data suggest that the M allele is protective against SLE disease (OR=0.73, 95% Confidence Interval 0.54-0.98). The 95% confidence interval of the odds ratio approaches 1, reflecting the borderline significance of this association. In addition, a significant effect of the L55M SNP on PON activity was seen in this study, with the L isoform associated with higher PON activity in white cases (p<0.0001), white controls (p<0.0001), and black cases (p=0.0009). The sample size of the black controls (n=38) may have been too small to detect statistically significant differences; however a trend is present with the L isoform having higher PON activity (LL homozygous individuals have highest PON activity [1284.98+/-698.99], followed by LM heterozygotes [1020.76+/-683.21] and finally MM homozygotes [710.72+/-499.89]). Although it has not has been previously shown to have a significant impact on PON activity, the L55M SNP has been shown to impact PON serum levels (reviewed in Costa et al., 2003). Since PON serum levels were not measured in this study, it is possible that individuals with the L isoform have higher

PON levels, resulting in higher PON activity, rather than an intrinsic difference in the catalytic ability of PON. Measurement of PON levels in serum in these patients could help to determine the mechanism behind this difference. Higher PON levels in individuals with the L isoform could explain the higher PON activity and would be consistent with previous studies. The association of the M allele with SLE disease status could be due to lower PON levels or PON activity associated with the M allele. SLE cases have been shown in this study to consistently have lower PON activity; it is possible that the lower activity M allele is a risk factor for SLE disease due to its effects on PON activity. However, due to the borderline association seen in this study, further study with a larger sample of SLE cases would be helpful at clarifying this association.

Another borderline association with SLE disease in whites was found at the -1739 position. The genotype distribution of the A-1739G SNP was significantly different in white cases vs. white controls (χ^2=6.43, p=0.040). In white cases, 23.4% were AA homozygotes (compared to 18.9% of controls), 40.2% were AG heterozygotes (compared to 49.3% of controls) and 36.3% were GG homozygotes (compared to 31.7% of controls). These data suggest that the heterozygous state is protective against SLE disease (Odds Ratio=0.66, 95% Confidence Interval 0.45-0.97). The 95% confidence interval of the odds ratio approaches 1, reflecting the borderline significance of this association. However, this would not fit into a model of lower PON activity conferring SLE disease risk since the AG genotype has intermediate activity in both cases and controls. Further study of this SNP with a greater number of cases and controls could help to clarify this association. To our knowledge, the impact of the A-1739G SNP on PON activity has not been reported. In this study, it showed a significant effect on PON activity in white cases

($p<0.0001$), white controls ($p<0.0001$) and black controls (0.0155). The sample size of the black cases (n=37) may have been too small to detect statistically significant differences; however a trend is present with the A isoform having higher PON activity. Although the A-1739G was in linkage disequilibrium with both the coding SNPs in both whites and blacks, interaction studies did not show significant interactions between the coding SNPs and A-1739G in whites or blacks. Therefore, the A-1739G SNP has an independent effect on PON activity. Future study to determine the cause of the impact of this SNP on PON activity could include PON serum level measurement (to determine whether the SNP impacts the serum level of PON or the catalytic properties of PON1) and molecular biological expression studies to determine how the SNP affects gene expression and, ultimately, PON activity. This could offer some additional information about how the SNP might contribute to SLE disease risk as well.

In blacks, a significant difference between the allele frequency in SLE cases and controls was found ($Z=-2.396$, $p=0.017$), with the A allele more frequent in the cases than controls. In addition, the genotype distribution between cases and controls was borderline significantly different ($\chi^2=5.98$, $p=0.050$). Black SLE cases were significantly more likely to be carriers of the A allele than controls ($\chi^2=5.28$, $p=0.022$). These data suggest that the -160A allele is a risk allele for SLE disease (Odds Ratio=2.91, 95% Confidence Interval 1.15-7.33). The wide 95% confidence interval of the odds ratio and the fact that the lower limit approaches 1 reflects the small sample size used in calculating the odds ratio. Larger sample size could make the confidence interval narrower to clarify the association with greater confidence. While no significant differences in PON activity were seen associated with the G-160A SNP, the trend in PON activity associated with the SNP was different in cases and controls. In black SLE cases,

the individuals with the -160GA genotype had the highest PON activity (1074.96+/-626.91), followed by the -160AA homozygotes (979.11+/-424.27) and finally the -160GG homozygotes (844.07+/-386.83). In black controls, however, the -160AA homozygotes had the highest PON activity (2028.88+/-286.07), followed by the -160AG heterozygotes (1126.42+/-751.58) and finally the -160GG homozygotes (1070.69+/-563.76). Larger black sample sizes would be helpful in clarifying the role of the G-160A SNP in PON activity in the SLE cases and controls. However, given the results of this study, it appears that there may be some gene-gene or gene-environment interaction affecting the PON activity of SLE cases carrying the A allele (perhaps the presence of APA) that alters the trend of PON activity when compared to controls.

PON activity has been shown to play a role in CHD risk, with low PON activity associated with greater risk for CHD. Significant differences between several of the PON1 SNPs and PON activity were seen in the present study. The Q192R SNP has been well-studied and has previously been shown to have a significant impact on PON activity (reviewed in Nakanishi, 2003). When paraoxon is used as a substrate, the R isoform is associated with greater PON activity. The results presented here show this pattern as well, and are consistent with previous studies (reviewed in Nakanishi, 2003). Although case-control studies have provided conflicting data on whether or not this SNP is a significant independent factor for CHD, in a high-risk population such as the SLE cohort, it may be possible to discern whether or not the SNP confers a risk for CHD. Further analysis of this data with information regarding cardiovascular events could establish whether the Q192R SNP could be used as a marker for CHD risk assessment in SLE patients. Although the Q192R SNP is significantly associated with PON activity and PON activity is significantly lower in SLE cases than controls, no association of this SNP was

observed with SLE disease risk. This further reinforces that SLE is most likely a multifactorial disease; while the Q192R SNP may affect PON activity and possibly influence disease risk, it cannot be seen as an independent risk factor for SLE disease.

Interaction studies showed that the Q192R SNP did have significant interaction with some of the promoter SNPs. In white controls, it interacted with the C-1432G SNP (p=0.017). In the initial PON activity analysis, the C-1432G SNP had a significant impact on PON activity (p=0.020). However, when multiple linear regression was performed for the C-1432G SNP to adjust for the effects of other significant SNPs, it was found not to be an independent factor (p=0.187). This suggests that the two SNPs may interact with each other, however the Q192R SNP has a greater impact on PON activity than the C-1432G SNP. Other significant interactions were seen in white cases. The Q192R SNP showed significant interactions with all of the promoter SNPs except for the A-1739G SNP. In addition, multiple linear regression showed that the A-1739G SNP has an independent effect on PON activity. As discussed above, further study of the biological mechanisms and importance of this SNP's impact on PON activity and SLE disease risk would help to clarify its role and possible clinical significance.

Haplotype analysis in the white population showed some significant differences between cases and controls. The -1739A,-1432C, -1074G, 55L, 192Q haplotype was seen significantly more often in controls (frequency = 1.58%) than in cases (frequency = 0.43%; p=0.0327). The -1739G,-1432G, -1074G, 55L, 192Q haplotype was seen significantly more often in cases (frequency = 6.19%) than in controls (frequency = 2.33%; p=0.0013). The -1739A,-1432C, -1074A, 55M, 192Q haplotype was seen significantly more often in controls (frequency = 3.93%)

than in cases (frequency = 1.79%; p=0.0199). The -1739G,-1432G, -1074A, 55M, 192Q haplotype was seen significantly more often in controls (frequency = 2.16%) than in cases (frequency = 0.35%; p=0.0022). Overall, the haplotype distribution in whites was borderline significantly different (p=0.051). In blacks, one haplotype had a significantly different frequency in SLE cases than controls. The -1739A,-1432C, -1074A, 55M, 192R haplotype was seen significantly more often in cases (frequency = 6.21%) than in controls (frequency = 0.00%; p=0.0313).

These differences in haplotype distribution indicate that PON1 may play a role the SLE risk. Larger sample size could help to determine the true significance of borderline associations. With further study, these markers may be informative for determining SLE risk. These results bring about the question of how PON1 could be involved in SLE etiology. Due to the borderline nature of the significant differences, it is unlikely that PON1 is an independent factor for development of SLE. As SLE is a complex disease, there are very likely a number other genetic and/or environmental factors that may be involved that cannot be determined from the present study.

The general lack of strong association between PON1 polymorphisms and SLE disease could be due to several reasons. One possibility is that, although PON1 is a candidate gene for SLE, these SNPs are unrelated to SLE disease risk. As discussed, there are nearly 200 SNPs in PON1 and this study focused on a small percentage of that genetic variation. Other SNPs in PON1 may play a role, or perhaps individuals with SLE have other, less common or yet-undiscovered changes in PON1 has affected their disease status. Gene-gene and gene-environment

interactions not investigated in this study are thought to play a large role in the development of complex diseases like SLE and CHD. Unfortunately, the number of factors involved could be infinite, where association studies such as this are very limited in scope and inability to identify other players in disease risk.

As discussed, individuals with SLE have a higher rate of APA than individuals in the general population. APA are significant risk factors for CHD. Very few significant associations were found between PON1 SNPs and the presence of APA. The allele frequency of the L55M SNP was significantly different between individuals positive for the ACL antibody (L: 0.603, M: 0.398) and individuals negative for all antibodies (L: 0.669, M: 0.331; p=0.030). The genotype distribution (χ^2=7.05, p=0.030) and allele frequency of the C-1432G SNP were significantly different between controls positive for all three antibodies (C: 0.917, G: 0.083) vs. controls negative for all antibodies (C: 0.594, G: 0.406; p=0.000). The allele frequency of the C-1432G SNP was also significantly different between controls positive for the β_2GPI antibody (C: 0.688, G: 0.313) vs. controls negative for all antibodies (C: 0.594, G: 0.406; p=0.000). Lastly, the allele frequency of the A-1074G SNP was significantly different between controls positive for the LAC antibody (A: 0.633, G: 0.367) and controls negative for all three antibodies (A: 0.744, G: 0.256; p=0.040). No discernable pattern was discerned between these significant differences; therefore it is unlikely that these differences represent an underlying biological difference by which PON1 affects the development of APA. Future study with larger sample sizes could help to determine whether these differences are meaningful or are statistical coincidence.

While PON1 has not been implicated in the presence of APA, APA have been shown to impact PON activity (Delgado Alves et al., 2002). Further analysis of available data could help to

determine the effect of APA on PON activity. Since, for the most part, SNP distributions were not significantly different between cases and controls, analysis of PON activity and antibody status could help to define the interaction between APA and PON activity. In addition, analysis of PON activity measurements between cases and controls in conjunction with cardiovascular events could help to establish a profile of SLE patients at high risk for CHD. This could have clinical implications for SLE patients and, with further study, for determining an individual's risk to develop CHD in the general population, when combined with other known risk factors. Genotype data may be useful in predicting PON activity, which in turn could help to predict CHD risk.

In summary, we have performed association studies between six polymorphisms in PON1 and SLE disease status, antibody status, and PON activity. Significant findings from this study include:

1. The PON1 polymorphisms examined in the present study have significantly different allele frequencies and genotype distributions in subjects of European descent and subjects of African descent.
2. The PON1 polymorphisms examined in the present study are not significantly associated with SLE disease status.
3. The PON1 polymorphisms examined in the present study are not significantly associated with the presence of APA.
4. Three PON1 polymorphisms (Q192R, L55M and A-1739G) significantly impact *in vitro* PON activity.

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